项目编号： 2018KW-041

管理类型： 项目类

项目类别： 国际科技合作计划项目-一般项目

陕西省重点研发计划
项目验收申请书

验申研字[2021]第0702号

项目名称： 川崎病冠脉病变的干细胞疗法

承担单位： 陕西省人民医院 （盖章）

项目负责人： 焦富勇

推荐部门： 陕西省卫生和计划生育委员会

申请验收日期： 2021-03-09

陕西省科学技术厅 制

一、项目信息情况

项目名称	川崎病冠脉病变的干细胞疗法	验收类型	正常验收
项目类别	国际科技合作计划项目-一般项目		
项目编号	2018KW-041		
项目起止时间	2018-01-01 至 2020-12-31	项目负责人	焦富勇
承担单位	陕西省人民医院	邮政编码	710068
单位地址	西安市碑林区友谊西路256号		
联系人	李盼	联系电话	029-68820605

项目概况：

1、主要研究内容与目标完成情况

主要研究内容：

1)使用干酪乳杆菌细胞壁萃取物成功建立川崎病冠脉病变小鼠模型；

2)成功分离、培养、冻存符合质量标准的脐带来源间充质干细胞；

3)动物实验中，验证脐带间充质干细胞修复动物模型病变冠脉存在积极作用；

目标完成情况：

本研究已经完成了川崎病冠脉病变动物模型的建模；成功分离、培养、冻存了符合质量标准的脐带来源间充质干细胞；同时将干细胞成功应用于川崎病冠脉病变动物模型，并且通过实验对照，采用高频超声仪器进行数据采集、数据对比、数据分析，获得干细胞对川崎病冠脉病变的积极作用结果。该项目的顺利进行，有望成为避免使用阿司匹林治疗引起胃粘膜损伤、肝损害、肾损害等问题的优选治疗方案；同时也有效解决了部分患者对阿司匹林不耐受或免疫球蛋白耐药的问题。

2、技术创新点与关键技术

创新点及关键技术：
1.建立了使用干酪乳杆菌细胞萃取物，成功建立川崎病冠脉病变小鼠模型；
2.用脐带间充质干细胞修复川崎病动物模型病变冠脉,为干细胞治疗川崎冠脉病变提供了临床依据。
关键技术：
1.干酪乳杆菌细胞提取物成功建立川崎病冠脉病变小鼠模型，国外曾有实验室成功建立川崎病冠脉病变模型，但是LC WE诱导川崎病小鼠模型的建立在西北尚属首次，实验中，我院团队克服重重困难，成功建立此模型为项目的成功打下坚实的理论基础。
2.脐带间充质干细胞修复川崎病动物模型病变冠脉。（1）脐带来源间充干细胞培养及鉴定，在临床前期的充分沟通及配合下，获得实验所需的材料进行细胞培养及鉴定的相关工作；（2）川崎病小鼠模型注射脐带间充干细胞疗效评价，评价工作中我们要测量小鼠的冠状动脉内径，观察其冠状动脉脉壁是否粗糙，有无增厚以及回音情况。但幼年小鼠冠脉内径较细给测量工作带来了极大困难，项目工作人员反复测量多次，实验中积累了大量LCWE诱导川崎病小鼠模 型的冠状动脉超声及病理结果的相关评价数据。

3、取得的成果与经济、社会效益

成果：（1）川崎病基础与临床研究获得了陕西省科技进步二等奖；（2）发表关于干细胞的学术论文三篇；（3）发表关于川崎病的论文20余篇，被国内外引用70余次；（4）建立了使用酪乳杆菌细胞萃取物成功建立川崎病冠状动脉小鼠病变模型；

社会效益：本项目有望寻找到新型方法——干细胞疗法治疗川崎病引起的冠脉病变。第一，可提高川崎病的诊断与治愈率；避免传统使用阿司匹林治疗可能会引起患者胃粘膜损伤、肝损害、肾损害等问题；有效解决部分患者阿司匹林不耐受以及耐免疫球蛋白的问题，对我国儿童后天性心脏病以及心血管疾病方面的研究有十分重要的意义。第二，项目的成功实施缩短了我国与发达国家在川崎病治疗方面的差距。项目相关成果转化为针对川崎病的干细胞药物可以推向市场，填补我国干细胞药品方面的不足。第三，传统使用丙种球蛋白治疗川崎病，花费较大，很多家庭无力承担，若项目可以成功实施，则有望减少川崎病治疗的花费减轻家庭和国家经济负担；针对川崎病的干细胞药物市场巨大，若能正式推向市场可以带来巨大的经济效益。

4、合同规定考核指标的完成情况

(1) 项目指标

① 项目负责人负责本课题总体控制，包括课题进展情况、实验结果汇总情况、经费使用情况及论文发表情况等。项目成员负责相关文献的查阅，动物实验及临床实验的结果准确、可靠；数据整理汇总；论文撰写等。严格按照项目分工完成项目任务。

② 动物实验考核

实验动物来源可靠；动物房规范化管理；动物实验操作规范。

④ 经费考核

建立了财务支出报表，专款专用。

(2) 成果

① 开发出川崎病引起的冠脉病变的干细胞疗法；

② 发表学术论文3篇；

④ 申报省部级科技进步奖1项。

二、项目人员情况

项目负责人					
姓名	焦富勇	性别	男	出生年月	1951-01-03
学历	本科生	职务	儿童病院名誉院长	职称	主任医师
从事专业	儿科学			手机	15398082028

项目组主要参与人员								
姓名	出生年月	性别	从事专业	职称	学历	所在单位	项目分工	签名
戴博	1977-05-10	男	干细胞生物学	教授	博士研究生	陕西九州生物医药科技	干细胞质控	
李盼	1993-02-16	女	干细胞生物学	未取得	硕士研究生	陕西九州生物医药科技	动物实验	
Lvova Olga	1974-05-07	女	儿科学	教授	博士研究生	Ural Federal University	项目技术优化	
郭康合	1970-07-05	男	干细胞生物学	高级工程师	硕士研究生	陕西九州生物医药科技	细胞库管理	
王杰民	1962-04-29	男	儿科学	主任医师	硕士研究生	陕西省人民医院	临床实验管理	
蔺婧	1985-01-27	女	儿科学	讲师(高校)	博士研究生	西安交通大学	动物模型构建优化	

三、项目经费情况

1、经费支出情况（万元）

明细指标	合同约定值	实际完成值	完成率
项目总投资	15.00	50000.00	333333.33%
专项经费	10.00	44414.00	444139.99%
项目投资支出情况（万元）			
支出科目	**总投资实际支出**	**专项经费实际支出**	**说明**
一、直接费用	50000.00	39414.00	
1、设备费	50000.00	0.00	
（1）购置设备费	30000.00	0.00	
（2）自制设备费	20000.00	0.00	
（3）设备改造与租赁	0.00	0.00	
2、材料费	0.00	12954.00	
3、测试化验加工费	0.00	0.00	
4、燃料动力费	0.00	0.00	
5、差旅费	0.00	0.00	
6、会议费	0.00	13500.00	
7、国际合作与交流费	0.00	0.00	
8、信息费（出版/文献/信息传播/知识产权事物费等）	0.00	2960.00	
9、专家咨询费	0.00	0.00	
10、劳务费	0.00	10000.00	
11、其他支出	0.00	0.00	
二、间接费用	0.00	5000.00	
1、管理费	0.00	0.00	
2、绩效支出	0.00	5000.00	
合计	50000.00	44414.00	

单位财务部门（盖章）

年　月　日

备注：此表需经本单位财务部门审核并盖章

2、需增添的主要仪器设备（使用专项经费购买价值5万元以上的设备）

序号	设备名称	型号	价格（万元）	增添理由

四、项目实现绩效

一级指标类别	二级指标类别	明细指标	绩效实现情况
产出类指标	知识产权	1、专利申请数（项）	2
		（1）申请发明专利	2
		（2）实用新型	0
		（3）外观设计	0
		2、专利授权数（项）	0
		（1）授权发明专利	0
		（2）实用新型	0
		（3）外观设计	0
		3、软件著作权授权数（项）	0
		4、发表论文（篇）	8
		（1）其中SCI索引收录数	0
		（2）其中EI索引收录数	0
		5、著作（部）	3
		6、制订标准数（项）	0
		（1）国际标准	0
		（2）国家标准	0
		（3）行业标准	0
		（4）地方标准	0
		（5）企业标准	0
	其他成果	1、填补技术空白数（项）	3
		（1）国际	1
		（2）国家	0
		（3）省级	2
		2、获奖项数	0
		（1）国家奖项	0
		（2）部、省奖项	0
		（3）地市级奖项	0
		3、其他科技成果产出	0
		（1）新工艺（或新方法模式）	0
		（2）新产品（含农业新品种）	0
		（3）新材料	0
		（4）新装备（装置）	0

		（5）平台/基地/示范点	0
		（6）中试线	0
		（7）生产线	0
		4、研究开发情况	\
		（1）小试	否
		（2）中试（样品样机）	否
		（3）小批量	否
		（4）规模化生产	否
	人才引育	1、引进高层次人才	0
		（1）博士、博士后	0
		（2）硕士	0
		2、培养高层次人才	1
		（1）博士、博士后	1
		（2）硕士	0
	产业化情况	新增产能（台/套/只等）	0
		新增产能利用率（%）	0
效果类指标	经济效益	1、新增产值（万元）	0
		2、新增销售（万元）	0
		3、新增出口创汇（万美元）	0
		4、新增利润（万元）	0
	社会效益	1、新增税收（万元）	0
		2、新增就业人数	0
		其中：本科以上就业人数	0
		3、就业培训（人次）	0
		4、带动农民增收（万元）	0
		5、农户培训（人次）	0
		6、新增产业带动情况（列举情况）	无
		7、技术集成示范（项）	0
		8、建立农业示范基地（亩数）	0
		9、节约资源能源（列举）	无
		10、环保效益	无
其他需要说明的情况		无	

五、项目有关资料目录

序号	资料名称	是否必备材料
1	计划项目合同书	是
2	项目实施总结报告	是
3	项目技术总结报告	是
4	财务审计报告	条件判断
5	其它有关的证明资料（论文、专著、专利、检测报告等）	否

六、审核意见表

申请单位意见： （单位盖章） 年　　月　　日
推荐部门审查意见： 盖章:
省科技厅业务处室审查意见： 负责人（签章）: 经办人（签章）:　　盖章:
省科技厅审查意见： 负责人（签章）: 经办人（签章）:　　盖章:

项目编号： 2018KW-041

管理类别： 项目类

陕西省重点研发计划

项目合同书

项目名称： 川崎病冠脉病变的干细胞疗法

承担单位： 陕西省人民医院 （盖章）

项目负责人： 焦富勇 电子邮箱： jiaofy@yeah.net

手机号码： 15398082028 联系电话： 029-85251331转3130

项目联系人： 李盼 电子邮箱： lipan@sxstemcell.com

手机号码： 15129010242 联系电话： 029-68820605

委托单位： 陕西省科学技术厅

推荐部门： 陕西省卫生和计划生育委员会

起止年限： 2018年01月01日 至 2020年12月31日

陕西省科学技术厅 制

填报说明

1、合同书通过“陕西省科技业务综合管理系统”，按照系统提示在线填写。

2、本合同书所列内容应实事求是填写，表达上要明确、严谨。

3、项目申请书是本合同书填报的重要依据，合同书填报不得降低考核指标，不得自行对主要研究内容作大的调整。项目申请书和本合同书将共同作为项目过程管理、验收和监督评估的重要依据。

4、项目预算表和预算科目

一、直接费用：直接费用是指在课题研究开发过程中发生的与之直接相关的费用，主要包括设备费、材料费、测试化验加工费、燃料动力费、差旅费、会议费、国际合作与交流费、出版/文献/信息传播/知识产权事务费、劳务费、专家咨询费和其他支出等。

（1）设备费：指项目实施过程中必需购置的专用仪器设备，对现有仪器设备进行升级改造，以及租赁外单位仪器设备而发生的费用。

（2）材料费：指科技项目研究开发或科技创新体系建设过程中所支付的原材料、燃料动力、低值易耗品等的采购及运输、装卸、整理等费用。专项经费不支持购买生产经营性材料、基建材料、普通办公材料。

（3）测试化验加工费：指项目实施过程中支付给外单位（包括项目承担单位内部独立经济核算单位）的试验、加工、测试、化验等费用。单项预算在5万元以上的测试化验加工项目，要重点说明与研究任务的相关性、必要性，以及选择测试化验加工单位的理由，次数、价格等测算依据。其他测试化验加工项目可结合课题研究任务进行合并说明。

（4）燃料动力费：是指在课题研究开发过程中相关大型仪器设备、专用科学装置等运行发生的可以单独计量的水、电、气、燃料消耗费用等。

（5）差旅费：指在科技项目研究开发或科技创新体系建设过程中，为科技项目研究开发或科技创新体系建设而进行国内调研考察、现场试验、学术交流等工作所发生的交通、住宿等费用。出境（含港澳台）差旅费只能通过申请国际科技合作与交流计划项目列支。

（6）会议费：指科技项目研究开发或科技创新体系建设过程中为组织开展学术研讨、咨询以及协调项目等活动而发生的会议费用。

（7）国际合作与交流费：是指在课题研究开发过程中课题研究人员出国及外国专家来华工作的费用。国际合作与交流费应当严格执行国家外事经费管理的有关规定。不同的国家的补助标准不一样，请参考目的地国家具体补助标准。

（8）信息费：指科技项目研究开发或科技创新体系建设过程中发生的信息检索费、著作出版印刷费、专用软件购买、论文版面费、数据调查费、专业通信费、知识产权事务费等。

（9）专家咨询费：指项目研究开发过程中支付给临时聘请的咨询专家的费用。

（10）劳务费：参与项目研究的研究生、博士后、访问学者以及项目聘用的研究人员、科研辅助人员等，均可开支劳务费。项目聘用人员的劳务费开支标准，参照当地科学研究和技术服务业从业人员平均工资水平，根据其在项目研究中承担的工作任务确定，其社会保险补助纳入劳务费科目列支。劳务费预算不设比例限制，由项目承担单位和科研人员据实编制。

（11）其他支出：指除上述费用之外与科技项目研究开发或科技创新体系建设有关的其他费用，需写具体费用名称。

二、间接费用：是指承担课题任务的单位在组织实施课题过程中发生的无法在直接费用中列支的相关费用。主要包括承担课题任务的单位为课题研究提供的现有仪器设备及房屋，水、电、气、暖消耗，有关管理费用的补助支出，以及绩效支出等。间接费用使用分段超额累退比例法计算并实行总额控制，按照不超过课题经费中直接费用扣除设备购置费后的一定比例核定，具体比例如下：

500万元及以下部分不超过25%；

超过500万元至1000万元的部分不超过15%；

超过1000万元的部分不超过13%。

（1）管理费：指科技项目承担单位及受托管理单位为组织管理科技项目而支出的相关费用。包括现有仪器设备和房屋使用费或折旧、直接管理人员费用和其他相关管理支出。

（2）绩效支出：是指承担课题任务的单位为提高科研工作绩效安排的相关支出。加大对科研人员的激励力度，取消绩效支出比例制。

陕西省重点研发计划项目合同书

甲方：陕西省科学技术厅（以下简称甲方）

乙方：陕西省人民医院（项目实施单位，以下简称乙方）

甲、乙双方根据国家有关法律法规的有关规定，为顺利完成乙方承担的“陕西省2018年重点研发计划”项目川崎病冠脉病变的干细胞疗法（项目编号：2018KW-041，以下简称本项目），特订立本合同。

本项目执行期自2018年01月01日 至 2020年12月31日 。

一、合作项目主要内容

川崎病（Kawasaki disease，KD）又称皮肤黏膜淋巴结综合征，该病以全身性中、小动脉炎为主要病理变化，冠状动脉病变是其严重并发症。近年来，川崎病发病率逐年增加，患儿心血管病变率已超过20%，川崎病成为儿童后天性心脏病的最重要原因之一，川崎病患儿因冠状动脉瘤破裂致死也时有报道。对川崎病疗法的研究愈发重要。

目前，临床上公认的川崎病治疗方法是静脉注射免疫球蛋白和口服阿斯匹林，从而控制全身非特异性血管炎，防止冠状动脉损害的发生。但临床治疗中仍存在部分病人阿斯匹林不耐受、有耐免疫球蛋白川崎病患者等问题。于是，人们把目光转向了如今炙手可热的干细胞疗法。

用脐带间充质干细胞治疗心肌梗塞、心衰等心脏疾病已是较为成熟的干细胞疗法，已有大量临床前动物实验、临床试验证实了脐带间充质干细胞治疗的安全性和有效性。因此，将干细胞疗法应用于川崎病引起的冠脉病变是合理可行的。此项目计划采用干酪乳杆菌细胞壁提取物（LCWE）诱导川崎病小鼠模型，给予脐带来源间充质干细胞治疗，观察并分析小鼠实验期间一般情况及冠状动脉病变情况，然后开展临床研究，最终评估干细胞疗法对于川崎病引起的冠脉病变的疗效。

二、合作方案、方式及知识产权归属

(1) 合作方案：

① 陕西省人民医院：

负责项目的总体设计、统筹安排、工作计划和实施。具体内容包括提供川崎病相关背景材料、川崎病动物模型构建技术、临床试验审批及实施、国内论外文撰写及专利申报等。

② 陕西九州生物医药科技集团有限公司：

利用现有的陕西省干细胞研究中心、细胞组织库等资源，按照GMP标准生产脐带来源间充质干细胞；通过提供的川崎病动物模型技术构建动物模型；完成脐带间充质干细胞对川崎病动物模型治疗实验；为开发间充质干细胞相关产品做临床前准备；国内论外文撰写及专利申报等。

③ 俄罗斯乌拉尔联邦大学：

利用儿科领域的研究优势，提供相关技术支持；多中心共同开展动物试验及临床试验；国内论外文撰写及专利申报等。

(2) 合作方式：

中俄双方同时开展实验，共同研究，开发出川崎病引起的冠脉病变的干细胞疗法。

(3) 知识产权归属：

本项目研究形成的论文及研究成果为三方共享，具体如下：

① 论文发表：共同通讯作者、共同第一作者均按实际研究工作贡献排序。

② 专利申请：本项目科研成果所形成的专利，由三方作为专利共同申请人共同申请，如专利申报成功，三方作为专利共同所有权人。

三、合作项目达到的技术指标及预期成果形态

(1) 预期目标：

本项目计划将干细胞疗法应用于儿科川崎病的临床治疗，并期待将项目成果产业化为川崎病的干细胞药品。所以，项目分步目标设定如下：

① 使用干酪乳杆菌细胞壁萃取物成功建立川崎病冠脉病变小鼠模型；

② 成功分离、培养、冻存符合质量标准的脐带来源间充质干细胞；

③ 动物实验中，验证脐带间充质干细胞修复动物模型病变冠脉存在积极作用；

④ 临床实验中，验证脐带间充质干细胞修复患者病变冠脉存在积极作用；

⑤ 试建立脐带间充质干细胞药品生产标准。

(2) 成果形态：

① 学术论文；

② 申请发明或实用新型专利；

③ 申报省级科技进步奖。

四、考核指标

本项目考核指标具体如下：

(1) 项目考核指标
① 团队考核
项目负责人负责本课题总体控制，包括课题进展情况、实验结果汇总情况、经费使用情况及论文发表情况等。项目成员负责相关文献的查阅，动物实验及临床实验的结果准确、可靠；数据整理汇总；论文撰写等。
② 动物实验考核
实验动物来源可靠；动物房规范化管理；动物实验操作规范化等。
③ 临床实验考核
筛选川崎病患者，签署本人及家属知情同意书，建立档案，各项指标入档。临床试验及复查时严谨记录，保证数据的真实可靠性。
④ 经费考核
建立财务支出报表，专款专用。

(2) 成果考核指标
① 开发出川崎病引起的冠脉病变的干细胞疗法；
② 发表学术论文1-2篇；
③ 申请发明或实用新型专利1-2项；
④ 申报省部级科技进步奖1项。

五、项目研发人员情况（含外方人员）

项目负责人					
姓名	焦富勇	性别	男	出生年月	1951-01-03
学历	本科生	职务	儿童病院名誉院长	手机	15398082028
从事专业	儿科学			职称	主任医师

项目组主要参与人员								
姓名	出生年月	性别	从事专业	职称	学历	所在单位	项目分工	签名
戴博	1977-05-10	男	干细胞生物学	教授	博士研究生	陕西九州生物医药科技集团有限公司	干细胞质控	
李盼	1993-02-16	女	干细胞生物学	未取得	硕士研究生	陕西九州生物医药科技集团有限公司	动物实验	
Lvova Olga	1974-05-07	女	儿科学	教授	博士研究生	Ural Federal University	项目技术优化	
郭康合	1970-07-05	男	干细胞生物学	高级工程师	硕士研究生	陕西九州生物医药科技集团有限公司	细胞库管理	
王杰民	1962-04-29	男	儿科学	主任医师	硕士研究生	陕西省人民医院	临床实验管理	
蔺婧	1985-01-27	女	儿科学	讲师(高校)	博士研究生	西安交通大学	动物模型构建优化	

六、项目经费情况

1、项目经费预算

项目总投资（万元）	15.00	已完成投资（万元）	0.00
计划新增投资（万元）	15.00	省级资助经费（万元）	10.00

已完成投资来源（万元）

合计	单位自筹	银行贷款	政府资助			其他来源
			国家	省级	市级	
0.00	0.00	0.00	0.00	0.00	0.00	0.00

计划新增投资来源（万元）

合计	单位自筹	银行贷款	政府资助			其他来源
			国家	省级	市级	
15.00	5.00	0.00	0.00	10.00	0.00	0.00

计划新增投资支出情况（万元）

支出科目	新增投资总额	省级资助经费	说明
一、直接费用	14.00	10.00	\
1、设备费	0.00	0.00	\
（1）购置设备费	0	0	
（2）试制设备费	0	0	
（3）设备改造与租赁	0	0	
2、材料费	4.50	4.00	购买实验试剂、动物等
3、测试化验加工费	3.00	3.00	数据分析、流式细胞术等
4、燃料动力费	1.50	1.00	大型仪器设备能源消耗
5、差旅费	1.00	0	参加国内外会议
6、会议费	0.50	0	会议注册
7、国际合作与交流费	0.80	0.50	第13届国际川崎病大会（美国）
8、信息费（出版/文献/信息传播/知识产权事物费等）	1.00	0.50	论文版面费、专利申请费等
9、专家咨询费	0.50	0	专业技术指导与支持
10、劳务费	1.20	1.00	参与课题人员科研补助
11、其他支出	0	0	
二、间接费用	1.00	0.00	\
1、管理费	0.50	0	占总预算3%
2、绩效支出	0.50	0	论文、专利等奖励
合计	15.00	10.00	\

单位财务部门（盖章） 年　　月　　日

备注：此表须经本单位财务部门审核并盖章。

2、需增添的主要仪器设备（使用省级资助经费购买价值5万元以上的设备）

序号	设备名称	型号	价格 （万元）	增添理由

七、项目进度计划（说明项目进度，包括实施方案、实施地点等内容）

序号	时间	计划完成内容
1	第1阶段 2018年01月01日 至 2018年12月31日	脐带间充质干细胞培养、鉴定（表面抗原鉴定、无菌检测、传染病检测、流式检测等）及冻存；制备LCWE，利用LCWE诱导川崎病小鼠模型。做好前期准备工作。
2	第2阶段 2019年01月01日 至 2019年12月31日	设计动物实验方案，开展动物实验。验证脐带间充质干细胞对川崎病小鼠模型病变冠脉的修复作用，为临床试验奠定理论基础。
3	第3阶段 2020年01月01日 至 2020年12月31日	设计临床试验方案，开展临床试验。选择冠脉病变不同程度的患者进行脐带来源的间充质干细胞治疗，定期复查并记录患者各项情况。最后，整理研究资料，申请结题，申报成果。

八、项目绩效目标

一级指标类别	二级指标类别	明细指标	预期绩效目标
产出类指标	知识产权	1、专利申请数（项）	2
		（1）申请发明专利	1
		（2）实用新型	1
		（3）外观设计	0
		2、专利授权数（项）	0
		（1）授权发明专利	0
		（2）实用新型	0
		（3）外观设计	0
		3、软件著作权授权数（项）	0
		4、发表论文（篇）	2
		（1）其中SCI索引收录数	1
		（2）其中EI索引收录数	0
		5、著作（部）	0
		6、制订标准数（项）	0
		（1）国际标准	0
		（2）国家标准	0
		（3）行业标准	0
		（4）地方标准	0
		（5）企业标准	0
	其他成果	1、填补技术空白数（项）	1
		（1）国际	1
		（2）国家	0
		（3）省级	0
		2、获奖项数	1
		（1）国家奖项	0
		（2）部、省奖项	1
		（3）地市级奖项	0
		3、其他科技成果产出	0
		（1）新工艺（或新方法模式）	0
		（2）新产品（含农业新品种）	0
		（3）新材料	0
		（4）新装备（装置）	0

		（5）平台/基地/示范点	0
		（6）中试线	0
		（7）生产线	0
		4、研究开发情况	\
		（1）小试	否
		（2）中试（样品样机）	否
		（3）小批量	否
		（4）规模化生产	否
	人才引育	1、引进高层次人才	0
		（1）博士、博士后	0
		（2）硕士	0
		2、培养高层次人才	0
		（1）博士、博士后	0
		（2）硕士	0
	产业化情况	新增产能（台/套/只等）	0
		新增产能利用率（%）	0
效果类指标	经济效益	1、新增产值（万元）	0
		2、新增销售（万元）	0
		3、新增出口创汇（万美元）	0
		4、新增利润（万元）	0
	社会效益	1、新增税收（万元）	0
		2、新增就业人数	0
		其中：本科以上就业人数	0
		3、就业培训（人次）	0
		4、带动农民增收（万元）	0
		5、农户培训（人次）	0
		6、新增产业带动情况（列举情况）	无
		7、技术集成示范（项）	0
		8、建立农业示范基地（亩数）	0
		9、节约资源能源（列举）	无
		10、环保效益	无
其他需要说明的情况		无	

九、附件清单

序号	附件名称	是否必备材料
□ 1	其他附件	否

十、其他条款

（一）、甲方（科技厅）

1、负责及时划拨项目经费给项目单位。

2、负责协调、监督项目实施，检查项目执行情况，审计项目经费使用情况。经检查审计，如发现违反合同，有权暂停或停止划拨经费。

3、按《陕西省科技计划暂行管理办法》等有关规定，依据本合同所规定的内容和要求对项目进行验收。

（二）、乙方（项目承担单位）

1、负责项目组织实施，进行项目日常管理及检查监督，并按规定向甲方报送项目年度执行情况报告。

2、乙方须呈交项目/课题科技报告。在项目/课题实施过程中提交进展报告和专题报告（包括试验/实验报告、分析/研究报告、工程/生产/运行报告、评价报告、技术节点报告、时间节点报告等），在项目/课题结题验收时提交最终报告。以上科技报告中，最终报告为必备报告，其他报告视项目/课题执行情况酌情提交。

3、按合同规定的开支范围，对甲方划拨项目经费实行专款专用。

4、负责提供应由本单位安排的基建、物资、自筹经费、人工等有关保证条件。

5、接受甲方对项目执行及经费使用等情况进行检查或审计。

6、项目完成后，负责提出项目总结报告，及时做出经费决算，接受甲方组织的项目验收。

7、项目完成后，必须进行成果登记；不进行成果登记的，项目负责人将不能承担省级各类科技计划项目；将会影响项目承担单位承担省级科技计划项目的信用。

（三）、项目推荐部门

1、负责项目实施过程中的组织协调、监督检查；对项目经费使用情况进行监督管理。

2、协助甲方对项目执行及经费使用情况进行检查或审计。项目完成后，协助甲方对项目进行验收。

3、负责解决应属本部门安排的基建、物资、配套资金等保证条件。

（四）、项目的转包、分包

1、非经甲方同意，乙方不得将合同项目及其权利和义务转包、分包给第三者。

2、本项目若转包、分包必须经甲方同意后另行签订合同，并将转包、分包合同副本作为本合同的正式附件，交甲方存查。

3、因第三方不能完成转包或分包合同的约定义务，影响乙方完成本合同应完成的义务，由乙方负责。

（五）、知识产权归属

凡使用甲方下达经费取得的研究成果及其形成的知识产权，除涉及国家安全、国家利益和重大社会公共利益的以外，授予科研项目承担单位。项目承担单位可以依法自主决定实施、许可他人实施、转让、作价入股等，并取得相应的收益。同时，在特定情况下，或根据合同中要求保留无偿使用、开发、使之有效利用和获取收益的权利。其它事宜按照科技部《关于国家科研计划项目研究成果知识产权管理的若干规定》执行。

（六）、技术资料的保密

1、非经双方同意，保密资料不得向第三方泄露。

2、对必须由保密审查部门审查后方能公开发表的保密资料，乙方不得擅自发表，擅自发表者要承担失密责任，直至依法对当事人追究刑事责任。

（七）、合同的变更或解除

1、任何一方提出变更合同或解除合同的要求，需与另一方协商，签订变更条款或协议，作为本合同的正式附件，方可执行。

2、一方因他方违反合同或发生不可抗力，或国家计划调整，致使合同履行成为不可能或不必要，有权通知另一方解除合同。

3、当事人一方逾期两个月不履行合同规定的义务，对方有权解除合同。

4、变更或解除合同造成的损失由双方协商或按责任原则分别承担。

（八）、不可抗力和风险责任的承担

1、任何一方因不可抗力或国家计划调整不能履行合同的全部或部分义务时，应及时通知另一方，并采取措施减少损失，在合理期限内提供合同不能履行的证明。

2、甲方不履行合同内容，导致项目失败或部分失败，所拨经费（无偿部分）和物资不得追回。乙方不履行合同内容，导致项目失败或部分失败，应全部退还或部分退还甲方所拨经费和物资，情节严重者要追究责任。

3、乙方在执行合同过程中发生风险情况，应及时通知甲方，并采取措施减少损失。乙方没有及时通知甲方并采取适当措施，导致损失扩大的，应就扩大的损失承担责任，甲方有权要求乙方支付违约金或赔偿经济损失。

（九）、科技报告

乙方需呈交项目科技报告。在项目实施过程中提交进展报告和专题报告（包括试验/实验报告、分析/研究报告、工程/生产/运行报告、评价报告、技术节点报告、时间节点报告等），在项目结题验收时提交最终报告。以上科技报告，最终报告为必备报告，其他报告视项目执行情况酌情提交。

（十）、合同文本的要求

本合同一式肆份，甲方存贰份，推荐部门存壹份，乙方存壹份，具有同等法律效力。

（十一）、其他附加条款

经双方协商订立的下列条款作为本合同正式内容的一部分。

十一、本合同签约各方

甲方：陕西省科学技术厅

业务处室负责人（签章）：

业务处室经办人（签章）：　　电话：81294887

通讯地址：丈八五路10号

邮编：710077　　盖章

年　月　日

乙方：陕西省人民医院

单位负责人（签章）：

项目负责人（签章）：

通讯地址：西安市友谊西路256号　　电话：029-85251331-2610

邮编：710068

盖章

年　月　日

推荐部门：陕西省卫生和计划生育委员会

负责人（签章）：

联系人：王琨　　电话：029-89620667

通讯地址：西安市莲湖路112号

邮编：710003　　盖章

年　月　日

项目实施报告

项目名称： 川崎病冠脉病变的干细胞疗法

项目编号：2018KW-041

概述

本项目于 2018 年经陕西省科学技术厅批准立项。项目批准后，主要进行了以下研究

1）利用干酪乳杆菌细胞壁提取物（LCWE）诱导川崎病（Kawasaki disease，KD）小鼠模型，2）通过高频小动物超声在 M 超切面记录小鼠左室室壁运动情况，并测量左室腔的大小，估算出左室射血分数（EF）和短轴缩短率（FS），用以评估川崎病对心功能的影响过程。3）初步探讨人脐带间充质干细胞(hUC-MSCs)对干酪乳杆菌壁提取物(LCWE)诱导的川崎病（KD)动物模型冠状动脉炎的积极影响。

立项后，项目主要负责人焦富勇教授组织建立科研团队儿童病院骨干医生、陕西省感染与免疫疾病重点实验室、陕西省细胞免疫工程技术研究中及肿瘤生物学国家重点实验室，西北大学生命科学院实验室、西安医学院第一附属医院 B 超科、空军医大学唐都医院超声科 、陕西省人民医院儿童病院实验室、B 超影像科、临床检验科等人员参加组成大约 15 人的多学科科研团队（MDT），其中有博士 3 位、硕士 5 位、学士 5 位、其他 2 位。

共分三组，第一组制模组（ 郭华 技师，杨勇，儿科博士 ），第二组动物实验管理组（穆志龙，硕士 ）第三组病理组织学组（张华），第四组 B 超影像学组 （负责人：张雪梅在读博士 ）。总负责焦富勇；人员协调，（严晓华 在读博士 ）

研究成果：

相关论文（详见附件）。

质量完成情况 ：

通过高频小鼠超声在 M 超切面记录小鼠左室室壁运动情况，并测量左室腔的大小，估算出左室射血分数（EF）和短轴缩短率（FS），用以评估川崎病对心功能的影响过程。**方法：**制备 LCWE，将 18 只 BALB/c 幼鼠随机分为 2 组：KD 模型组 15 只，正常对照组 3 只。经腹腔单次注射 0.5ml LCWE 于模型组诱导 KD 模型，分别于注射后 2d、15d 和 30d 用 M 超记录小鼠左室室壁的运动情况，并测量左室收缩末期及舒张末期内径，计算出 EF、FS。利用高频小动物超声能够清晰获得小鼠心脏图像，利用 M 超动态、连续的观察左室壁运动情况，部分 KD 模型组小鼠出现节段性室壁运动异常，心功能明显减低，验证动物建模成功；利用干细胞介入川崎动物模型，准确测定了 EF,FS 及室壁运动情况。精确对比干预前后的指标差异，准确获取干细胞在动物模型的治疗数据基础。

项目进度 ：

2018 年：实验动物的准备，川崎病小鼠模型的建立。

2019 年：小鼠建模后全麻备皮，由西安医学院第一附属医院 B 超科、空军医大学唐都医院超声科、陕西省人民医院 B 超影像科 三家 B 超科专家联手 B 超测量，于西北大学生命科学院实验室进行小鼠川崎病冠状动脉的组织病理学检查。

2020 年：搜集整理实验数据，分析总结发表论文。

工作评价及经验总结：

严格执行科学研究规范及守则，对实验数据进行认真记录分析，在整个项目实施及实验过程中做到严谨负责。本项目实施是由陕西省人民医院组织，联合组织西北大学生命科学院实验室、空军医大学唐都医院超声科、西安医学院第一附属医院 B 超科、陕西省人民医院 B

超影像科多学科联系。从动物模型制作、影像 B 超、病理学改变等多个方面动态检测、研究川崎病冠脉病变的干细胞疗法的疗效。川崎病小鼠模型国内外鲜少，技术难度系数大，但项目团队能够克服重重困难，首次在西北地区使用干酪乳杆菌细胞壁萃取物成功建立川崎病冠脉病变小鼠模型，并对小鼠模型进行 B 超、病理学动态监测。由于上班期间门诊、住院部工作不能请假，团队人员大多利用下班后休息时间，时常实验至晚上 11 点才结束返家，项目负责人焦富勇主任医师虽已年过 70 岁，但仍与团队一起工作至夜深才从实验室返回，焦富勇主任医师精益求精，论文修改近 10 余次始终不放弃终于在国内外发表，填补了我省乃至西北地区此方面的空白，儿童病院川崎病课题组（项目名称：川崎病的基础与临床研究）荣获“陕西省科学技术进步奖”二等奖。博士、硕士、学士的参与，带动了学术研究，亦使年轻人得到了锻炼，学会了如何进行科学研究。

总结

第一，特点：陕西省感染与免疫疾病重点实验室、陕西省细胞免疫工程技术研究中及肿瘤生物学国家重点实验室，西北大学生命科学院实验室、西安医学院第一附属医院 B 超科、空军医大学唐都医院超声科 、陕西省人民医院儿童病院实验室、B 超影像科、临床检验科多学科紧密配合、协同合作，才能使科研工作圆满完成。

第二，优势：由多学科博士、硕士、学士等高学位人员参与组成科研团队，整体提高了学术研究水平。

第三，缺点：设计要求太高，SCIJ 成果需要有较长周期，这个设计周期太短，3 年很难发表出 SCI ，科研经费少 。

第四，风险：受国内疫情影响，硕士等一些研究人员放假离开医院，返院时隔离了一段时间，导致动物实验受到了一定的影响。

第五，仍需解决的问题：干细胞疗法于 20 世纪末提出，临床治疗经验较普通治疗还较缺乏，远期预后资料较少，仍尚待研究。因此，不能排除干细胞治疗的风险性，如：体外培养干细胞可能造成细菌、病毒污染等，会影响干细胞的正常功能；间充质干细胞注射入体内后，其生长分化程度较难。另外，干细胞要在人体进行实验要求很高，需要进行更多的动物实验后才能在人体进行。

项 目 技 术 总 结 报 告

项目编号：2018KW-041
项目名称：川崎病冠脉病变的干细胞疗法
承担单位：陕西省人民医院
2018KW-041
陕西省人民医院
2018 年
川崎病冠脉病变的干细胞疗法技术总结

1.研究背景

川崎病（Kawasaki disease，KD）又称皮肤黏膜淋巴结综合征，是一种发生于小儿的急性发热性疾病。主要以全身性中、小动脉炎为主要病理变化，冠状动脉病变是其严重并发症，以冠状动脉瘤（CAA）和冠状动脉狭窄为主[1]。根据陕西省川崎病流行病学调查研究[2]：1992 年 1 月-1997 年 12 月，5 年间陕西省共报告川崎病患者 376 例，年平均约 75 例。陕西省的川崎病发病率为 2.34/10 万，与全国相比偏高，川崎病为我省儿童较常见疾病。其中，376 例川崎病患儿中患有心脏后遗症者 70 例，占报告患儿数的 18.62%。严晓华等[3]对 2011 年 1 月至 2015 年 1 月于陕西省人民医院儿科住院并确诊为川崎病的 170 例患儿的临床情况进行分析，其中冠脉扩张 38 例（22.4%，38/170 例），冠脉瘤 6 例（3.5%，6/170 例），巨大冠脉瘤 2 例（1.1%，2/170 例）。近年来，川崎病发病率逐年增加，患儿心血管病变率已超过 20%。目前，该病已取代风湿热成为儿童后天性心脏病的最重要原因之一。同时，川崎病还存在死亡的风险，川崎病患儿因冠状动脉瘤破裂致死时有报道。因此，如今川崎病研究为儿科重要课题之一。

陕西省川崎病的流行病学调查[2]显示，1992-1997 年间，陕西省 5 年共检出川崎病例 376 例，发病率 2.34/10 万人，死亡率 20%，心脏后遗症 70 例（18.62%）。陕西省人民医院于 2011 年至 2015 年间收治的 170 人次川崎病患者[3]中，冠脉扩张及冠脉瘤为 46 例（27.1%，46/170 例）。根据 2013 年中国台湾统计资料，每 10 万人中 5 岁以下人群年平均发病率为 67.3/10 万人。到 2030 年，中国台湾有川崎病史的人口比例将达到 1/700。陕西作为人口较密集地区，川崎病发生率或将存在类似上升风险。然而，川崎病对儿童的危害并不在于发烧、皮疹、淋巴结肿大等外在症状，而其真正的危险在于对心脏等脏器的危害。川崎病并发心血管损害的类型包括冠状动脉瘤、冠状动脉扩张、冠状动脉狭窄或闭塞等。动脉瘤可单发或多发，大多可于 1—2 年内消退；但局部管壁的纤维化可导致冠状动脉不能有效扩张，严重者可形成血栓、管腔狭窄闭塞乃至心肌梗死。川崎病患儿因冠状动脉瘤破裂致死也时有报道[8]。如何挽救因川崎病引起的冠脉瘤致死的患儿，降低川崎病死亡率是当前棘手的问题。所以，川崎病的冠脉病变应当得到重视，其治疗方法的研究十分必要。

2.研究思路

目前，临床上公认的川崎病治疗方法是静脉注射免疫球蛋白或称丙种球蛋白（IVIG）和口服阿斯匹林。这种联合治疗方案的广泛应用使川崎病死亡率和冠状动脉并发症明显下降，但经研究发现，丙种球蛋白耐药病例逐年增多。为此，我们把目光转向了干细胞疗法。20 世纪末以来，干细胞治疗成为研究热点，以干细胞技术为核心的再生医学成为了人们治愈疾病的新希望。

2.1 实施方式

本项目实施是由陕西省人民医院组织，联合组织西北大学生命科学院实验室、空军医大

学唐都医院超声科、西安医学院第一附属医院 B 超科、陕西省人民医院 B 超影像科多学科联系。从动物模型制作、影像 B 超、病理学改变这几个方面动态检测、研究川崎病冠脉病变的干细胞疗法的疗效。致力于研发出疗效更加，副作用更少，能解决现在丙种球蛋白耐药困境的全新的治疗方案，填补我省乃至西北地区在此方面的空白。

2.2 主要技术指标

本研究通过 LCWE 诱导小鼠川崎模型，利用高频小动物心脏超声仪，用 M 超记录室壁运动情况，并测量小鼠心脏左室收缩末期内径（leftventricularend-systolicdiameterLVESD）及左室舒张末期内径（leftventricularend-diastolicdiameter,LVEDD），计算出左室射血分数（Ejectionfraction,EF）及左室短轴缩短率（Fractionalshortening,FS），来评价川崎病对心脏功能的影响，尤其是对冠状动脉的影响。

2.3 阶段进度计划

2018 年：实验动物的准备，川崎病小鼠模型的建立。

2019 年：小鼠建模后全麻备皮，由西安医学院第一附属医院 B 超科、空军医大学唐都医院超声科、陕西省人民医院 B 超影像科 三家 B 超科专家联手 B 超测量，于西北大学生命科学院实验室进行小鼠川崎病冠状动脉的组织学病理检查。

2020 年：搜集整理实验数据，分析总结发表论文。

3. 核心技术及创新点

3.1 核心技术

1、使用干酪乳杆菌细胞壁萃取物成功建立川崎病冠脉病变小鼠模型；
2、分离、培养、冻存符合质量标准的脐带来源间充质干细胞;
3、动物实验中，验证脐带间充质干细胞修复动物模型病变冠脉存在积极作用；

3.2、首次在西北建立川崎病小鼠模型

1、首次对小鼠模型进行 B 超、病理动态监测。
2、干酪乳杆菌壁提取物(LCWE)对川崎病小鼠冠状动脉损伤模型建立
3、人脐带间充质干细胞(hUC-MSCs)对冠脉损伤作用
4、人脐带间充质干细胞(hUC-MSCs)对冠脉损伤病理改变
5、人脐带间充质干细胞(hUC-MSCs)对冠脉损伤超声改变

4.国内外主要技术性能指标对比分析及本项目产品的技术水平评价

国内西北地区缺乏对此类的研究报告，干细胞治疗的实验学数据对探索 KD 新的治疗方法及动物实验都有重要价值和意义。本研究探索的新型方法——干细胞疗法治疗川崎病引起的冠脉病变若成功实施可缩短我国与发达国家在川崎病治疗方面的差距，对我国儿童后天性心脏病的治疗有十分重要的意义，在心血管疾病方面的研究也具有非常重要的经济和社会价值。

5.应用情况、需要继续解决的问题

5.1 推广应用情况

1、在国内外发表多篇相关学术文章；
2、利用陕西省儿内科医学临床研究中心及九个省级分中心和 40 个网络协同中心推动

宣传；

3、召开国内外相关大会，向各参会国介绍推广该疗法对川崎病的意义。

5.2 需要继续解决的问题

干细胞疗法于 20 世纪末提出，临床治疗经验较普通治疗还较缺乏，远期预后资料较少，仍尚待研究。因此，不能排除干细胞治疗的风险性，如：体外培养干细胞可能造成细菌、病毒污染等，会影响干细胞的正常功能；间充质干细胞注射入体内后，其生长分化程度较难控制，治疗作用有局限性等。

Annals of Pediatric Research

Research Article
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Clinical Analysis of Occurrence of Coronary Artery Aneurysm in 426 Children with Kawasaki Disease

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Abstract

Objectives: To analyze the clinical features of children with Kawasaki Disease (KD) complicated with coronary artery aneurysm.

Methods: The clinical data, laboratory examination, echocardiography and treatment of 17 children were retrospectively analyzed, who were diagnosed as coronary artery aneurysm in the 426 children with KD admitted to the Children's Hospital of Shaanxi Provincial People's Hospital from December 2013 to March 2018.

Results: 1). The 17 (4.0%) children of the 426 KD children had coronary artery aneurysm. The mean age of onset was 2.0 ± 2.5 years old, and the incidence of coronary artery aneurysm in children aged 1 to 3 was the highest, accounting for 29.4%. The morbidity of males was significantly higher than that of females, and the ratio of males to females was 4:1. 2). In the distribution of coronary artery involvement, bilateral coronary artery involvement was the most common (47.1%), left coronary artery involvement alone accounted for 23.5%, and right coronary artery involvement alone accounted for 29.4%. 3). The time of finding coronary artery aneurysm was mostly within 2 weeks, and the average time of finding coronary artery aneurysm was 9.9 ± 4.4 days. 4). There was no significant difference between the degree of increase of ESR within 1 week and 2 weeks of the course of disease. After admission, the CRP level in 8 cases was significantly higher than normal, and in 10 cases became normal at discharge, the CRP level at discharge was significantly lower than that after admission. Coronary artery aneurysms were detected by echocardiography mostly within 2 weeks; coronary artery aneurysms appeared to be mostly affected by both coronary arteries; the proportion of primary infusion of gamma globulin was the highest.

Conclusion: For males and 1 to 3 years old children with KD should be alert to the occurrence of coronary artery aneurysm, and Intravenous Gamma Globulin (IVIG) should be applied as soon as possible.

Keywords: Kawasaki disease; Coronary artery aneurysm; Echocardiography; Mucocutaneous lymphnode syndrome

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Introduction

Kawasaki Disease (KD), also known as muco-cutaneous lymph node syndrome, is an acute febrile rash disease characterized by systemic vasculitis, which mainly occurs in children under 5 years old. Tomisaku Kawasaki first reported KD in Japan in 1967 by descriptive statistics of the clinical manifestations of 50 children and the etiology and pathogenesis of KD are still unclear. KD has been reported among children of almost all races and has increased year by year in recent years. Undiagnosed and untreated KD in childhood may affect health care delivery systems in developing countries in the long term [1]. Coronary artery damage is the most serious complication of KD, and the incidence of coronary artery injury in children with untreated or untimely treated KD is up to 20% to 25% [2]. In developed countries, KD has replaced rheumatic fever as the leading cause of acquired heart disease in children. This study retrospectively analyzed the clinical data of children with KD complicated with coronary artery aneurysm, summarized its clinical features to improve the clinician's understanding of KD with coronary artery aneurysm.

Materials and Methods

General information

A total of 426 cases of children with KD admitted to the Children's Hospital of Shaanxi

Provincial People's Hospital from December 2013 to March 2018 was selected. Among them, 17 patients were diagnosed with coronary artery aneurysm by echocardiography (including the newly diagnosed children in our hospital and the children with coronary artery aneurysm diagnosed in other hospitals for further treatment in our hospital). Clinical data of KD children with coronary aneurysm were collected.

Methods

The age, gender, major clinical manifestations, laboratory findings (including CRP, ESR, PLT), ultrasound examination results (including coronary artery aneurysm detection time and coronary artery involvement), IVIG treatment methods and effects were retrospectively recorded and analyzed in KD children with coronary artery aneurysm.

Diagnostic criteria for KD and coronary artery aneurysm

The diagnosis of KD is based on the diagnosis, treatment and long-term management guideline for Kawasaki disease issued by the American Heart Association in 2004 [3], that is, clinical diagnosis can be made with at least 5 of the 6 main clinical manifestations; if 5 items are not satisfied, but echocardiography or cardiovascular angiography confirms coronary artery aneurysm or coronary artery dilatation, except for other diseases at the same time. Complete KD can also be diagnosed. Unexplained fever for 5 days or more combined with 2 or 3 typical KD clinical features may be considered incomplete KD. The diagnosis criteria for coronary artery aneurysm: The ratio of the inner diameter of the coronary artery expansion segment to the adjacent segment is greater than 1.5, among which, the inner diameter of the aneurysm <5 mm is a small coronary artery aneurysm, 5 mm to 8 mm is a medium coronary artery aneurysm and >8 mm is a giant coronary artery aneurysm [4].

Statistical processing

EpiDate software was used to establish a data base and the data was entered in parallel and checked. Statistical analysis was performed using SPSS 20.0 software and the significance test level was $P<0.05$. The measurement data in accordance with the normal distribution were expressed as mean ± standard deviation ($x \pm s$) and the mean value was compared by t test. The counting data was expressed by the number of cases (percentage) and compared by χ^2 test.

Results

Basic information

There were 13 males and 4 females in 17 patients and the ratio of male to female was 4/1; the age of onset was from 3 months to 7 years old and the average age was 2.0 ± 2.5 years old, among which, 5 cases (29.4%) were less than 1 year old, 8 cases (47.1%) were 1 to 3 years old, 1 case (5.9%) was 4 to 5 years old, 3 cases (17.6%) were >5 years old.

Clinical manifestations

There were 13 cases of fever ≥ 5 days, 9 cases of bilateral conjunctival congestion (no exudates), 8 cases of lip and oral manifestations (labial redness and chapped, bayberry tongue, diffuse hyperemia), 7 cases of limb changes (redness of palm, plantar and finger tip, toe end nail bed and skin membrane like peeling during recovery), 9 cases of cervical lymph node enlargement, 11 cases of skin manifestation (pleomorphic erythema, scarlet fever rash and perianal skin redness, desquamation).

Laboratory test results

ESR: A 1 week after admission (50 to 100 mm/h 4 cases, >100 mm/h 2 cases) and within 2 weeks of the disease (50 to 100 mm/h 3 cases, >100 mm/h 1 case), the rest were not examined.

CRP: After admission <50 mg/L 3 cases, 50 to 100 mg/L 3 cases, >100 mg/L 5 cases and the rest were not examined. At the time of discharge <50 mg/L 10 cases and others were not examined.

Among the 17 children with Kawasaki disease with coronary artery aneurysm, there was no significant difference in the degree of increase of ESR between 1 week and 2 weeks. After admission, 8 cases of CRP were significantly higher than normal, 6 cases were not examined, 10 cases were normal at discharge and 7 cases were not examined. The CRP level at discharge was significantly lower than that after admission.

Ultrasound examination results

Time of detection of coronary artery aneurysm in the course of disease: 1 case within 1 week, 12 cases within 2 weeks and 4 cases more than 1 month. Coronary artery aneurysms were usually detected within 2 weeks of the course of disease. Coronary artery involvement: There were 8 cases of bilateral coronary aneurysms, 4 cases of left coronary aneurysm alone, 5 cases of right coronary aneurysm alone. Bilateral coronary artery involvement was the most common.

Treatment of gamma globulin

The 7 cases were injected with gamma globulin once, 5 cases were injected with gamma globulin twice, 3 cases were given glucocorticoids after the first dose of gamma globulin were insensitive and 2 cases were given glucocorticoids after the second dose of gamma globulin were insensitive. The proportion of primary infusion of gamma globulin was the highest.

Discussion

Kawasaki disease is a muco-cutaneous lymph node syndrome which commonly occurs in children under 5 years old, and more sick boys than girls, which may be related to the male specific FCGR2A susceptibility gene [5]. The essence of Kawasaki disease is systemic vasculitis which has not yet been diagnosed in developing countries [6]. The most serious and common complication of Kawasaki disease is coronary artery lesions and the incidence of coronary artery lesions in untreated KD is 20% to 25% [2]. Coronary artery dilatation and coronary artery aneurysm both belong to coronary artery lesions and coronary artery aneurysm is severe coronary artery dilatation. The incidence of coronary artery aneurysm decreased significantly after high dose Intravenous Gamma Globulin (IVIG) was used in the treatment of KD in the acute stage. According to the investigation data of KD epidemiology in Beijing from 2000 to 2004, the incidence of coronary artery aneurysm has decreased to 4.3% [3]. It has been reported in the literature that age ≤ 1 year old and male patients are risk factors for coronary artery lesions in Kawasaki disease [7]. Children with incomplete KD with a duration of fever >10 days are more likely to develop coronary artery aneurysm, which is consistent with the characteristics of coronary artery injury in KD children [8]. In 1993, a survey for children with Kawasaki disease in 652 hospitals in Japan found that children who received IVIG after 9 days of disease had a higher risk of cardiac sequelae [9], and there is constant research to confirm this point of view [10]. The 2017 AHA Guideline recommends that IVIG should be actively used to treat Kawasaki disease within 10 days of the course of disease [11]. This study showed that there were significantly more males than females in KD children complicated with coronary artery aneurysm, with a male to female ratio of 4:1. From the age of onset, children aged 1 to 3

were significantly more than other age groups. There were 17 children complicated with coronary artery aneurysm in 426 children with Kawasaki disease, accounting for 4% of the total. Most of the 17 cases of KD with coronary artery aneurysm were found within 2 weeks of the course of disease. Although the incidence of coronary artery aneurysm has significantly reduced after the application of IVIG in the acute phase of KD, once coronary artery aneurysm occurs, it will affect the physical and mental health of the child in different degrees. Identifying risk factors for coronary artery aneurysm early in the acute phase of KD will help prevent coronary artery aneurysm. Therefore, timely diagnosis and treatment of Kawasaki disease is one of the important means to reduce the occurrence of coronary artery aneurysm.

Conclusion

In summary, medical staff should improve their understanding of Kawasaki disease, improve the diagnostic procedure for Kawasaki disease and perform cardiac ultrasound early to determine coronary artery conditions. Special attention should be paid to the early identification and treatment of incomplete Kawasaki disease, which is one of the important means to reduce the occurrence of coronary artery aneurysm. There were few cases of coronary artery aneurysm in this study and the follow-up time was short. It is expected that large sample, multicenter, long-term follow-up clinical studies.

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Research Article
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Establishment of a model of coronary artery injury by Lactobacillus casei wall extract in mice with Kawasaki disease and study of the effect of human umbilical cord mesenchymal stem cells on coronary artery injury and pathological and ultrasound changes

Abstract

Abstract: Objective To investigate the establishment of a model of Kawasaki disease mouse coronary artery injury caused by Lactobacillus casei wall extract (LCWE) and the effect of human umbilical cord mesenchymal stem cells (hUC-MSCs) on coronary artery injury and pathological and ultrasound changes.

Methods: Sixty BalB/C mice were randomly assigned to 3 groups, each with 20 mice. The control group was given phosphate buffered saline (PBS) intraperitoneally for 2 consecutive days; the model group and the stem cell group were immunized with the prepared LCWE for 2 consecutive days. From the 16th day of the immunological model, the control group and model group were injected with 300 μL PBS daily; the stem cell group was injected with 300 μL (105/mL) of human umbilical cord mesenchymal stem cells daily for 10 consecutive days. The mice were killed in batches on the 4th, 15th, 26th, and 32nd day of immunization, and the morphological changes of the coronary arteries of the mice were observed by echocardiography and histopathology.

Results: LCWE can induce coronary artery damage similar to Kawasaki disease in mice. B-ultrasound showed that 57.5% (23/40) mice had coronary artery disease, of which 5% (2/40) had right coronary artery aneurysm and 27.5% (11). /40) Thickening of the coronary artery wall, widening of the inner diameter of the left coronary artery trunk, and thickening of the intima; histopathology showed mild swelling of the epicardium of the aortic valve, mitral valve, right ventricle and atrium, and a few neutral Granulocytes were scattered and infiltrated, the coronary artery lumen within it was dilated and a small amount of myocardial cells died and collapsed, and the local fibrous connective tissue was significantly proliferated and accompanied by solid calcium salt deposition. After hUC-MSCs intervention treatment, ultrasound showed that the inner diameter of the main coronary artery was reduced. Histopathology showed that there was a large amount of lymphocytes, eosinophils, and monocytes in the outer membrane of the left atrial appendage of the mouse. There was no obvious vasculitis and other infiltrations. Obviously abnormal.

Conclusion: Lactobacillus casei wall extract (LCWE) can induce coronary arteritis in an animal model of Kawasaki disease (KD). 2. Human umbilical cord mesenchymal stem cells have a therapeutic effect on coronary artery disease in animal models of Kawasaki disease.

Keywords: Kawasaki disease; human umbilical cord mesenchymal stem cells; Lactobacillus casei wall extract; coronary artery damage

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Introduction

Kawasaki disease (Kawasaki disease, KD) occurs in children under 5 years of age. It is a disease that seriously threatens the physical and mental health of children. It is also an acute, self-limiting systemic disease with inflammatory lesions of the middle and small arterioles as the main pathological changes. Vasculitis. Coronary artery disease is the most serious complication of Kawasaki disease and is related to coronary artery obstruction and arteriosclerosis in adulthood. Up to 25% of untreated children with Kawasaki disease can develop coronary artery disease, leading to myocardial infarction, sudden death, and ischemic heart disease. It is the first cause of acquired heart disease in children. Some children develop huge coronary artery tumors.[1,2] It has been found in clinical practice that most children with Kawasaki disease can achieve good therapeutic effects after conventional treatment, but some children have the first dose of gamma resistance and need to be injected intravenously with human immunoglobulin and/or hormone therapy again; some of them Children with severe coronary artery damage (such as coronary artery aneurysm, etc.) require long-term or lifelong anticoagulation drugs, and the effect of coronary artery disease improvement is not good, and always be alert to cardiovascular critical events, even leading to myocardial infarction in adults Coronary aneurysms also exist (cases occur at 37 years of age). Therefore, how to identify the disease

itself early and find coronary artery disease early, so as to give more effective treatment, is a hot research issue to reduce the occurrence of cardiovascular sequelae. Umbilical cord mesenchymal stem cells are derived from the Walton's glue in the fetal umbilical cord. They have the advantages of unethical disputes, wide sources, easy access, low antigenicity, and multi-directional differentiation potential. They are one of the most important seed cells in tissue engineering. One.[3] According to different induction conditions, umbilical cord mesenchymal stem cells can differentiate into bone cells, chondrocytes, cardiomyocytes, hepatocytes, pancreatic islet cells, etc., bringing new treatments for neurological diseases, cardiovascular diseases, diabetes, liver diseases, etc. Direction and means.[4,5] At present, the animals that have established Kawasaki disease models at home and abroad include mice, rabbits, pigs and dogs, among which the commonly used strains of mice include C57BL/6J, C3H/HeN, CDI, DBA/2N and Balb/c, etc., mouse model induction The main agents are Lactobacillus casei cell wall extract and Candida albicans extract.[6] According to comprehensive literature reports, this laboratory selected Lactobacillus casei cell wall extract as an inducer to construct a mouse model of Kawasaki disease for further research on Kawasaki disease The treatment method provides experimental materials. To this end, this study intends to create a mouse model of immune coronary arteritis and observe histopathological changes under an optical microscope, so as to provide a basis for exploring the mechanism of KD coronary artery damage.

At present, the pathogenesis of Kawasaki disease is not fully understood. Experimental animal models can be used to observe the therapeutic effects of different drugs on vasculitis and help find more effective treatment options. Qin Lijun et al. used lactobacillus casei cell wall extract (LCWE) to induce coronary arteritis in mice, and Yan Jingru et al. used Candida albicans water soluble fraction (CAWS) to induce coronary arteries in mice. The pathological changes of Kawasaki disease are similar to coronary arteritis. These studies have laid a solid experimental foundation for the research and treatment of Kawasaki disease. Human umbilical cord mesenchymal stem cells (hUC-MSCs) are derived from the Walton's glue in the fetal umbilical cord. They have no ethical controversy, a wide range of sources, easy access, low antigenicity, and multi-directional differentiation potential. And other advantages, it is one of the very important seed cells in tissue engineering [Muheremu]. According to different induction conditions, umbilical cord mesenchymal stem cells can differentiate into bone cells, chondrocytes, cardiomyocytes, hepatocytes, pancreatic islet cells, etc., bringing new treatments for neurological diseases, cardiovascular diseases, diabetes, liver diseases, etc. Direction and means [Li L, Perea-Gil]. Therefore, this study used LCWE as an inducer to construct a BalB/C mouse model of Kawasaki disease to observe the inhibitory effect of umbilical cord mesenchymal stem cells on coronary arteritis, in order to explore the use of umbilical cord mesenchymal stem cells for clinical treatment of coronary artery damage in patients with Kawasaki disease The mechanism provides experimental basis.

Materials and methods

Experimental animals: female 10-week-old BalB/C mice [production license SCXK (Beijing) 2016-0006, sales license SYXK (Shaan) 2012-005], purchased from Beijing Weitong Lihua Laboratory Animal Technology Co., Ltd., The weight range is 17g ± 0.5g.

Reagents: Lactobacillus casei was purchased from (Bina Cell Biological Collection Management Center, No.: 134415); MRS broth medium (Hangzhou Best Biotechnology Co., Ltd.); ribonuclease (RNAse), deoxyribonuclease I (DNAse I)), Trypsin, Rhamnose standard, Sodium Dodecyl Sulfonate (SDS) (Beijing Soleibao Biological Company); Umbilical Cord Mesenchymal Stem Cell Injection (CD73, CD90 and CD105 positive The rate is not less than 95%), Department of Hematology, Shaanxi Provincial People's Hospital, production batch number: 20190702.

Instruments: MALDI-TOF mass spectrometer (Bruck, USA), VisualSonics 2100 small animal high-resolution ultrasound imaging system (Fujifilm (China) invested company), pathology graphics processing system (Nikon Instruments (Shanghai) Co., Ltd.), BONDRX and more Functional dyeing machine (Shanghai Leica Co., Ltd.).

Method

Preparation of Lactobacillus casei cell wall components (LCWE): Lactobacillus casei is routinely cultured in lactic acid bacteria broth medium, and identified as Lactobacillus casei-like cheese species by mass spectrometer. The bacteria in the logarithmic growth phase are collected at the Add 4% SDS to lyse overnight. After washing and centrifugation with phosphate buffered saline (PBS), add 250 μg/mL RNase, DNase and trypsin successively and incubate at 37°C for 4 h. After washing and centrifugation with PBS, collect bacteria The fragments were added with 1 g wet weight in 5 mL PBS, and then subjected to ultrasonic lysis for 2 h in a dry ice water bath. After centrifugation, the supernatant was collected as LCWE. The rhamnose content was determined by the sulfuric acid phenol colorimetric method, and the concentration was adjusted to 1 mg/mL with PBS. BALB/C mice were injected with a single injection of 0.3 mL of LCWE into the abdominal cavity to induce the Kawasaki disease model twice.

Establishment of Kawasaki disease mouse model and experimental grouping: 60 BalB/C mice were randomly divided into three groups, the control group, the model group and the stem cell group, each with 20 mice. Mice in the model group and stem cell group were intraperitoneally injected with LCWE at a concentration of 1 mg/mL, 0.3 mL/time/mouse, for 2 consecutive days. The control group was injected with phosphate buffered saline (PBS) at the same time. With reference to previous studies on the pathological changes of KD model mice, the echocardiograms of the control group, model group and stem cell group were observed on 4d, 15d, 26d and 32d after intraperitoneal injection of LCWE and the pathological changes of the acute coronary arteries of mice.

Observation indicators of mouse appearance: daily observation and recording of the general condition of mice. Specific evaluation indicators include body weight (g), food intake (g), water consumption (ml), grip (g), hair changes, etc. In the event of sudden death, immediately perform an autopsy and take the heart to make pathological sections to observe whether there is coronary artery aneurysm, thrombosis, etc.[6-16]

High-resolution echocardiography detection of mouse heart: After anesthetizing the mouse with isoflurane gas, apply the depilatory cream to the left chest area, and wait 1 minute to wipe off the hair in this area with a sanitary swab; In the supine position, the chest was coated with ultrasound coupling agent for ultrasound detection, and the parasternal left ventricular long-axis and short-axis views were selected for examination; the left coronary artery trunk at the end of diastole was measured and recorded 4d, 15d, 26d and 32d after intraperitoneal injection of LCWE And the ultrasound measurement of the inner diameter of the right coronary artery trunk. Measure the

extent and size of the tumor when there is coronary aneurysm-like expansion. The diagnostic criteria for coronary aneurysms refer to the clinical criteria for coronary aneurysms established by the American Heart Association, that is, the ratio of the inner diameter of the dilated coronary artery to the inner diameter of the adjacent segment>1.5. Choose 3 consecutive cardiac cycles. The mouse ultrasound storage parameters were extracted and measured and analyzed in Vevo770 software.

Data collation and statistical analysis method Measure the left and right coronary artery inner diameters of mice on the 4th, 15th, 26d, and 60d days. To test the homogeneity of the measurement data, the normal distribution is expressed as the mean ± standard deviation, and the difference between the two groups is analyzed by grouping or paired T test, and the two groups are tested by analysis of variance at different time points.

Statistical processing: SSPS 20.0 software was used for statistical analysis of the results, Pearson chi-square test or Fisher exact probability test, two-sided test was used to compare the differences between groups, P<0.05 was considered statistically significant.

Results

General conditions and analysis After injection of CAWS, mice in the experimental group showed changes in activity, food intake, weight loss, hair disorder, irritability and other appearance changes. By 28 days after the injection, neither the experimental group nor the control group died. The appearance changes of mice in the experimental group were most obvious at 3 and 7 days after the last stimulus injection. The changes in body weight and heart weight of mice in the experimental group were significantly different from those in the control group at 24 h, 3 d, and 7 d after injection ($P<0.05$). 14 d, 28 d after injection of human umbilical cord mesenchymal stem cells At that time, the body weight and heart weight of the experimental group mice gradually recovered, and the difference was not statistically significant compared with the control group.

B-ultrasound results after mouse modeling and intervention

High-frequency small animal echocardiography showed that 57.5% (23/40) of the modeled mice had coronary artery disease, and 5% (2/40) had right coronary artery aneurysms with an internal diameter of 0.918 mm, so an aneurysm was considered; 27.5 %(11/40) The coronary artery wall is thickened, the inner diameter of the left coronary artery trunk is widened, and the intima is thickened. After stem cell intervention, the inner diameter of the left main coronary artery decreased (see Figure 1).

A: It shows that the inner diameter of the left coronary artery trunk widens, about 0.255 mm, and the intima is thickened by 0.105 mm after 4 days of immunization; B: It shows that the coronary artery is significantly widened after 15 days of immunization; C: It shows that the right coronary artery aneurysm appears in the 24 days of immunization; D: Shows that the inner diameter of the left coronary artery trunk decreases after 10 days of stem cell intervention.

Histopathological changes of coronary artery

After intraperitoneal injection of LCWE, mouse coronary inflammatory reaction can be seen, neutrophils, macrophages and a small amount of lymphocytes infiltrate, the inner and outer elastic layers of blood vessels are destroyed, and focal fibrinoid necrosis and fibers of varying degrees can be seen in the outer membrane (See Figure 2).

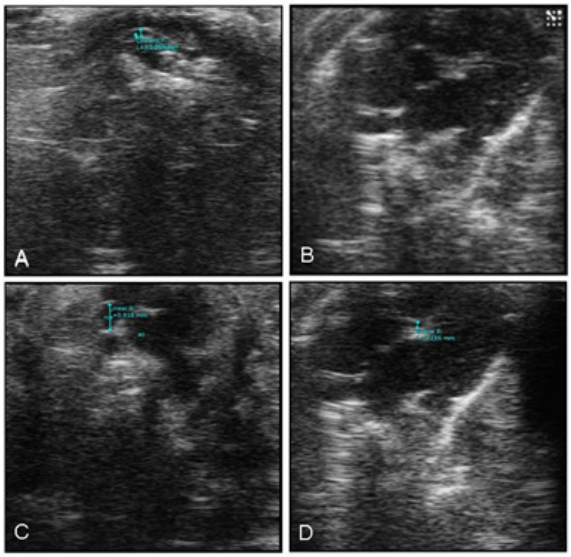

Figure 1 Echocardiogram.

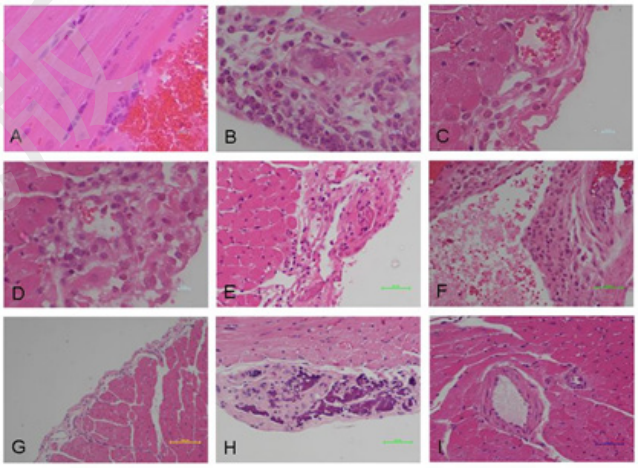

Figure 2 The results of physical staining of mouse heart disease (A-E, 40×; F, H, 20×; G, 10×; I, 200×).

4 days after LCWE injection, local endothelial cells of the left ventricle fell off, and white thrombus formed on it. A small number of neutrophil infiltration was occasionally seen between the intima and muscle wall (A); the epicardium of the right ventricle and atrium was highly swollen. A large number of neutrophils and a small number of monocytes (B, C, E) can be seen. The coronary artery lumen within (right) dilates and a small amount of myocardial cell necrosis and collapse (C, D); a coronary artery wall thickens, the lumen is narrow, and white thrombosis can be seen in the cavity (E); the aortic valve and mitral valve are slightly swollen, and a few neutrophils are scattered infiltration (F). 15 days after LCWE injection, the epicardial interstitial edema, a small amount of lymphocyte infiltration and diffuse and mild hyperplasia of fibrous connective tissue (G), and significant local fibrous connective tissue proliferation accompanied by solid calcium salt deposition (H). After hUC-MSCs intervened for 10 days, a large number of lymphocytes, eosinophils, and monocytes were infiltrated locally in the outer membrane of the left atrial appendage (I), and no obvious vasculitis and other obvious abnormalities were seen.

Citation: Zhang S, Guo H, Zhang X, et al. Establishment of a model of coronary artery injury by Lactobacillus casei wall extract in mice with Kawasaki disease and study of the effect of human umbilical cord mesenchymal stem cells on coronary artery injury and pathological and ultrasound changes. *J Pediatr Neonatal Care.* 2021;11(1):1–5. DOI: 10.15406/jpnc.2021.11.00433

Discussion

Current studies believe that small and medium vascular inflammation is the main pathological change of KD. The long-term existence of vascular inflammation is the basis for the eventual complications of coronary atherosclerosis. However, the pathogenesis of KD at this stage is not very clear. It may be related to infection, environment, genetic susceptibility, T cell-mediated immune response, endothelial cell dysfunction, etc.[12] At present, the animals for establishing Kawasaki disease models include mice, rabbits, pigs and dogs, among which the commonly used strains of mice include C57BL/6J, C3H/HeN, CDI, DBA/2N and Balb/c, etc., mouse model inducers Mainly Lactobacillus casei cell wall extract and Candida albicans extract etc.[13] which provide valuable experimental materials for the research of Kawasaki disease.

In this study, a mouse immune vasculitis model was constructed to simulate the changes of coronary artery inflammation during KD and observe the dynamic changes of coronary vascular endothelium. Vascular endothelial cells cover the surface of the vascular intima and are the part where vascular tissue interacts most closely with inflammatory factors in the blood. The damage and dysfunction of endothelial cells have an early indication of the deepening and outcome of KD. The further development of KD vascular inflammation can cause damage to the smooth muscle and inner elastic layer of the vascular wall, causing coronary artery dilation and aneurysm.[14]

The clinically accepted treatment for Kawasaki disease is intravenous immunoglobulin or IVIG and oral aspirin. This combined treatment plan significantly reduces the mortality and coronary complications of Kawasaki disease. However, studies have found that Gamma The number of globulin-resistant cases is increasing year by year, and myocardial infarction has become a major public health problem worldwide. Clinical trials have shown that stem cell therapy can improve heart function, reduce infarct size and mortality, and has become a hot spot in cardiovascular disease research.[15] Stem cell is a cell with strong self-renewal ability and multiple differentiation potentials, and has unique biological characteristics. Human umbilical cord mesenchymal stem cells (hUC-MSCs) have gradually gained the favor of clinical researchers due to their wide sources, low immune response, and multi-differentiation potential, and they have been able to grow into osteoblasts, adipocytes, fibroblasts, Differentiation of endothelial cells, cardiomyocytes and other cells.[16]

In this study, after intraperitoneal injection of LCWE in mice, echocardiography showed that 57.5% (23/40) of the mice had coronary artery disease, of which 5% (2/40) had right coronary artery aneurysm and 27.5% (11/40) had coronary artery disease. Thickening of the wall, widening of the inner diameter of the left coronary artery trunk, and thickening of the intima; histopathological examination showed that the epicardium of the aortic valve, mitral valve, right ventricle and atrium was slightly swollen, and a few neutrophils scattered infiltration , The (right) coronary artery lumen is dilated and a small amount of myocardial cells die and disintegrate, and the local fibrous connective tissue is significantly proliferated and accompanied by solid calcium salt deposition. After the intervention of stem cells, ultrasound showed that the inner diameter of the main coronary artery was reduced. Pathological examination showed that there was a large amount of lymphocytes, eosinophils, and monocytes in the outer membrane of the left atrial appendage. There was no obvious vasculitis and other obvious abnormalities. The results suggest that hUC-MSCs intervention can significantly reduce the Kawasaki disease-like coronary artery injury in mice caused by LCWE injection. The advantage of echocardiography is that it is fast and non-invasive, and has higher accuracy for coronary arteries running in the wall, which is higher than that of CT and angiography. Histopathology is the "gold standard" of coronary artery examination. It is combined with echocardiography to examine the pathological changes of the coronary artery in mice after modeling and intervention. It can be combined with speed and slow, accurate and efficient, and is the prevention and treatment of Kawasaki disease. The research provides sufficient technical support.

Conclusion

In conclusion, this study not only successfully induced the experimental mice to produce coronary artery pathological changes similar to Kawasaki disease, but also confirmed that the intervention of umbilical cord mesenchymal stem cells can reduce the degree of inflammation of the coronary artery disease in mice, which is a clinical treatment for umbilical cord mesenchymal stem cells The pathological changes of coronary artery damage and ultrasound detection of Kawasaki disease provide laboratory evidence.

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Review Article

First Retrospective Analysis of 448 Pediatric Cases of Kawasaki Disease of Two Cohorts from China and Russia

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Abstract

Kawasaki disease (KD) is an acute multisystemic vasculitis of unknown etiology that occurs in infants and children. Diagnosis is based on clinical criteria that include fever, exanthema, conjunctivitis, changes in the extremities, erythema of oral mucosa and lips and cervical lymphadenopathy. We investigated the clinical signs and therapy efficacy of patients with KD who admitted to our department. We reviewed 22 patients in Russia, with an age range of 2,5 months to 12 years. Moreover, 426 cases of children with KD admitted to the Children's Hospital of Shaanxi Provincial People's Hospital (China) from December 2013 to March 2018 were selected and compared. In case of hospitalization within 10 days of fever we reviewed incomplete KD in 5 patients (25%). In 3 patients KD was finally diagnosed either after antibiotics had been ineffective or when other symptoms of KD emerged, in 70% of cases the diagnosis of KD was undoubted. There was also no doubt of diagnosis in all patients admitted to our department after 10 days of fever. 77% of patients recovered after one dose (2 g/kg) of intravenous immunoglobulin (IVIG) and in 23% of cases it was necessary to insert the second dose. Clinicians should consider the KD diagnosis in young children with persistent inexplicable fever to start IVIG treatment within 10 days to prevent development of coronary aneurysms.

Introduction

The Kawasaki disease (KD) or mucous and skin lymphonodular syndrome – an acute general disease with primary damage of average and small arteries and development of a destructive and proliferative vasculitis. Clinically KD proceeds with fever, modifications of mucous membranes, skin, lymph nodes, possible defeat of coronary and other visceral arteries [1]. For the first time this disease is described by T. Kawasaki in Japan in 1967 [2]. Since 1961, he examined children with persistent fever and such symptoms as cervical lymphadenitis, conjunctivitis, rash, edema of brushes and feet with the subsequent peeling of fingers. This symptom complex did not match any other known disease. T. Kawasaki described about 50 cases, then this state had the name "febrile oculo-oro-kutaneo-acrodesquamative syndrome with not purulent cervical lymphadenitis". In 1992 the disease as independent nosologically unit was allocated in the most important reference book of the American pediatricians -"Nelson text book" and received a name of its discoverer – Kawasaki disease. Clinical guidelines for the diagnosis and treatment of Kawasaki syndrome have been developed in Russia [3].

Patients and Methods

Russia

In Office of Diagnostics and Recovery Treatment of National medical research center of children health (Russian Federation) we

observed 22 children with KD. One of them was 12 years old, other children – aged from 2.5 up to 36 months (middle age – 12 months). The ratio of boys and girls was 1:1,2. In case of unclear diagnosis for children with persistent fever in addition to a standard complex of the examinations, conducted at fever without the infection site, ultrasound examination of heart and coronary vessels was conducted. In case of KD confirmation heart ultrasonography was carried out repeatedly after 10th day of diseases.

China

A total of 426 cases of children with KD admitted to the Children's Hospital of Shaanxi Provincial People's Hospital at Xiatong University in Xian from December 2013 to March 2018 were selected. Among them, 17 patients were diagnosed with coronary artery aneurysm by echo-cardio-graphy (including the newly diagnosed children in our hospital and the children with coronary artery aneurysm diagnosed in other hospitals for further treatment in our hospital). Clinical data of KD children with coronary aneurysm were collected. The age, gender, major clinical manifestations, laboratory findings (including CRP, ESR, PLT), ultrasound examination results (including coronary artery aneurysm detection time and coronary artery involvement), IVIG treatment methods and effects were retrospectively recorded and analyzed in KD children with coronary artery aneurysm.

The diagnosis of KD is based on the diagnosis, treatment and long-term management guideline for Kawasaki disease issued by the American Heart Association in 2004 [3], that is, clinical diagnosis can be made with at least 5 of the 6 main clinical manifestations; if 5 items are not satisfied, but echocardiography or cardiovascular angiography confirms coronary artery aneurysm or coronary artery dilatation, except for other diseases at the same time complete KD can also be diagnosed. Unexplained fever for 5 days or more combined with 2 or 3 typical KD clinical features may be considered incomplete KD. The diagnosis criteria for coronary artery aneurysm [4]. the ratio of the inner diameter of the coronary artery expansion segment to the adjacent segment is greater than 1.5, among which, the inner diameter of the aneurysm <5mm is a small coronary artery aneurysm, 5-8mm is a medium coronary artery aneurysm and >8mm is a giant coronary artery aneurysm.

Epidemiological Aspects of Kawasaki Disease

The disease of Kawasaki is widespread among babies and infants. The incidence considerably varies between ethnic groups. The incidence of KD is equal to 5-10 cases per 100,000 children in recent European researches, under 5 years old [4-7]. In the Asian countries it is reported about much higher level [8,9]. The highest incidence is registered in Japan; the last national research in 2012 reports about incidence - 265/100.000 children, under 5 years old and assumes that the KD level still increases. Most of patients are babies from 6 months to 5 years old, in spite of the fact that incidence cases also take place among children of elder age and adults. A ratio of men/women - about 1,5 to 1 [7]. In the USA about 3000 new cases of KD are annually described – frequency 20,8 per 100,000 children, younger than 5 years old (dispersion from 18,5 to 23,1 in different years). 76.8% from all KD cases were diagnosed at this age, the ratio of boys and girls was about 3:2 [10,11]. For the 15-year period of observations in Irkutsk region the average level of KD incidence was only 6,6 cases per 100,000 children under 5 year old and 2,7 cases per 100,000 children under 17 year old. Thus, patients under 5 year old were 67,5% of the diseased, out of them 50,8% – under 3 year old. Infants bear KD most severely, in Russia their share is 23,5% [12].

According to an epidemiological study conducted in Russia (Irkutsk location) from 2005 to 2009 KD was in all seasons of the year [13]. The disease was more often recorded in spring - 30.2% and in winter - 28%, less often in autumn - 22.8% and in summer - 19%. A separate analysis of the seasonal incidence of BC among boys and girls found that in boys the disease was also more often recorded in the winter-spring period, moreover, more cases were recorded in boys (44.5%) from January to April than in girls (34%). Among girls, a more pronounced increase in the incidence rate was found than among boys, in the autumn-winter season with the largest number of cases from October to December (girls - 32%, boys - 17.7%). An interesting fact is that the risk of BC in these months in girls is 2 times higher than in boys (RR = 2.19, 95% -DI 1.29-3.72).

In February, 2.7 times fewer cases of BC were registered among girls than among boys. A decrease in the incidence of BC in the summer months is observed in patients of both sexes, slightly more pronounced in girls (girls - 20.7%, boys - 17%). The unambiguous opinion on the cause of KD development does not exist. In favor of infectious nature, general inflammatory symptoms, an increase in the level of inflammation markers, seasonality and epidemic outbreaks are said. In this regard Epstein-Barre's virus, retro and parvoviruses, streptococci, staphylococcus and also toxins were considered. Multiple viral infectious triggers have been suggested, including coxsackie virus, para influenza virus, respiratory syncytial virus, human metapneumovirus, chikungunya, and cytomegalovirus. The fact that KD is rare in children under 4 months could indicate passive maternal immunity, and the extreme rarity in adults – to reflect artificial immunity. The geographical sequence of incidence growth (Asia – Japan – Hawaii – the West bank of the USA could be explained by proliferation of the KD pathogens due to the influence of the atmospheric phenomena [14,15].

In fact, two recent studies showed that up to about half of all KD patients had one of more respiratory viruses detected by PCR, but their etiological role is unproven [16,17]. Also, the possibility of a respiratory RNA virus has been suggested by ultrastructural studies of autopsy specimens [18,19]. Bacteria have also been suggested as the trigger of KD, with research mainly focusing on bacterial s

uperantigens. Superantigens produced by several bacteria are able to stimulate a large percentage of T cells by binding to the Vβ region of T cell receptors and so stimulate the production of pro-inflammatory cytokines. One study looking at five superantigens (streptococcal pyrogenic exotoxin (SPE)-A, C, G, and J, and toxic shock syndrome toxin-1 (TSST-1)), found these in 70% of stool samples collected from acute KD patients as opposed to 27% in healthy controls visiting the same center for vaccinations [20]. Another study found significantly increased IgM antibodies against five superantigens (staphylococcal enterotoxin A, B, and C, TSST-1, and SPE-A) [21]. Nevertheless, the role of superantigens in KD remains unclear. An immune response plays role in KD pathogenesis.

The encounter of a susceptible individual with the unknown agent probably leads to an (exaggerated) immune response involving innate and adaptive pathways. Multiple studies have been performed, both evaluating animal models and immune response in the peripheral blood as well as immune infiltration in the coronary arteries [22,23]. The general paradigm of the immune response is an imbalance between pro-inflammatory and anti-inflammatory pathways. For example, regulatory T cells, a subset of T cells limiting inflammation, have been shown to be important in the vascular inflammation [24]. Also, the IL-1 signaling pathway is upregulated, with upregulated IL-1 pathway genes and increased IL-1 concentrations in peripheral blood of KD patients during the acute phase [25,26]. Recently, it has become clear that inflammasomes, multiproteins that are part of the innate immune system, are induced by the NLRP3 gene and promote the production of IL-1β and IL-18, play a role in KD [27]. In the coronary arteries, immune infiltration of the arterial wall with neutrophils, CD8+ cytotoxic T cells, Ig-A producing plasma cells, and macrophages have been found, accompanied by pro-inflammatory cytokines which may vary in proportion and contribution over time [28].

Genetics are considered to contribute to susceptibility to KD, and probably to CAA and response to treatment [29,30]. A number of genome-wide association studies (GWAS) have been performed [31-37]. Apart from the GWAS, multiple studies have identified specific single nucleotide polymorphisms (SNPs) in several genes. Most of these candidate genes have an immune regulatory function. Pathomorphologically, in KD arteries of average and small size are infected, involvement in process of the coronary arteries (CA) – up to 20% of cases is of particular importance. At autopsy in CA hypostasis of endothelial and smooth muscle cells with inflammatory infiltration of all 3 layers of a vascular wall is revealed. It leads to fragmentation of an inner elastic membrane with forming of aneurysms. Neutrophils in infiltrates quickly give way to macrophages, lymphocytes (mostly, CD8 cells) and plasmocytes, which produce A immunoglobulin in its turn. Fibrosis with the vessel intima proliferation at the recovering stage eventually leads to stenosis or full occlusion of its gleam [38].

Clinical Aspects of Kawasaki Disease

The clinical picture of Kawasaki disease conditionally is divided into 3 stages. The acute feverish stage lasts 1-2 weeks, and without appropriate treatment – up to 3-4 weeks that increases risk of coronary arteries disease [39]. It is characterized by persistent febrile fever, lasting more than 5 days, but most often without treatment – 2-3 weeks. Lesion of mucous membranes usually is characterized with not purulent conjunctivitis, expressed by an injection of conjunctiva vessels (in 95% of cases) (more bulbar - episcleritis) (Figure 1). Cheilosis, changes of oral cavity and tongue' mucous membrane ("crimson-coloured" tongue), cracks of lips, which we observed at 15 children (75%) (Figure 2), are also usually seen. Lesion of skin is usually followed with rash on body appearance, limbs, inguinal area.

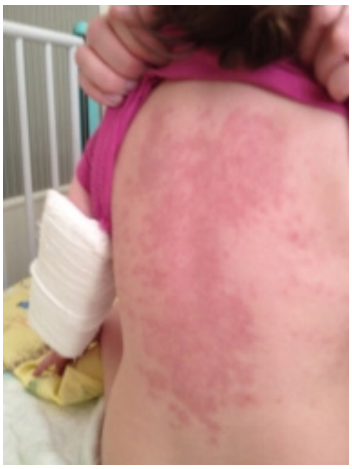

Figure 1: Typical rash in Kawasaki Disease on the back

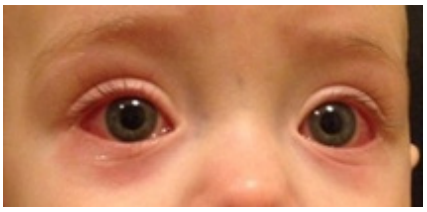

Figure 2: Bilateral conjunctivitis

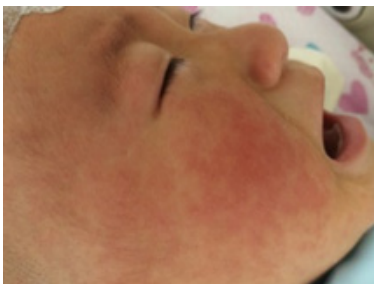

Figure 3: Typical rash in Kawasaki Disease in the face

The exanthema, sometimes drain, is mostly often characterized with papules and macules, though also urticarius and micropustulous elements could be seen. Bullas and vesicles were

never described. We observed rash at 90% of patients, not always profuse, sometimes ephemeral; rash appeared on 2nd-6th days of a disease and died away in 2-4 days in all cases (Figure 3). Increase (unilateral) of front and cervical lymph nodes was diagnosed by us in half of cases, two children had a mask of purulent lymphadenitis with a local dermahemia and fluctuation. However, the puncture was not carried by these children, because existence of other KD signs allowed to prescribe immunoglobulin for intravenous administration, then changes of lymph nodes altogether with fever disappeared within 24 hours. Plasticity of brushes and, more often, feet, typical for KD (Figure 4) were observed at 12 patients in the first days of a disease, the morbidity of movements in joints and dermahemia above them was defined at 7 children. These symptoms were also stopped due to introduction of IVIG (Figure 5).

Figure 4: Typical rash in the face

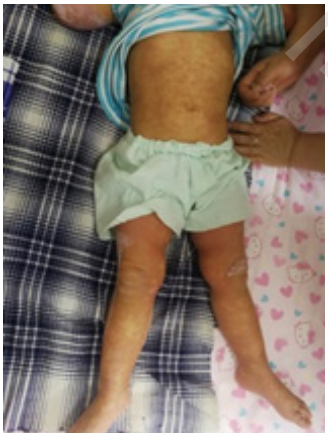

Figure 5: Typical rash in Kawasaki oft he whole body

For KD irritability of the patient is typical; the child is usually whining and inconsolable, it also could be a symptom of aseptic meningitis (the lymphocytic pleocytosis up to 25-100 in 1 μl at the normal level of glucose and protein appears in cerebrospinal fluid). A typical clinical symptom at small children is reddening and consolidation of BCG injection place (patients with KD have a cross-responsiveness between proteins of thermal shock and T-cells). The subacute stage covers the 3-5th week of a disease, skin peeling, which begins with fingers of hands and legs and also in a groin, is typical for it with the background of the persistent fever. Since the end of the 2nd disease week macrolaminar peeling was observed by us at 11 (50%) children, the same - the marked peeling of a red border of lips. In 1-2 weeks after stopping of fever it is possible to observe cross deep lines on nails at some children (lines Beau). Recovery, as a rule, occurs in 6-10 weeks from disease appearance, all clinical symptoms disappear, SOE is gradually normalized. Changes from heart are manifested in the form of arrhythmias, heart noises («machine» noise), caused by regurgitation.

Echographic signs of a coronaritisis with a thickening of walls or expansion of coronary arteries' gleam and also ST-T changes of ischemic type on an ECG are registered more than at a half of patients; at a quarter of the patients, who were not receiving treatment in the KD acute period, symptoms of the postponed myocardial infarction reveal [40]. It is considered that forming of CA aneurysms happens on the 7-9th day of a disease. It is directly connected with destruction of their elastic frame. Aneurysms at the patients, who had KD, are found not only in CA, but in 2,2% of patients in the axillary, common and internal iliac, renal, subclavian, upper brychee, inner thoracic, femoral arteries [41]. We revealed signs of coronaritisis, such as thickening of walls and dilatation of coronary arteries, at 13 (65%) patients, much more often at 3 of 4 children, during hospitalization after the 10th day of a disease. One child of 4 months, hospitalized on the 31st day of fever, expansion of the left and right coronary arteries up to 4,5 mm was diagnosed. He responded to treatment of IVIG, and within 6 months the echographic picture was normalized. Without treatment in the acute period symptoms of heart failure, myocardium ischemia are often registered. According to the Russian authors, the heart failure (HF) develops in 56,3% of cases (50% – HF of the 1-2nd functional class, 6,3% – the 3-4th).

In the same research myocardium dysfunction are met considerably more often at patients with CA dilatation, than without it – 78,3% and 43,8% respectively [18]. Huge aneurysms of CA (the internal diameter more than 8 mm) are rarely met, however are fraught with risk of a cardiac tamponade, thrombosis or myocardial infarction. Other manifestations of KD. Tonsillitis, symptoms of pneumonia, gastroenterocolitis, liver failure, urinary and central nervous system failure are revealed quite often in a debut of KD. In these cases, especially in KD incomplete picture, naturally, fever are supposed to be like a bacterial infection in the beginning; but persistence of temperature after antibiotic prescription to the child with symptoms of an urine system or pneumonia infection allows to doubt the diagnosis, because it could delay KD diagnostics no more than for 2-3 days. The orchitis, pulmonary infiltrates, pleural exudate, haemo phagocytal syndrome [42-44] and also transient sensoneural deafness are considered as rare manifestations of a disease [45,46].

The example of doubts in the diagnosis was when we faced the child, who came to hospital for the 3rd day of fever with a leukecyturia, one kidney increase in the sizes, leukocytosis and

increase in level of bacterial inflammation markers. Persistence of fever for the 3rd day of therapy with ceftriaxone, then meropeny and also appearance of generalized rash (in the beginning to be regarded as allergic), increase of sclerite, cheilitis and pastosity of feet expressiveness from the 5th day of a disease made us to diagnose KD. Fever was stopped after IVIG introduction during the 6-7th day of a disease in a dose of 2 g/kg, the same day all other symptoms began to regress and macrolaminar peeling of hands' fingers appeared. Two more children, hospitalized till 10th day of diseases, the diagnosis of KD competed with suspicion of a severe bacterial infection with increase in inflammation markers. One of them had watery diarrhea. It made to the diagnosis in favor of bacterial intestinal infection, and second one – for lack of the infection site – in favor of bacteremia. These suspicions were kind of confirmed by decrease in temperature after 36-48 hours of antibiotics therapy, however its new increase in a day with gaining of KD symptoms, became a reason for IVIG introduction, which gave fast effect.

Laboratory Results (Moskau, Russia)

The acute period reveal, as a rule, acute and inflammatory changes – a neutrophylic leukocytosis, increase in SOE, high level of C-jet protein and also normochromic normocytic anemia, but any of these tests are not pathognomonic for KD. Laboratory parameters usually come back to norm in 6-8 weeks. Data on pro-calcitonin differ a little: its level in the first days of a disease is high (3 and more ng/ml), but it is quickly normalized, so it usually comes to norm on the 2nd week. Level of thrombocytes increases on the 1st week of a disease, considerably increases on the 2nd, reaches peak on the 3rd week and gradually normalized on 6-10th week of a disease. Also other changes in the system of hemostasis, in particular, hyper aggregation of thrombocytes, exhaustion of a fibrinolytic system and also physiological anticoagulants appear [47].

It makes necessary their introduction to therapy. Predictors of KD severe state, increasing risk of cardiogenic complications, is increase in level of transaminases, general bilirubin, decrease in level of albumine and serumal sodium. Gammaglutamil-transpeptidase raises at 67% of patients [48]. In the clinical analysis of urine approximately in a third of cases the sterile proteinuria and proteinuria is revealed. The analysis of thousand of proteinaceous molecules showed that about 190 of them could be found in urine of children with KD. 2 proteins, associated with failure of endothelial and muscle cells, were identified: filamin C and immunoregulator meprin A, which are considerably raised at the patients, who did not respond to starting therapy in comparison with the patients, who ceased to be in a fever after the first dose of IVIG. These indicators could be regarded as markers of disease activity, and increase of their level at children with KD incomplete form could theoretically be additional criterion of diagnosis [49].

Laboratory Results (Xian, China)

ESR:1 week after admission (50-100mm/h 4 cases, >100mm/h 2 cases) and within 2 weeks of the disease (50-100mm/h 3 cases,>100 mm/h 1 case), the rest were not examined. CRP: After admission, < 50mg/L 3 cases, 50-100mg/L 3 cases, >100mg/L 5 cases and the rest were not examined. At the time of discharge, < 50mg/L 10 cases and others were not examined. Among the 17 children with Kawasaki disease with coronary artery aneurysm, there was no significant difference in the degree of increase of ESR between 1 week and 2 weeks. After admission, 8 cases of CRP were significantly higher than normal, 6 cases were not examined, 10 cases were normal at discharge and 7 cases were not examined. The CRP level at discharge was significantly lower than that after admission.

Time of detection of coronary artery aneurysm in the course of disease: 1 case within 1 week, 12 cases within 2 weeks and 4 cases more than 1 month. Coronary artery aneurysms were usually detected within 2 weeks of the course of disease. Coronary artery involvement: there were 8 cases of bilateral coronary aneurysms, 4 cases of left coronary aneurysm alone,5 cases of right coronary aneurysm alone. Bilateral coronary artery involvement was the most common.

Diagnosis of Kawasaki Disease

The diagnosis of KD is based on the presence of clinical features of persistent fever in combination with a polymorphous exanthema, cervical lymphadenopathy, non-purulent conjunctival injection, changes of the lips and oral cavity (including strawberry tongue, cracked lips, redness of the mucosae), and changes in extremities (swelling and redness of the palms, desquamation in the subacute phase) [50]. In the most recent American Heart Association (AHA) guidelines, persistent fever is classified as ≥5 days, but in the presence of four or more symptoms, the diagnosis can be made with only 4 days of fever [50,51]. "Complete" KD is defined as fever and ≥4 out of the 5 symptoms. It is important to appreciate that criteria may present successively instead of simultaneously. The AHA has created an algorithm to increase the possibility of "incomplete" KD in case ≤3 criteria are present. This algorithm includes CA abnormalities on echocardiography and/or laboratory abnormalities [50]. There is no diagnostic test for KD, and the diagnosis may be delayed or overlooked. To improve diagnosis, multiple new biomarkers have been studied, but none has so far proved specific for KD [52]. Classification tools have been developed to aid in the differentiation between KD and other febrile illness, although the utility as a point-of-care diagnostic test remains unproven [53,54].

Russian Experience with Kawasaki with Patients

In our examinations the diagnosis of KD was made in the 1st day of hospitalization in 70% of the children, who came till 10th day. The diagnosis of all children, hospitalized after 10th day of disease, also did not make any doubts. Incomplete KD was diagnosed for 5 children (25%). The girl of 3 months with fever within 9 days -

the dermahemia over all interphalanx and to lesser extent – over metatarsal joints, disputable swelling of palms and feet and almost inevitable sclerite draw our attention. The diagnosis was made after identification of coronaritisis signs at heart ultrasonography. The dose of IVIG of 2 g/kg did not make effect, after infusion expansion of right CA to 3,5 mm was seen. After the second dose of IVIG fever was stopped, right CA diameter decreased up to 2,4mm. The girl of 12 months for the 8th day of fever had only 2 signs out of 5 – swelling of brushes with a dermahemia over interphalangeal joints and expressed sclerite without signs of a coronaritisis. At introduction of IVIG in a dose of 2 g/kg fever was stopped in the 1st day.

The diagnosis of 5 months- girl was difficult with a periodic disease in the family anamnesis and carriage of the same gene mutation to distinguish. She came for the 32nd day of fever; due to careful interrogation of mother we succeeded to know only about moderate conjunctivitis and light swelling of brushes in disease beginning; identification of left CA expansion up to 3,4 mm and right – up to 3,2 mm on ultrasonography helped to make the diagnosis. Introduction of IVIG in a dose of 4 g/kg led knocks to apyrexia with the subsequent reduction of CA diameter up to 2,6 mm on the 3rd day. The boy of 22 months came on the 18th day of fever with sclerite and rash in the disease anamnesis. Identification of right CA expansion to 5,5 mm with consolidation of walls also confirmed diagnosis. Continuous 4 g/kg of IVIG with stopping of fever for 2 days, after which fever and sclerite came back. Pulsotherapy with methylprednisolonum in a dose of 30 mg/kg within 3 days gave positive effect with reduction of CA diameter up to 3 mm and normalization of hematologic indicators.

In the next 2 weeks the sclerite and subfebrile condition up to 37,7 °C with a further absolute recovery periodically appeared. The girl of 1 year with a picture of retropharyngeal abscess (fever, morbidity at the movements of the head, lockjaw) and hypoechoic (20-30 pieces of N), lenticular, not accumulating contrast excrescence in retropharyngeal area, revealed in computer tomography. The lack of pus during opening the place of hypostasis on throat back wall helped to suspect KD and persistence of temperature despite antibacterial therapy. Besides, from mother's interrogation we succeeded to find out existence of a one-day injection of scleras and ephemeral rash in the 1-2nd days of a disease. Introduction of IVIG stopped fever, and on the 10th day peeling of fingers appeared. We found the description of the few similar observations in literature [55]. Incomplete KD meets more often in infants; therefore, as well as foreign authors, we recommend echocardiography carrying out to all children with unclear fever and laboratory markers of inflammation [39,56].

Differential Diagnosis

A number of diseases with exanthema and changes of joints (the infection, which are followed by exanthema, syndromes of toxic shock and "the scalded skin", juvenile pseudorheumatism, Stephens-Johnson's syndrome, etc.) has signs, similar to KD. Part of them is excluded according to clinical data even with persistent fever less than 5 days (including – effect on antibiotic therapy). At infectious mononucleosis in 10-15% of cases there is maculo-papular rash, but fever is kept usually less than a week and laboratory data help with the diagnosis verification. Stephens-Johnson's syndrome differs from KD with violent and erosive elements. Juvenile idiopathic arthritis is quite often shown only by fever, generalized lymphadenopathy and spotty pale "flying" rash within several weeks. This diagnosis, as well as a nodular polyarteritis, must be meant at patients with suspicion on KD, whom were carried out treatment with an adequate dose of IVIG without effect. We observed a similar picture at the infant, that altogether with the instruction on "possible changes of coronary arteries walls" on heart ultrasonography, made to do IVIG, which did not give the expected improvement; further juvenile idiopathic arthritis was diagnosed for the child.

Treatment and Prognosis of Kawasaki Patients in Russia

Starting therapy of both full and incomplete KD assumes introduction of 2 g/kg of IVIG given over 8–12 h [57]. Treatment with IVIG significantly reduces the incidence of CAA [58]. IVIG is preferably given within the first 10 days after disease onset [50]. Acetylsalicylic acid (as anti-inflammatory and antiaggregant drug) in a dose of 80-100 mg/kg/day in 4 receptions. Apart from IVIG, high-dose aspirin is advised by the AHA, although evidence for further risk reduction for CAA is lacking [58,59]. Additional prescription of anticoagulants (warfarin, dipyridamolum) could be required by children with huge coronary aneurysms. In Japan there is an experience of treatment by smaller doses of acetylsalicylic acid – 30-50 mg/kg/day – for the purpose of hepatotoxic effect prevention [58,60]. Most of children respond to IVIG introduction with fever stopping and considerable improvement of health within the first 24 hours, at the same time the risk of vessels and heart failure, forming of aneurysms authentically decreases (from 20% to 5%). About 10-20% of the patients, who received IVIG course, have repeated fever [7].

In that case immunoglobulin is given repeatedly in the same dose (2 g/kg). In Japan, risk-scores have been developed to identify patients with a higher risk of IVIG resistance [61- 63]. Unfortunately, these risk-scores do not perform adequately in Western, ethnically mixed, and in Chinese populations [64-68]. A possible method to decrease IVIG resistance is to intensify the initial treatment. A recent meta-analysis showed a beneficial effect of adding corticosteroids to the initial treatment with IVIG, yet this effect was only found in Japanese studies and not in two studies conducted in the USA [69]. Only in case of inefficiency of IVIG repeated therapy the question of infliximabum prescription is

considered. Though there are data on its efficiency as starting drug at KD treatment [70]. Patients are considered to be resistant to IVIG, if in 2 days and more after an initial dose it was necessary to give a repeated dose, methylprednisolonum, rituximab or infliximabum. Resistant KD, according to researches, is met in 16,3% of cases with ratio between clinics of 8,0-26,8%. More often cases of resistance are registered among the Afro-Americans [71]. After normalization of temperature, the AHA advises ongoing aspirin in a low dose until no evidence of CA dilation are present at by 4 to 6 weeks after the acute illness [50]. If CAA are present and persisting around that time, aspirin is continued as anti-thrombotic therapy.

In case of large (around a z-score ≥10) or complex abnormalities, additional anticoagulation therapy should be administered to prevent clotting due to turbulence in these pro-coagulatory large coronary artery lesions [50,72]. In case of possible inactivation of live vaccines after IVIG treatment, vaccination against measles, epidemic parotitis and rubella must be postponed for 11 months. There is also a risk of development of Ray syndrome in children, who receive acetylsalicylic acid for a long time, especially at flu or chicken pox disease. In case of the child contact or disease of these infections temporary cancellation of acetylsalicylic acid is possible, and to children with existence of aneurysm – prescription of dipyridamolum on this time. KD is the leading reason of heart acquired diseases at children, increasing risk of development of coronary heart disease and myocardial infarction at young age. Male, aged under 1 year old, persistent fever and also KD recurrence are to be predictively adverse factors of CA aneurysm development. Thrombocytopenia, hypoalbuminemia and also low level of hemoglobin, G-class immunoglobulins are predictors of KD severe current with a possible outcome in CA aneurysm forming [2,39].

In case of KD timely adequate treatment, for example in the USA, lethality is less than 0,01%. Follow-up observation of 546 KD cases showed that 5 of them died. Cause of death in 1 case was CA failure, in 1 case – sudden death without symptoms of thrombosis or myocardial infarction, in 1 case – an acute coronary syndrome as a result of aneurysm thrombosis, in 2 – other options of an acute coronary syndrome. In 50% of cases death came within a month after an acute state [73]. Approximately 2% of patients have KD recurrence in several months and even years after the 1st disease. Among the children, observed by us, one 6-month-old child had KD recurrence in 2 months after the 1st episode, treated in other hospital. The girl came on the 1st day of fever with vivid clinical manifestations of a disease and expansion of coronary vessels. Fever was stopped after the 1st IVIG dose with further regression of clinical symptoms and normalization of ultrasonic changes.

Nowadays many researches is devoted to studying of KD role in development of periodic feverish syndromes and some autoimmune diseases [74]. All children with KD, observed by us, were treated by IVIG, in 77% of cases we got lasting effect after single dose of 2 g/kg in the form of temperature normalization, considerable improvement of state. In 23% of cases (5 children) it was necessary to repeat IVIG therapy, it significantly raised the price of treatment. Use of acetylsalicylic acid in a dose of 80-100 mg/kg/day in 4 receptions did not cause by-effects in our observations, as well as its further prolonged use in a low dose (3-5 mg/kg/day).

Conclusion

We had a possibility of 22 children with KD examination, both on the earliest and at late stages of a disease. All these children were hospitalized with one persistent complaint – persistence of fever, which was not stopped before specific treatment. The lack of several typical symptoms, which after all appeared in later terms at most of patients, was one of the main difficulties of KD diagnostics at early stages of a disease before emergence of coronaritisis' signs. Besides, it was possible to find out in parents' interrogation existence of some typical symptoms of a disease, which appeared at the very beginning of a disease and disappeared until hospitalization. Measures on timely diagnostics and adequate therapy of KD, according to the international standards and recommendations, include the focused attitude towards children with the persistent fever, which is especially not stopped with antibiotics, structured interrogation of parents about existence of KD symptoms at the very beginning of a disease and timely qualified ultrasonic examination of coronary arteries.

After discharge from a hospital long follow-up observation with control of heart ultrasonography and hematologic indicators for patients is carried out.

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Review

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Kawasaki Disease: Global Burden and Genetic Background

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Abstract

Kawasaki disease (KD) is a childhood vasculitides associated with serious coronary artery lesions. It is the most common cause of pediatric acquired heart disease in developed countries, and is increasingly reported from many rapidly industrializing developing countries. The incidence varies widely among different nations and is highest in North-East Asian countries, where almost 1 in 100 children in Japan having the disease by age of 5, where the lowest incidence reported in sub-Saharan Africa. The etiology of KD is still uncertain; interaction between a genetic predisposition and several environmental and immunological factors has been hypothesized. Several susceptibility genes were identified to be associated with the development of KD and increased risk of coronary artery lesions. Gene-gene associations and alteration of deoxyribonucleic acid (DNA) methylation are also found to play key roles in the pathogenesis and prognosis of KD. This article will focus on the global epidemiological patterns of KD, and the currently known genetic predisposition.

Keywords: Kawasaki disease; Epidemiology; Genetics

Introduction

Kawasaki disease (KD), first reported by the Japanese physician Tomisaku Kawasaki, is considered the most common childhood vasculitides in developed countries [1, 2]. Various epidemiologic reports showed a significantly increasing incidence of KD in rapidly industrializing developing countries as well, such as China, India, and Latin American countries. This may be due to an actual increase in incident cases or the improvements in health facilities, and the widespread use of antimicrobials and vaccines that helped to eliminate infectious diseases with their similar fever and rash allowing more awareness and ascertainment of KD [2, 3].

The disease causes inflammatory changes to the vessel walls of small and medium-sized arteries of any area of the body. However, coronary arteries are predominantly involved, and coronary artery lesions can occur in up to 25% of untreated children with KD resulting in serious complications as coronary artery ectasia/dilatation, coronary artery aneurysm, and acute myocardial infarction [4]. Therefore, early detection and prompt treatment are crucial, since treatment with intravenous immunoglobulin (IVIG) within 10 days after disease onset can lower the incidence of aneurysm to < 5% [5, 6]. The peak age incidence of KD generally range between 6 months and 5 years, and usually presents with fever, skin rash, diffuse mucosal inflammation, non-exudative conjunctivitis, cervical lymphadenopathy and extremities changes [7, 8].

The etiology and pathogenesis of KD remain uncertain. The presence of familial aggregation and the increased incidence by 10 times in Asian population suggest a strong genetic origin [9]. However, genetic factors alone cannot explain seasonal variations or geographic and temporal clustering of KD cases [10]. Additional factors, such as infectious agents (bacteria, viruses, fungi, etc) [11], environmental rigger or autoimmune reactions [12] are needed for the onset of the disease, but their definite role remains unclear. In addition, epidemics of KD have been reported, most known in Japan in 1982 and 1986 [13]. Several studies have also documented a correlation between higher socioeconomic status, smaller family size, and urbanization and increased KD incidence [14, 15].

Since 1980s, KD attained a significant public health concern. As with the control of infection, availability of antimicrobial agents, and enhanced general hygiene; the incidence of rheumatic fever and other infectious diseases declined in developed countries. Meanwhile the incidence of KD remarkably increased and became the commonest cause of pediatric acquired heart disease, particularly in Japan, Korea, the USA, and many other developed countries [16].

Global Epidemiology

KD has been documented in more than 60 countries and cross all ethnicities [4, 17] (Fig. 1, [2]). The incidence of KD is in-

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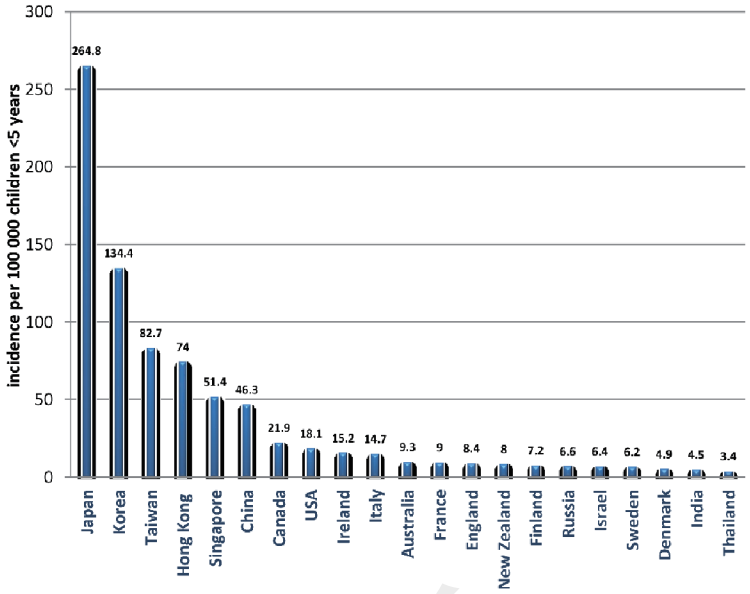

Figure 1. The global incidence of Kawasaki disease per 100,000 children under 5 years old [2].

creasing in several countries especially rapidly industrializing nations, which may reflect a role of air pollution in the pathogenesis of KD [3]. The diagnosis of KD till now is based only on the characteristic clinical features with the presence of a proportion of incomplete or atypical KD cases that have no standard criteria for their diagnosis, which is usually achieved through clinical and laboratory data supported by expert opinion [4, 18]. This difficulty in diagnosis makes the true incidence and burden of the disease to be still unknown [2].

Epidemiological distributions of KD

The incidence of KD varies greatly among different regions. In North America, Europe, and Australia, the incidence of KD ranges from 5 - 22 per 100,000 children < 5 years old. In these countries the incidence was increasing, partly as a result of increased ascertainment, until the past 10 years, when the incidence becomes nearly constant [2, 17]. Conversely, North-Eastern Asian countries, especially Japan, Korea, and Taiwan report an incidence of more than 10 times greater than North America, Australia and Europe; and it continues to increase as reported during the last two decades [19-21]. In the rapidly growing economics like China and India, although the epidemiological reports are less accurate, the incidence appears to be significantly growing and follows the epidemiological patterns of the North-Eastern Asian countries [22, 23].

KD in North America, Europe and Australia

KD incidence in these countries is nearly constant with the lowest incidence in Scandinavian countries, and the highest incidence in North America [2, 17]. In USA, the incidence is estimated to be 17 - 21 per 100,000 children < 5 years old [17]. Both data from the USA and Canada showed significant ethnic variation, with a markedly higher rate among American Asians and peoples of the Pacific Islands supporting the genetic background of KD [17, 24]. Most of the European countries report an incidence rate < 16 per 100,000 children < 5 years old; there were also noticeable ethnic variations, with higher incidence reported in children of Eastern Asian descent [25]. In Australia, the incidence rate ranges between 8 and 10 per 100,000 children < 5 years, and it is not determined whether the incidence has plateaued or is still increasing [25].

KD in Japan, South Korea and Taiwan

The incidence rates of KD in these countries are the highest worldwide (> 50 per 100,000 children < 5 years old) and the number continues to increase [19-21]. The highest rate globally is reported in Japan with an estimate of 264 per 100,000 children < 5 years. Recurrence rate of KD was 3.5% of cases, mortality rate was < 0.02%, and resistance to IVIG reported in 17.0% of cases [21]. South Korea reported the second highest incidence rate (134.4 per 100,000 children < 5 years) [20]. Data on KD in Taiwan are extracted from the records of the national health insurance program with an estimated incidence of (82.8 per 100,000 children < 5 years) and reported coronary complications in 5.4% of the cases [21, 26].

KD in China

The incidence data of KD are available from studies conducted in different provinces, as an accurate nationwide study is difficult to conduct in the most populous country in the world. The reported incidence varies between 7.06 and 55.1 per 100,000 children < 5 years [27-29]. For example, in Beijing the inci-

dence rate increased from 40.9 per 100,000 children in 2000 to 55 per 100,000 in 2004, and in Shanghai the rate increased 3 times in less than 20 years (from 16.8 per 100,000 in 1998 to 50.5 per 100,000 children in 2012). On the other hand, in the lower income Sichuan province, the reported incidence was 7 per 100,000 children, largely lower than other provinces which may reflect a lack of access to medical care services due to relative lower social status. In Hong Kong, the incidence rate is the highest in China (74 per 100,000 children < 5 years) [30]. Despite the shortage of a nationwide data in China, it appears that it follows the trend of the increasing incidence of KD similar to that of other East Asian countries.

KD in other Asian countries

A gradual increase in the incidence of KD has been reported in India, Thailand, and many other countries across Asia [23, 30]. The lack of nationwide epidemiological data makes it difficult to determine whether this is a true increase due to environmental and climate changes associated with industrialization or just an increase ascertainment resulting from more awareness by health care professionals and increased access to medical care following the rapid economic growth [23]. Before 1990, there were only three available reports of KD in India. After this time, KD has been reported in almost all regions of the country with incidence data of 4.5 cases per 100,000 children less than 15 years old [31].

KD in the Middle East and Sub-Saharan Africa

Except for some studies conducted in Turkey and Iran, limited data have been reported from other Middle Eastern countries [32, 33]. A reason of shortage of incidence reports from this region could be due to the lower socioeconomic status and a lack of census data for children < 5 years of age. A study from Egypt reported coronary aneurysms denoting missed childhood KD cases when they reviewed a series of 580 patients ≤ 40 years of age presenting with symptoms of coronary artery ischemia [34]. Also, Agha et al reported two cases with incomplete KD in children < 5 years old complicated with coronary aneurysm [35]. The data for KD incidence are lacking in sub-Saharan Africa, however several sporadic cases have been reported across many countries in the region [36]. These findings raise the possibility that KD is not that uncommon in the Middle East and sub-Saharan Africa as previously thought, and increased awareness and diagnosis may reveal the true incidence of KD.

KD in Latin America

Prior to the formation of the Latin American Kawasaki Disease Network (REKAMLATINA), which included 20 countries, little was known about the epidemiology and prevalence of KD in these countries [37]. In Chile as an example, there were several reports of increasing cases of KD, which also may be a result of the increased awareness and ascertainment of the disease [38].

Seasonality of KD

Many Asian countries such as Japan and South Korea had reported distinctive seasonal patterns of KD, where they reported a summer peak in July and a winter peak in January [19, 20]. In USA, Canada, Europe, and temperate regions of Australia, they reported peak of incidence of KD occurring in the winter season except for Hawaii, which has different seasonality [14-17]. It has been hypothesized that tropospheric wind patterns arising from north-eastern China are associated with KD peaks in Japan, Hawaii, and southern California suggesting that KD could be induced by an airborne pathogen arising from this area [39].

Genetic Background

Ethnicity significantly affects the genetic ability to develop KD. Not only as mentioned before that the incidence is increased by 10 times in Asian children [9], but also the genes associated with KD and their degree of expression seem to vary among different ethnicities [40]. In response to this situation, several susceptibility genes including single-nucleotide polymorphisms (SNP) for the *ITPKC*, *CASP3*, *FCGR2A*, *BLK*, *ORAI*, and *CD40* genes have been recognized through genome-wide association and genome-wide linkage analysis employed in different ethnic populations to have an association with the etiology and prognosis of KD, and also with the development of coronary artery aneurysms (CAA) [41-43].

Gene-gene associations

Recently, several studies have indicated an important role of gene-gene interactions in KD pathogenesis, and have identified that varying gene-gene associations could predict the development of KD or the increased risk of its complications of CAA; and its prediction power is greater than that of individual SNPs [44-46]. Kuo and colleagues examined 384 SNPs for 159 immune-related candidate genes in of deoxyribonucleic acid (DNA) samples collected from 73 KD patients with CAA (73 patients), KD patients without CAA (153 patients), and 575 healthy controls. By logistic regression analysis, they identified that the combined acquisition of *PDE2A* gene (rs341058) and *CYFIP2* gene (rs767007) significantly increased KD susceptibility (odds ratio (OR) = 3.54; $P = 4.14 \times 10^{-7}$), while the combined acquisition of *LOC100133214* gene (rs2517892) and *IL2RA* gene (rs3118470) significantly increased the risk of CAA in KD patients (OR = 5.35; P < 0.001) [9].

In another study conducted by Kuo et al, they noticed that high-risk genotype patients with combined acquisition of *ITPKC* gene (rs28493229) and *CASP3* gene (rs113420705) were more associated with CAA formation (P = 0.0227, OR = 3.06)

and had a higher IVIG resistance rate in comparison to patients with individual susceptible SNP [47].

Gene-expression patterns associated with KD

During the past years, gene expression signatures have generated new clues for the diagnosis and pathogenesis of various infectious and inflammatory diseases with unclear etiology [48, 49]. According to the current guidelines [4, 18], the diagnosis of KD relies on clinical features that are usually similar to other infectious or inflammatory diseases, leading to delayed or missed diagnosis and treatment in many cases with an increased liability to complications.

In a study performed by Wright et al [50], they presented a rapid diagnostic blood test dependent on the measurement of small numbers of host gene transcripts, which would help an early diagnosis of KD and definite differentiation from other infectious or inflammatory diseases. They recognized a 13-transcript gene expression signature with high sensitivity and specificity for KD that included eight genes (*CACNA1E*, *DDIAS*, *KLHL2*, *PYROXD2*, *SMOX*, *ZNF185*, *LINC02035*, and *CLIC3*) with an increased expression in KD relative to other conditions, and five other genes (*S100P*, *IFI27*, *HS.553068*, *CD163*, and *RTN1*) that showed a decreased expression in KD. These findings, supported by other earlier studies [51, 52], might form the basis of a fast and easy applicable diagnostic laboratory test for KD.

DNA methyltransferases expression in KD

Methylation is the principal epigenetic modification in mammals' genome, and in general, the DNA methylation alteration of CpG islands present in the promoter region of the genes can lead to a powerful transcription inhibition [53]. DNA methylation is controlled by two groups of enzymes, the first are DNA methyltransferases (DNMTs) and the second are the Ten-eleven translocation (TET) family [53, 54].

In 2018 Chen et al [55] compared the CpG methylation status in KD patients and controls, and found that most of CpG loci (97%) revealed hypomethylation with only 3% showed hypermethylation; a finding suggesting that the majority of genes in KD patients have a hypomethylation status which results in an over-expression of these genes with increased activity of varies immune mechanisms including T helper 1 (Th1), Th2, Th17, innate immunity, acquired immunity, cytokines, etc. The exact reason why most genes are activated remains uncertain. DNA hypomethylation by imbalanced DNMTs and TET activities is hypothesized to be a key factor in the pathogenesis of this condition.

In another study Huang [56] et al identified differential expressions of DNMTs and TETs' mRNA levels in KD patients when compared to both febrile and healthy controls, with a significant decrease in the mRNA levels of DNMT1 and DNMT3A, and a significant increase in TET2 levels in KD patients. In addition, following an IVIG treatment, they noted a decrease in the mRNA level of TET2 and significantly lower DMNT1 mRNA levels between patients with CAA and those without. These studies emphasize the hypothesis of altered DNA methylation to be among the first pathogenic changes during the acute phase of KD.

Conclusions

KD is an acute febrile vasculitic disease in children and is the most common cause of acquired heart disease in children and young adults in developed counties. It is also increasingly reported in several developing countries, including China, India, Middle East and Latin American countries. The etiology of KD is still unclear; interaction between a genetic predisposition and several environmental and immunological factors has been hypothesized. Several susceptibility genes were recognized to have an association with the development of KD and an increased predisposition to its compilations. Gene-gene interactions and alteration of DNA methylation are also found to play key roles in the pathogenesis and prognosis of KD.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

Author Contributions

Karim Elakabawi and Fuyong Jiao contributed to the conception and design of the work; Karim Elakabawi and Jing Lin contributed to the literature search, and drafting the manuscript; Fuyong Jiao, Ning Guo, and Zuyi Yuan critically revised the manuscript.

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Update on Study of Kawasaki Disease

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Abstract

Kawasaki disease is an acute, self-limited vasculitis of unknown etiology that occurs predominantly in infants and children. It is rapidly becoming the most common cause of acquired heart disease in children in both the developed and developing world. Manifested initially by high fever, mucocutaneous inflammation, and cervical lymphadenopathy, KD targets the coronary arteries and other cardiovascular structures. Diagnosis is based on the presence of fever in addition to four out of five other clinical criteria, but it is complicated by the quarter of the Kawasaki disease patients with "incomplete" presentation. Standard first-line therapy for Kawasaki disease consists of intravenous immunoglobulin (IVIG) and aspirin. Current guidelines recommend 2g/kg of IVIG and 80 to 100mg/kg of aspirin administered within the first 10 days of illness. Here we review recent advances in epidemiology, possible aetiologies, genetics, imaging, diagnosis and treatment.

Keywords: Vasculitis; Aneurysm; Aetiologies; Diagnosis; Therapy

Introduction

First described in Japan in l967 by Tomisaku Kawasaki, the disease is now known to occur in both endemic and community-wide epidemic forms in the Americas, Europe, and Asia in children of all races. Kawasaki disease is characterized by fever, bilateral nonexudative conjunctivitis, erythema of the lips and oralmucosa, changes in the extremities, rash, and cervical lymphadenopathy. Coronary artery aneurysms or ectasia develop in »15% to 25% of untreated children with the disease and may lead to myocardial infarction (MI), sudden death, orischemic heart disease [1]. In Japan, Europe and the North America, Kawasaki disease has surpassed acute rheumatic fever as the leading cause of acquired heart disease in children [2-4].

In2004,the American Heart Association (AHA) published guidelines for the diagnosis, treatment, and long-term management of KD [5]. The current scientific statement incorporates new evidence regarding underlying pathological processes, an algorithm to ensure capture of incomplete KD during the effective window of therapy, improved management of the acute illness that includes the use of additional therapies for IVIG-refractory patients, greater use of *Z*-scores for classifying coronary artery involvement, greater specification of long-term management based on both initial and current coronary artery involvement, and acknowledgment of the care needs of a growing population of adults with a previous history of KD and coronary artery aneurysms.

The American Heart Association published online the 2017 edition of "diagnosis, treatment and Long-term Management of Kawasaki Disease-American Cardiology", 2017-03-29 Scientific statements for Medical Professionals. Compared with the 2004 edition, this Declaration has more specialties (paediatrics and adult heart disease, infectious diseases, pathology, rheumatism immunity, etc.). Nursing, surgery, anaesthesia, epidemiology, preventive medicine, etc.) and expert participation, with a richer content, and evidence levels that increase the rating of benefits and risks, and make the statement more evidence-based. The new statement mainly updates the following: New evidence of process; diagnostic procedures for incomplete Kawasaki disease; acute improvement of stage treatment (especially in patients with high risk of coronary artery disease and IVIG response); classification of coronary artery involvement with Z value; more standardized long-term management based on coronary artery involvement; Cardiac health care needs of KD and coronary artery aneurysms in adults [6].

Epidemiology

Kawasaki disease was shown to account for 23% of all paediatric vasculitides in a United States rheumatology clinic population study and is the second most common multisystem vasculitis of infancy and childhood behind Henoch-Schonlein purpura [7-9].

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Kawasaki disease is markedly more prevalent in Japan and in children of Japanese ancestry, with an annual incidence of »112 cases per 100 000 children <5 years old [10]. Recent reports have emphasized the occurrence of Kawasaki disease in older children, who may have a higher prevalence of cardiovascular complications related to late diagnosis. In Japan, the recurrence rate of Kawasaki disease has been reported to be »3%. The proportion of cases with a positive family history is »1% [11-12]. Within 1 year after onset of the first case in a family, the rate in a sibling is 2.1%, which is a relative risk of »10-fold as compared with the unaffected Japanese population; »50% of the second cases develop within 10 days of the first case. The risk of occurrence in twins is »13% [13-15]. Higher rates of Kawasaki disease in the siblings of index cases and twins suggest a possible role for genetic predisposition that interacts with exposure to the etiologic agent or agents in the environment [13.14.16.17].

The reported occurrence of Kawasaki disease in children of parents who themselves had the illness in childhood also supports the contribution of genetic factors [18-21]. Reported associations of Kawasaki disease with antecedent respiratory illness and exposure to carpet-cleaning fluids have not been consistently confirmed [22-27]. Virtually all deaths in patients with Kawasaki disease result from its cardiac sequelae [28]. The peak mortality occurs 15 to 45 days after the onset of fever; during this time well-established coronary vasculitis occurs concomitantly with a marked elevation of the platelet count and a hypercoagulable state [29]. However, sudden death from MI may occur many years later in individuals who as children had coronary artery aneurysms and stenoses. Many cases of fatal and nonfatal MI in young adults have been attributed to "missed" Kawasaki disease in childhood [30].

Etiology and Pathogenesis

Although the etiology of KD is unknown, the current consensus is that it is likely caused by an (infectious)trigger initiating an abnormal immune response in genetically predisposed children. Striking immune perturbations occur in acute Kawasaki disease, including marked cytokine cascade stimulation and endothelial cell activation. The key steps leading to coronary arteritis are still being clarified, but endothelial cell activation, CD68+ monocyte/macrophages, CD8+ (cytotoxic) lymphocytes, and oligoclinal IgA plasma cells appear to be involved.

Although early studies provided evidence for an immune response triggered by a super antigen, subsequent studies favored a canonical response to a conventional antigen. Activation of the innate immune system is an early event, with high numbers of activated, circulating neutrophils and evidence for activation of the interleukin (IL) 1, IL-6, and tumor necrosis factor (TNF) signaling pathways [31]. Study of the adaptive immune response demonstrated that both proinflammatory and regulatory T cells can be found in the circulation in the first week after fever onset [32]. Expansion of the regulatory T-cell population after IVIG administration is associated with cessation of fever and clinical improvement [33]. The self- limited nature of the disease coupled with a low rate of recurrence suggests emergence of T- and B-cell memory that is protective against future encounters with the KD agent.

Principal Clinical Findings

The clinical course of KD consist of four phases: (1) acute, the period lasting 1-2 weeks if untreated, when the child has a spiking ,often remittet 4^0 Celsius fever and principle symptomatic features and may present with cardiac manifectations including valvitis, pericarditis, and myocarditis; (2) subacute, the approximately 2 week period following the abatement of fever when the child is at the greatest risk of sudden death due to myocardial infarction; (3) convalescent, the clinically invisible period following the cessation of symptoms and continuing until acute-phase reactants return to normal serum levels; (4) chronic, which describes patients who require follow-up management due to coronary artery involvement [34-36].

Fever

The diagnosis of classic KD is based on the presence of ≥5 days of fever (first calendar day of fever is illness day1) and the presence of ≥4 of the 5 principal clinical features. In the presence of >4 principal clinical criteria, particularly when redness and swelling of the hands and feet are present, the diagnosis may be made with only 4 days of fever. Typically the clinical features are not all present at a single point in time, and it is generally not possible to establish the diagnosis very early in the course. Similarly, some clinical features may have abated in patients who present after 1 to 2 weeks of fever, and a careful review of prior signs and symptoms can help establish the diagnosis.

Extremity changes

Changes in the extremities are distinctive. Erythema of the palms and soles and firm and sometimes painful in- duration of the hands or feet often occur in the acute phase. Desquamation of the fingers and toes usually begins in the periungual region within 2 to 3 weeks after the onset of fever and may extend to involve the palms and soles. At 1 to 2 months after fever onset, deep transverse grooves across the nails (Beau's lines) may be noted.

Conjunctivitis

Bilateral bulbar nonexudative conjunctival injection usually begins shortly after fever onset and often spares the limbus, and a vascular zone around the iris. Anterior uveitis is often observed by slit-lamp examination during the first week of fever [37-38].

Subconjunctival hemorrhage and punctate keratitis are occasionally observed.

Oral changes

Changes of the lips and oral cavity include (1) erythema, dryness, fissuring, peeling, cracking, and bleeding of the lips; a "strawberry tongue," with erythema and prominent fungiform papillae; and (3) diffuse erythema of the oropharyngeal mucosa. Oral ulcers and pharyngeal exudates are not consistent with KD.

Cervical lymphadenopathy

Cervical lymphadenopathy is the least common of the principal clinical features. Lymph node swelling is usually unilateral, ≥1.5cm in diameter, and confined to the anterior cervical triangle. In a small subset of patients, lymph node findings may be the most notable and sometimes only initial clinical finding, prompting a clinical diagnosis of bacterial lymphadenitis and significantly delaying KD diagnosis [39]. In such cases, fever persists, and other typical KD features, such as rash and conjunctival injection, will follow. Imaging studies including ultrasound and computed tomography (CT) can be helpful in differentiating KD lymphadenopathy from bacterial lymphadenitis. In KD, multiple lymph nodes are enlarged, and retrophoryngeal edema or phlegmon is common. In contrast, bacterial lymphadenitis is most frequently associated with a single node with a hypoechoic core. It has been increasingly recognized that cervical lymphadenopathy can be associated with deep neck

inflammation leading to parapharyngeal and retropharyngeal edema and nonsuppurative phlegmon.

Conary arteyry aneurysms

Multiple criteria have been used for diagnosis of CAA. The criteria of the Japanese Circulation Society (JSC) State that an aneurysm is an artery of >3mm in a child under the age of 5 and an artery of>4mm in a child ≥5 years or when an arterial segment is 1.5 times its adjacent segment. A giant CAA is classified as ≥8mm or > times its adjacent segment. Conversely, over the past years, it has become clear that ζ-scores, diameters adjusted for basal-surface-area, may be better indication of abnormality. Multiple ζ-score classification exist. Unfortunately, the ζ-scores using different classifications can vary, mainly at larger dimensions. The threshold for abnormality is a ζ-score ≥2.5, although a ζ-score between 2 and 2.5 can be classified as a dilation. A small-sized CAA has a ζ-score of 2.5—5, a medium-sized CAA of 5—10, and a giant CAA of ≥10.

Diagnose

The diagnosis of KD was made using the criteria defined by the American Heart Association. A child was diagnosed with typical KD if he or she had fever for 5 days or more, with at least 4 of the following 5 signs or symptoms: (1) acute changes in the extremities, including erythema and edema of the hands and feet and membranous desquamation of the fingertips; (2) polymorphous exanthema; (3) bilateral, painless bulbar conjunctivitis without exudate; (4) changes in the lips and oral cavity, including erythema and cracking of lips, a strawberry tongue, and diffuse injection of the oral and pharyngeal mucosa; and (5) cervical lymphadenopathy (≥1.5cm in diameter), which is usually unilateral.

The diagnosis of incomplete KD was also made using the criteria of the American Heart Association. A child was diagnosed with incomplete KD (or atypical KD) if he or she had fever for more than 5 days but had fewer than 4 of the 5 clinical manifestations mentioned above [40-41].

Treatment of Kawasaki Disease in Children

The best treatment for Kawasaki disease is aspirin combined with IVIG, which reduces the risk of coronary artery disease. If IVIG effect is not good, need to use IVIG again or use glucocorticoid, immunosuppressant and so on.

Treatment with aspirin

Through the application of aspirin, cyclooxygenase and synthesis can be seriously inhibited, platelet aggregation is reduced, the activity of cyclooxygenase is lost, and the purpose of inhibiting thrombosis is achieved. In addition, aspirin has an ideal anti-inflammatory effect and antioxidation, which can promote the production of nitric oxide, resist atherosclerosis, and further reduce the recurrence rate of progressive cerebral infarction. In the acute phase of pediatric Kawasaki disease, the dosage of the drug is 30 ~ 50 mg/ (kg d). After 3 days of heat regression, the dose can be reduced to 3 ~ 5 mg/. (kg.D). No changes in the coronary artery, oral aspirin 6~8 weeks, if the coronary artery abnormalities, oral aspirin until the coronary artery returned to normal after 6~8 weeks. Some experts found that during the acute treatment of Kawasaki disease (Kawasaki disease), aspirin was used and 30 children were selected to be divided into two different groups. The first group was given a dose of 50 to 80 mg per kg BW per day, with appropriate reductions after half a month's treatment. The whole course of treatment was 60 days. The second group was given a dose of 20mg/kg BW per day. According to the final statistical results, the therapeutic effect of the two groups with aspirin was much better than that with basic treatment. The effect of aspirin was confirmed.

Treatment of intravenous human immunoglobulin

Intravenous injection of Human Immunoglobulin is also an essential method for the treatment of Kawasaki disease. For children who have been diagnosed with Kawasaki disease, early introduction can be introduced to reduce the risk of cardiovascular complications. According to the results of the study, it is found that the antibodies can be effectively provided and all the pathogenic microbes are killed at the same time. Inhibiting the immune response, avoiding inflammation and neutralizing toxins. At the younger age (age < 1 year), the white blood cells of the boys increased significantly. High platelet count and elevated C reactive protein > 100mg/L, liver injury and low protein were high risk factors for coronary artery aneurysm. For patients at risk of coronary aneurysm (2g/kg), intravenous drip was given once or twice.

Between 11% and 23% of patients may present with IVIG resistance diagnosed if a patient exhibits persistent or recurrent fever at least 36 hours after the first IVIG does has been infused. IVIG resistance is problematic because recalcitrant fever is indicative of ongoing arteritis, which places patient patients at a higher risk of developing coronary artery aneurysms. It is recommended that refractory disease is first treated with a second dose of IVIG 2g/kg, though the efficacy of a number of other therapeutic options, including intravenous corticosteroid pulse therapy, anti-TNF-alpha antibodies, and cytotoxic agents, is an ongoing area of research.

Treatment of glucocorticoid

At present, the etiology of Kawasaki disease is not clear, combined with the results of the study can be found that the main way of clinical treatment is oral aspirin and single intravenous injection of high-dose gamma globulin. After this treatment, nearly 10% of the children still have no response to the drug, so it is feasible to introduce glucocorticoid. According to the results of relevant expert studies, in the analysis of hormone therapy, because the etiology of the disease is not fully described, only in clinical identification of streptococcus and mycoplasma pneumoniae are associated. Therefore, 15 cases were selected for treatment. Seven of the ineffectual children were treated with methylprednisolone intravenously, while the remaining children were treated again. According to the final treatment results, the choice of intravenous methylprednisolone treatment is more satisfactory.

Prevention and treatment of coronary artery thrombosis in Kawasaki disease

Treatment of clopidogrel: In children, clopidogrel has no significant symptoms of adaptation to clopidogrel. In the event of multiple coronary artery involvement or aneurysm, choose clopidogrel combined with aspirin in the course of clinical treatment. A small number of data and information studies have shown that the combined use of two drugs can easily cause skin damage and, based on the combination of warfarin and clopidogrel, can lead to severe gastrointestinal bleeding problems .

Treatment of dipyridamole: It is well known that dipyridamole is an antiplatelet drug. In general, the dosage of dipyridamole is 2 to 5 mg/(kg d), but not alone, but in combination with aspirin.

Anticoagulant therapy: Low-dose aspirin combined with

warfarin is an effective strategy for anticoagulant and thrombus prevention. In general, treatment of children with Kawasaki disease is limited, and the dose used should be guaranteed at a dose of 0.05g/ 0.12mg/ (kg d).

Conclusion

Although many aspects of KD are unknown, there is increasing knowledge on the origin and treatment of KDs well as the development and classification of CAA. Since children with previous KD are entering adulthood, long-term follow-up, with appropriate imaging modalities and awareness of the long-term effects, is increasingly important.

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Update Research of Stem Cell Treatment of Coronary Artery Lesion of Kawasaki Disease

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Abstract

Kawasaki Disease (KD) is an acute, self-limited systemic vasculitis that affects the middle and small arteries, especially the coronary arteries. The incidence is currently on the rise, with about 15 to 25 per cent of children without systemic disease eventually developing coronary artery damage, becoming the most common cause of child-acquired heart disease in the countries where it occurs. At present, stem cell therapy for Kawasaki Disease in mice has made important progress. This article reviews the formation of stem cell therapy for Kawasaki disease.

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Introduction

Kawasaki Disease (Kawasaki Diseases KD) is a autoimmune disease, also known as lymph node syndrome, skin and mucosa in 1967 by a pediatrician Kawasaki rich covered in Japan for the first time, develops in children under the age of five, the main is a kind of small artery inflammatory lesions in the body, the main pathological changes of acute febrile disease and systemic vasculitis [1], more invasion of the heart coronary artery and cause serious complications. Coronary Artery Injury (CAL) is a serious complication of Kawasaki disease, which has the risk of myocardial infarction and sudden death, and has become the primary pathogenic factor of acquired heart disease in children in developed countries. Up to 15-20% of patients with Kawasaki disease do not respond to high-dose intravenous immunoglobulin therapy, and these patients have a significantly increased incidence of coronary aneurysms [2].

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The mechanism of coronary artery lesion caused by kawasaki disease

So far, scholars on the pathogenesis and pathogenesis of Kawasaki disease is not very clear, but through nearly half a century of research support genetic factors play a key role in its occurrence and development, and emerged two university theories: infection theory and immunity theory. Kawasaki disease is a self-limited disease that causes inflammatory changes in blood vessels. It occurs most frequently in East Asian populations and occurs most frequently in winter and spring. The clinical manifestations of Kawasaki disease overlap with other infectious diseases [3], its clinical biochemical indicators, such as increased white blood cell /CRP and rapid erythrocyte sedimentation rate, are enough to prove that the underlying lesions are caused by inflammatory cells. During the acute onset of Kawasaki disease, a large number of inflammatory cytokines are released. After the activation of immune cells, the activated immune cells can release a large number of inflammatory cytokines [4], is a vicious cycle, the formation of waterfall reaction, so that the body loses the control of the inflammatory response, resulting in endothelial damage. Secondly, a large amount of Tumor Necrosis Factor (TNF) is produced to damage vascular endothelial cells, resulting in increased endothelial permeability, and then the vascular endothelial cells release chemokines, resulting in the aggregation of immune cells to the surface of the damaged vessels. The number of Endothelial Progenitor Cells (EPCs) is reduced by tumor warth cells, thereby reducing the repair of damaged endothelial cells and leading to coronary artery damage. Thirdly, during the acute period of Kawasaki's disease, the body releases a large amount of interleukin, which enables cells to detect the toxic effect, inhibit the expression of P53 gene, reduce the apoptosis of lymphocytes, continuously produce more inflammatory factors and adhesion molecules, and mediate the damage of vascular endothelial cells [5]. Abnormal activation of T cells is the initial link and key step of immune system activation in Kawasaki disease, which leads to vascular immune injury [6]. A large number of T cells make the body in a state of immune activation. Activated T cells act on humoral immunity and induce vascular inflammatory response. Vascular endothelial growth factor (Vascular Dendothelial Growth Factor, VEGF) can cause excessive increased Vascular permeability, children with kawasaki disease can be detected in blood of Vascular endothelial growth factor, Vascular VEGF promote the white blood cells gathered in coronary inflammation of stimulation, the increased Vascular permeability, and stimulate the activation of peripheral blood mononuclear cell chemotaxis to endothelial injury and lead to local produce again producing VEGF, form a vicious circle, coronary blood vessels to participate in and lead to damage. Rao Xiao Hong [7]. According to the study of et al., platelets are involved in vascular damage of Kawasaki disease. After thrombocytosis and enhanced activation, thrombocytosis causes thrombosis in the damaged coronary arteries, intima thickening, and release a variety of angioconstrictors and clotting substances, leading to myocardial function impairment, increased hypercoagulability and vascular blockage, resulting in ischemic necrosis of the myocardium. At present, there are many studies on gene fragments such as ITPKC, Casp3, TGF-S, BLK, CD40 and FCGR2a at home and abroad, suggesting that many genes are involved in the occurrence of Kawasaki disease and the development of CAL [8]. In conclusion, the mechanism of KD disease is a complex process, but studies have shown that it is the abnormal activation of immune cells and the large release of various cytokines, leading to vascular endothelial damage and dysfunction, and the occurrence of cascading damage of coronary arteries.

Pathological basis of coronary artery lesion in kawasaki disease

The United States Lehman etc. [9]. The coronary arteritis model was induced by Lactobacillus Casei Cell Wall Extract (LCWE). Pathological sections of the coronary artery showed thickening of the vascular wall and invasion of inflammatory cells around the heart tissue, and plaques may appear. LCWE induced Kawasaki disease vasculitis is characterized by inflammatory cell infiltration in the aortic root, necrotic coronary artery arteritis, and then complete coronary artery stenosis due to lumen occlusion caused by LMP [10]. Philip [11]. In 2004, through pig model, it was found that the coronary arteries of pig heart were dilated differently. Pathologically, it was found that the inner elastic membrane was broken and the intimal hyperplasia changed, and the perivascular inflammatory infiltration was observed. Through the analysis and observation of the clinical data of children with Kawasaki disease, combined with a large number of animal models of Kawasaki disease theoretical research basis and clinical research to improve the understanding of Kawasaki disease induced coronary artery damage. It shows that Kawasaki disease is an inflammation of small and medium blood vessels, and long-term vascular inflammation eventually leads to coronary atherosclerosis. The coronary artery wall was thickened, the inner diameter of the main artery was widened, the intima was thickened, the valve and the intima of the atrium were slightly swollen, the neutrophils were scattered and infiltrated, the coronary artery lumen was expanded and the cardiomyocytes were necrotic and dissolved, and local fibers replaced the calcium salt deposition of tissue hyperplasia. The extravascular interstitium of the heart is edema, with a few lymphocyte infiltrates and diffuse hyperplasia of fibrous connective tissue. The muscular layer of blood vessels was destroyed, and the adventitia showed focal fibrinoid necrosis and fibrosis. White thrombosis is seen in the lumen. Severe and persistent coronary thrombosis with endothelial cell damage and myocardial cell necrosis results in irreversible heart changes. Through the study of animal model and pathological changes, the inflammatory theory and immune theory of damage mechanism of coronary artery injury were confirmed.

Mechanism and effect of stem cell therapy

Functionally, stem cells have unique biological characteristics, such as the ability to self-renew, expand and limit the potential of differentiation, maintain and control cell regeneration, and then repair necrotic or damaged tissues. Through the theory of infection, as long as the inflammatory response caused by Kawasaki disease can be controlled or weakened, Kawasaki disease can be controlled or recovered. Therefore, a therapeutic measure is needed to inhibit or block the inflammatory response. In addition to promoting functional tissue regeneration and repair, stem cells also have a wide range of immunomodulatory and anti-fibrosis activities [12]. Macrophages in tissues are involved in the multiple roles of anti-inflammation and tissue repair in inflammatory injury. M1-type macrophages are dominant in the early stage of myocardial infarction, and M1-type macrophages lead to myocardial injury by phagocytosis, dissolution and digestion of necrotic myocardial tissue [13]. Type M2 macrophages participate in the anti-inflammatory response in the late stage of acute myocardial infarction and promote the effect of heart repair. Studies have shown that stem cell therapy can significantly increase the invasion of type

M2 macrophages in the area of myocardial infarction, reduce fibrosis, increase angiogenesis, and improve cardiac function. A mouse model of myocardial infarction treated with stem cells showed that after one week of treatment, the number of M1 type in myocardial cells was less, the number of M2 type was increased, the number of residual myocardial cells was more, the infiltration of lymphocytes was reduced, the apoptotic index of myocardial cells and the deposition of myocardial collagen were decreased [14]. Peng Y [15,16]. Studies have shown that stem cells reduce the expression of inflammatory factors and increase the expression of anti-inflammatory factors by adjusting the inflammatory response, so as to avoid excessive inflammatory response and tissue damage caused by inflammation. Mesenchymal stem cell-derived exosomes can enhance the M2 polarization effect of macrophages in myocardial tissue of myocardial infarction model, and inhibit the inflammation and apoptosis of myocardial tissue [17]. The transition from inflammatory injury tissue to pro-tissue repair in the inflammatory response is induced by the transformation of macrophages from M1 type to M2 type. The injection of mesenchymal stem cells induces the transformation of a large number of macrophages from M1 to M2, thereby reducing the inflammatory response of Kawasaki disease and promoting the repair of damaged heart tissue.

Stem cells have the ability to self-replicate, renew and effectively repair damaged tissues or organs [18]. Stem cells are a kind of relatively special cells, which are easy to obtain and do not involve many ethical issues [19,20]. Stem cells have homing properties and can be used as carrier tools to deliver biological agents in a directional manner, thus realizing the treatment of related diseases. Zhao L [21]. The research of et al. demonstrated that stem cell DNA carries some gene points that determine the differentiation of muscle lines, thereby promoting the differentiation of stem cells into cardiomyocytes. Animal experiments have shown that the application of stem cells in the treatment of damaged myocardial cells can effectively improve the situation of myocardial blood supply and improve the body's cardiac function [22,23]. Human umbilical cord mesenchymal stem cells can differentiate into endothelium cardiomyocytes, and they have a wide range of sources with little immune response, so they have been used in the treatment of coronary artery damage caused by Kawasaki disease. After the injection of stem cells in animal models, the inner diameter of the main coronary artery was reduced, and a large amount of lymphocytes and eosinophils were infiltrated, but no obvious inflammatory response and injury changes were observed. Stem cells can successfully differentiate into vascular endothelial cells and some cardiomyocytes to repair heart tissue damaged by Kawasaki disease.

Summary and prospect

Traditional treatments for Kawasaki disease are gamma globulin injections or oral aspirin. However, the 2004 American College of Cardiology guidelines state that the use of gamma globulin within five days of fever does not reduce the incidence of coronary damage, and there is a risk of developing resistance [24]. In addition, the price of gamma globulin is expensive, which increases the cost for the families of children. Stem cells play a key role in the regeneration and repair of coronary artery lesion caused by Kawasaki disease, providing a new treatment method and idea to get rid of the limitations of traditional treatment, which is expected to successfully prevent the occurrence of CAL and improve the prognosis of patients with Kawasaki disease. However, it faces many problems. Stem cell therapy for coronary artery injury is still in the research stage, and the safety and effectiveness of stem cell extraction need to be further studied and solved. However, with continuous efforts, stem cell therapy will become a more effective way to treat coronary artery lesion.

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专家讲座

川崎病——儿童 COVID-19 的一种新的表现形式

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［摘要］ 欧美国家发现，2019 冠状病毒病（COVID-19）盛行以来，川崎病的发生率明显上升，引发儿科医师与家长的担忧。COVID-19 会引起全身多个器官出现炎症反应，这点与川崎病的全身系统血管炎性改变类似，甚至 COVID-19 也会造成四肢末端的皮肤红疹，这点也与川崎病类似。川崎病的病因目前尚不明确，现在也不能除外 COVID-19 与川崎病的发病率增加有关。因此，在 COVID-19 流行期间，如果患儿出现类似于川崎病样症状，应尽早使用静脉注射免疫球蛋白治疗，降低冠状动脉损害的发生。

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［关键词］ 2019 冠状病毒病；川崎病；丙种球蛋白；儿童

Kawasaki disease — New manifestation of COVID-19 in children

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Abstract: Since coronavirus disease 2019 (COVID-19) has been prevalent in Europe and America, the incidence of Kawasaki disease has significartly increased, which has aroused concern among pediatricians and parents. COVID-19 can cause inflammation reactions of multiple organs, which is similar to the systemic vasculitis of Kawasaki disease, and even COVID-19 can cause skin rash on the extremities of the limbs, which is also similar to Kawasaki disease. The cause of Kawasaki disease is currently unclear, and it cannot be ruled out that COVID-19 is associated with an increased incidence of Kawasaki disease. Therefore, during the epidemic period of COVID-19, if children have symptoms similar to Kawasaki disease, intravenous immunogloblin is recommended as early as possible to reduce the incidence of coronary artery lesions.

[Chin J Contemp Pediatr, 2020, 22(7): XXXXX]

Key words: COVID-19; Kawasaki disease; Intravenous Immunogloblin; Child

近期英国、意大利、法国、西班牙、瑞士等欧洲国家报告儿童发生类似川崎病样综合征的病例，截至 2020 年 4 月 29 日，全球病例数量已接近 100 例，川崎病（Kawasaki disease, KD）是一种目前病因尚不清楚，但可能与感染及免疫介导参与及遗传易感因素有关的全身中小血管为主要病理改变的炎性损害性疾病，KD 自 1967 年日本川崎富作医师报道 50 个病例以来，全世界都有报道，已成为目前儿童后天性心脏病最重要的原因 [1]。

KD 又称黏膜皮肤淋巴结综合征，是一种以全身性血管炎为主要病理改变的小儿急性发热性疾病，多见于 5 岁以下儿童，通常表现为发热、皮疹、弥漫性黏膜炎症、非渗出性结膜炎、颈部淋巴结病变和肢体改变 [2]。这种疾病会导致身体任何部位的中小型动脉血管壁发生炎性变化，其中冠状动脉主要受累。在未经治疗的 KD 患儿中，高达 25% 可能发生冠状动脉病变，从而导致严重的并发症，如冠状动脉扩张、冠状动脉瘤和急性心肌梗死 [3]。因此，早期发现和及时治疗至关重要，在发病后 10 d 内静脉注射免疫球蛋白（IVIG）可以将动脉瘤的发生率降低到 <5%[4]。

KD 的病因和发病机制尚不清楚，流行病学显示，在几个亚洲国家，如日本、韩国和印度，KD 的发病率有所上升。并且在这些亚洲人群中，家

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族性发病率增加了 10 倍，表明有很强的遗传起源[5]。然而，仅靠遗传因素不能解释 KD 的季节变化或地理和时间的聚集性。其他因素，如感染性因素（细菌、病毒、真菌等）、环境操纵者或自身免疫反应[6]都是该病发生所必需的，但其确切作用尚不清楚。

冠状病毒是一种非节段性正链 RNA 病毒，基因组约 30 kb，被蛋白包膜包围。大多数冠状病毒在其特定的宿主物种中引起疾病[7]；少数可以通过跨物种传播感染人类的冠状病毒已经成为公共健康的重要威胁。有研究表明，KD 与冠状病毒感染性疾病，包括重症急性呼吸综合征、中东呼吸综合征、2019 冠状病毒（COVID-19）在流行病学分布模式上有很大的相似点。（1）KD 首先主要流行于东亚国家，其次为西欧和北美，印度及南美地区的一些快速发展中国家，以及土耳其和伊朗等一些中东国家的报告人数开始增加；COVID-19 最初在中国爆发流行，然后主要传播在西欧和北美。（2）KD 在北半球的热带以外维度有一个明显的季节模式，其特征是报告病例的高峰出现在 1~3 月的冬季；COVID-19 于 2019 年 12 月在中国湖北首次报道，自 2020 年 2 月下旬以来在全球广泛传播，这两种疾病在季节模式上有明显的相似性，似乎都与冬季及寒冷季节有关。

近期，美国有线新闻（CNN）报道[8]，英国报告了 COVID-19 儿童患者出现了一种罕见的综合征，包括腹痛、胃肠症状和心脏炎症等，症状与 KD 很相似，数量很少但是与日俱增 . 报道指出，英国儿科重症监护室协会发布紧急警报称，危重儿童病例略有增加，部分儿童患者 COVID-19 检测阳性，出现“中毒性休克综合征和非典型 KD 的共同特征”，这些症状被认为与最近的 COVID-19 感染有关。意大利和英国医学专家正在调查冠状病毒大流行与儿童炎症性疾病之间的关系，儿科心脏病专家马蒂奥·齐费雷达说，意大利贝尔加莫的一家医院在过去的 1 个月中出现了 20 多例严重的血管炎症病例，是一年内预期的 6 倍。纽约州州长安德鲁库莫宣布，3 名儿童死于一场可能与 COVID-19 有关的严重疾病，这种疾病的症状类似川崎病和中毒性休克综合征；同期他们正在调查 85 例被称为“与 COVID-19 相关的小儿多系统炎性综合征”的病例，这些儿童的 SARS-CoV-2（导致 COVID-19 的冠状病毒）核酸检测呈阳性，或者被发现有针对该病毒的抗体，但表现出不同的症状[9]。雪莱·里法根表示在 2020 年 4 月中旬的 10 d 内，他们注意到 8 名儿童出现了前所未有的高炎症性休克，表现出与非典型川崎病、川崎病休克综合征或中毒性休克综合征类似的特征，其中有两名患儿 SARS-CoV-2 核酸检测阳性，表明对于此类患儿应早期得到社区的关注，并优化早期识别[10]。

在 COVID-19 流行期间，若患儿发热时间超过 5 d，合并 KD 类似的症状，炎症指数较高的情况下，一定要考虑到 KD 的发生，因为 KD 会造成冠状动脉病变，形成冠状动脉瘤危及患儿生命，且与 COVID-19 的治疗有显著的不同，所以要高度警惕。因此，COVID-19 流行期间出现 KD 样症状或者对于危重症患儿，应尽早使用 IVIG，以减少 KD 冠状动脉损害的发生。

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Hans汉斯

川崎病冠脉损害的干细胞治疗研究进展

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摘　要

川崎病是一种具有明显季节性和区域流行性的疾病，好发于5岁以下儿童，表现为急性自限性的全身性血管炎，累及中小动脉，特别是冠状动脉病变。目前发病呈增高趋势，大约15%~25%未经系统治疗的儿童最终发展成冠状动脉损害，成为发达国家儿童获得性心脏病的最常见原因。目前干细胞治疗小鼠川崎病模型所致的冠脉损害取得重要进展，现就干细胞治疗川崎病冠脉损害的形成进行简要综述。

关键词

川崎病，冠脉损害，干细胞

Research Progress of Stem Cell Therapy for Coronary Artery Damage in Kawasaki Disease

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Abstract

Kawasaki Disease (KD) is an acute, self-limited systemic vasculitis that affects the middle and small arteries, especially the coronary arteries. The incidence is currently on the rise, with about 15 to 25 per cent of children without systemic disease eventually developed coronary artery damage, becoming the most common cause of child-acquired heart disease in the countries where it occurs. At present, stem cell therapy for Kawasaki Disease in mice has made important progress. This article reviews the formation of stem cell therapy for Kawasaki disease.

Keywords

Kawasaki Disease, Coronary Artery Lesion, Stem Cell

1. 前言

川崎病(Kawasaki disease, KD)是自身免疫系统疾病，又称皮肤粘膜淋巴结综合征，1967 年由儿科医生川崎富作首次在日本报道，多发生于五岁以下儿童，其主要是一种以全身中、小动脉炎性病变为主要病理变化的急性发热性疾病和全身性血管炎[1]。多侵犯心脏冠状动脉而引起严重的并发症。川崎病冠脉损害(coronary artery lesion, CAL)是严重的并发症，有心肌梗塞及猝死的风险，成为发达国家儿童获得性心脏病的首要治病因素。高达 15%~20%的川崎病患者对大剂量静脉注射免疫球蛋白治疗没有反应，这些患者的冠状动脉瘤发生率明显增加[2]。

2. 川崎病病因及发病机制

到目前为止，学者们对川崎病的发病原因及发病机制尚不十分清楚，但是通过近半个世纪的研究支持遗传因素在其发生及发展中起关键作用，并出现两大学说：感染学说及免疫学说。川崎病是一种致血管炎症性改变的自限性疾病，好发于东亚人群及冬春季，与其他感染性疾病的临床表现有重叠[3]，其临床生化指标，如白细胞/CRP 增高及血沉增快，足以证明其基础病变为炎性细胞所致。川崎病急性发病期释放大量的炎性细胞因子，激活免疫细胞以后，能使活化的免疫细胞释放大量的炎性细胞因子[4]，呈恶性循环，形成瀑布反应，使机体失去对炎性反应的控制，造成内皮损伤。其次大量的肿瘤坏死因子产生，损害血管内皮细胞，造成内皮通透性增加，继而血管内皮细胞释放趋化因子，使免疫细胞向受损的血管表面聚集。肿瘤坏死细胞减少了内皮祖细胞数量，从而减低了对受损内皮细胞的修复作用，导致冠脉损害。在川崎病急性期内，机体大量释放白细胞介素，白细胞介素使细胞发生毒性作用，抑制 *P53* 基因表达，使淋巴细胞凋亡减少，持续产生更多的炎性因子和黏附分子，介导血管内皮细胞损伤[5]。T 细胞异常活化是川崎病免疫系统激活导致血管免疫损伤的始动环节和关节步骤[6]。大量的 T 细胞使机体呈免疫活化状态，活化的 T 细胞作用于体液免疫，诱发血管发生炎性反应。血管内皮生长因子(Vascular endothelial growth factor, VEGF)可导致血管通透性增高，川崎病患者血液中可检测到大量的血管内皮生长因子，血管 VEGF 促进白细胞聚集到冠脉炎症部位进行刺激，活化的外周血单核细胞趋化到内皮损伤部位，

导致局部产生 VEGF，参与冠脉血管壁的损害。通过饶晓红[7]等人的研究，血小板参与川崎病血管损害，血小板增多及活化增强后，在受损的冠脉引起血栓形成，内膜增厚，并且其释放多种血管收缩素及凝血物质，导致心肌功能受损，增加高凝状态。目前，国内外对 *ITPKC/CASP3/ TGF-S/BLK/CD40/FCGR2A* 等研究较多，表明众多基因参与川崎病的发生及 CAL 发展过程[8]。总之，KD 病变机制是一个复杂的过程，但研究表示其是免疫细胞异常活化及各种细胞因子的大量释放，导致血管内皮损害及功能障碍。

3. 川崎病冠脉损害的病理基础

美国 Lehman 等[9]通过提取乳酸菌胞壁取物(lactobacillus casei cell wall extract, LCWE)诱发冠状动脉炎模型，通过其病理切片显示心脏组织可见血管壁增粗且周围有炎症细胞浸润，可出现斑块。LCWE 诱导的川崎病血管炎的特征是主动脉根部炎性细胞浸润，冠状动脉发生坏死动脉炎，然后由于 LMP 导致管腔堵塞，导致冠状动脉完全狭窄[10]。Philip [11]等于 2004 年通过猪模型发现小猪心脏冠脉不同扩张，病理学见内层弹力膜断裂内膜增生改变，血管周围炎性浸润等。学者们通过对患儿的临床数据分析及观察，结合大量的川崎病动物模型的理论研究基础及临床研究提高对川崎病诱发冠脉损害的认识。其表明川崎病是中小血管的炎症，长期的血管炎症存在最终导致冠状动脉粥样硬化。冠状动脉血管壁增厚，主干内径增宽，内膜增厚，瓣膜及心房内膜轻度肿胀，中性粒细胞散在浸润，冠状动脉管腔扩张及心肌细胞坏死溶解，局部纤维接替组织增生伴钙盐沉积。心脏外膜间质水肿，可见少量淋巴细胞浸润及纤维结缔组织弥漫增生。血管肌层被破坏，外膜可见局灶性纤维素样坏死及不同程度纤维化。管腔内可见白色血栓形成。严重持续性的冠脉血栓形成内皮细胞损伤及心肌细胞坏死，造成心脏不可逆性改变。

4. 干细胞治疗的机制及效果

干细胞具有独特的生物学特性，无限的或永生的自我更新能力，其功能是维持及控制细胞再生，具有扩增能力和限制分化潜能。从功能上讲，干细胞具有多项分化潜能及自我更新能力，从而修复坏死或受损的组织。通过感染学说，只要能控制或减弱川崎病所致的炎症反应，就能使川崎病得到控制或康复，因此需要一种治疗措施能够抑制或阻断严重反应。干细胞可促进组织功能性再生修复外，还有广泛的免疫调节及抗纤维化活性的作用[12]。组织内巨噬细胞有参与炎性损伤抗炎和组织修复的多重作用，心肌梗死早期以 M1 性巨噬细胞为主，M1 型巨噬细胞通过吞噬溶解及消化坏死心肌组织，同时导致心肌损伤[13]。M2 型巨噬细胞在急性心肌梗死后期参与抗炎症反应，参与促进心脏修复效应。研究表明，干细胞移植可显著增加心肌梗塞区域 M2 型巨噬细胞的浸润，减少纤维化，增加血管生成，改善心脏功能。干细胞治疗心肌梗死小鼠模型研究发现，治疗一周后，心肌细胞内 M1 型数量较少，M2 型增加，残存心肌细胞较多，淋巴细胞浸润减少，心肌细胞凋亡指数和心肌胶原沉积降低[14]。Peng Y [14] [15]等研究表明干细胞通过调整炎症反应，降低炎性因子的表达，提高抗炎因子的表达，避免过度的炎症反应和炎症导致的组织损伤。间充质干细胞来源的外泌体可增强心肌梗死模型心肌组织中巨噬细胞 M2 极化效应，抑制心肌组织炎症和心肌细胞凋亡[16]。炎症反应中炎症性损伤组织向促组织修复转折的过程，是巨噬细胞 M1 型向 M2 型转化所诱导，间充质干细胞的注射诱导大量巨噬细胞 M1 向 M2 转化，从而减少川崎病的炎症反应，促进受川崎病受损心脏组织的修复。并且在《Science Translational Medicine》上发表的文献显示[17]，日本研究团队启动了心肌球来源细胞(CDC)治疗扩张型心肌病的临床试验，共涉及到 5 名儿童患者。在注射心肌球来源细胞一年后，患者没有出现严重副作用，且心脏功能有改善的迹象。

干细胞具有自我复制更新能力，能够有效修复损害的组织或器官[18]。干细胞是一类相对特殊的细胞该类细胞取材比较方便，且不涉及伦理等诸多问题[19] [20]。干细胞具有归巢性，能够作为载体工具定向运送生物制剂，从而实现相关疾病的治疗。Zhao L [21]等人通过研究证明：干细胞 DNA 携带的一些决定

肌系分化的基因点，从而促进干细胞向心肌细胞分化。通过动物实验研究显示，应用干细胞治疗受损的心肌细胞，能够有效改善心肌供血情况，提高机体心脏功能[22] [23]。人脐带间充质干细胞能够向内皮细胞心肌细胞分化，加之其来源广泛免疫反应少，其具有应用于治疗川崎病造成的冠脉损害的作用。对动物模型注射干细胞后，血管冠状动脉主干内径减少，大量的淋巴细胞及嗜酸性粒细胞单核细胞浸润，未见明显的血管炎性反应及其损伤改变。干细胞可以成功分化为血管内皮细胞及部分心肌细胞，修复被川崎病所受损的心脏组织。

5. 小结与展望

川崎病的传统治疗是注射丙种球蛋白或口服阿司匹林。但 2004 年美国心脏病学会指南指出，发热五天内应用丙种球蛋白不能降低冠脉损害的发生率，并且有发生耐药性的风险；另外丙种球蛋白价格昂贵，对患儿家庭来说增加花费。干细胞对川崎病所致的冠脉损害的再生和修复具有关键作用，提供一种新的治疗方法和思路，摆脱传统治疗的局限性，有望成功阻止 CAL 的发生，改善川崎病患者的预后。然而其面临不少难题，干细胞治疗冠脉损害仍处于研究阶段，干细胞提取的安全性和有效性等有待进一步研究及解决。但是通过不懈的努力，干细胞治疗将成为治疗冠脉损害更加有效的新途径。

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