Investigater Manual:

Effects of carrimycin on biomarkers of inflammation and immune function in tumor patients with sepsis

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Foreword

Carrimycin (trade name is Bite) is a physiological active substance with various medicinal value, which is jointly developed by Shenyang Tonglian Group Co., Ltd. and Institute of Pharmaceutical Biotechnology, Chinese Academy of Medical Sciences. It belongs to the national 1.1 new drug, which has Chinese independent intellectual property rights and is the key research project of 863 "Tenth Five-Year Plan".

Carrimycin belongs to macrolide antibiotics in a broad sense. It is a multi-component antibiotic, which is directly fermented by modified genetically engineered bacteria. It is a physiological active substance with 16-element cyclolide bond.The main components were Isovalerylspiramycin (ISP) I, II and III.It inhibits protein synthesis by binding to the large subunit of the bacterial ribosome 50S.In terms of anti-infection, it has inhibitory activity against Gram-positive bacteria, some Gram-negative bacteria, Legionella, especially mycoplasma and chlamydia.The completed phase III clinical trials have confirmed that carrimycin is safe to take, with low oral dosage and low adverse reaction rate. It has a good effect on upper respiratory tract bacterial infection, and there is no complete cross-resistance with similar drugs. The clinical efficacy and safety of carrimycin were better than that of the control drug azithromycin. In addition, carrimycin also showed good prospects in the anti-tuberculosis infection.

With the deepening of the research, it was found that the role of carrimycin has been beyond the definition of antibiotic -- only as an anti-bacterial infection scope, but a wide range of bioactive substances produced by genetically engineered microorganisms, which can regulate the physiological, proliferation, development and immune functions of biological cells. For example, carrimycin has the function of anti-liver fibrosis, which is mainly characterized by the large amount of expression and secretion of type I collagen in the liver. Using the screening model of type I collagen gene promoter - luciferase reporter gene, it was found that carrimycin has significant inhibitory activity. The rat model of liver fibrosis induced by conventional bile duct

ligation was tested, and it was found that the liver fibrosis induced by bile duct ligation was also significantly improved by carrimycin. In addition, pharmacological studies have shown that carrimycin can reduce blood glucose in mice. It can prolong the life of nematodes and other new pharmacological effects.

The latest study showed that the expression of mTOR and p-mTOR could be inhibited by treating A549 cells with carrimycin for 48 h. The expression of P70S6K1 (ribosomal protein S6 kinase) and p-P70S6K1 downstream of mTOR was inhibited. Some studies showed that the levels of IL-6 and IL-8 were significantly decreased after mTOR was inhibited. Carrimycin (0.05,5μg/ml) could significantly reduce the expression of PD-L1 on A549 cell membrane, and PD-L1 plays an important role in inducing tumor-specific T cell apoptosis and tumor immune escape. Carrimycin significantly reduced IL-4 in the lungs, kidneys, liver and spleen of infected mice, especially in the liver and spleen. Carrimycin significantly reduced IL-1β in small intestine, lung, spleen, liver and kidney, especially in small intestine and lung.In mice, carrimycin could significantly promote the increase of total T cells (CD3 positive cells), and CD4 and CD8 positive cells increased. Carrimycin can potentially inhibit the function of M2-type macrophages, improve the function of M1-type macrophages, and strongly induce the transformation of M2-type macrophages into M1-type macrophages. Existing studies have shown that M2-type macrophages promote inflammation and tumorgenesis, and are positively correlated with tumor immune escape.

Pharmacological and toxicological studies showed that carrimycin had no effect on the autonomic activity of mice. It had no synergistic effect with pentobarbital sodium and had no obvious effect on cardiac function and respiratory system. No obvious side effects were observed in the blood system after five days of continuous administration of carrimycin. Mutagenesis test results showed that the possibility of mutagenesis and teratogenesis was small. The results of reproductive toxicity test showed that the reproductive toxicity and perinatal toxicity of carrimycin were small, and the teratogenic effect of carrimycin was small at the therapeutic dose. Long-term toxicity tests showed that carrimycin was safe and well tolerated at therapeutic doses.

Non-clinical pharmacokinetic studies have shown that after oral administration of

carrimycin, it is absorbed rapidly and completely in the body. After entering the body, it is mainly metabolized into active spiramycin and rapidly enters the tissues, while a part of it is retained as isopentyl prototype. Before spiramycin is absorbed into the body, it begins to be decomposed into inactive new spiramycin in the gastrointestinal tract. In most tissues, the total concentration of the drug after oral administration of the drug was higher than that after the administration of the same dose of spiramycin, and the ratio of tissue concentration to plasma concentration was also higher than that after the administration of the same dose of spiramycin, indicating that the drug has a higher tissue affinity than that of spiramycin.

After carrimycin was introduced into animals, the plasma concentration of the main component of spiramycin was low, but it could be detected in most tissues, and the concentration of spiramycin was higher in liver, small intestine, lung, stomach, spleen, uterus and other tissues.This indicates that after being absorbed into the body through the gastrointestinal tract, isovalyl spiramycin distributes rapidly to the tissues, but is slowly eliminated and accumulates in some tissues.

The AUC of the parent drug isovalyl spiramycin I, II, III and the active metabolites spiramycin I, II, III$_{0\text{-}12h}$The total oral absolute bioavailability was 96.7%. carrimycin was widely distributed in tissues, the tissue concentration was higher than the blood concentration, the tissue concentration was maintained for a long time, and it had a long post-antibiotic effect.

The Phase I safety and tolerability trials of carrimycin showed that carrimycin stimulated the gastrointestinal tract and caused a slight increase in ALT and AST.According to the phase I pharmacokinetic study of carrimycin , the oral absorption of carrimycin tablets was rapid, the drug was widely distributed in the body, and the original drug was rapidly transformed into active metabolites.The elimination of the original drug was slow, and the T of each component in a single dose$_{1/2\beta}$ Between 23 and 27h.There is some accumulation in the body when multiple doses are administered. Food had no effect on the substitution of carrimycin, so in the course of clinical trials, patients took the medicine after meals, which greatly reduced the incidence of adverse reactions.

The phase II and III clinical trials of carrimycin were conducted in 18 clinical research units in different regions of the country, using double blind double simulation trials. Enrolled patients were male and female patients aged 18-65 years old. The control drug was azithromycin, which was also a macrolide antibiotic. The results

showed that the experimental group was not inferior to the control group in the treatment of acute tracheal bronchitis, acute bacterial sinusitis and so on.

According to the completed phase II and phase III clinical trials of this product, a total of 649 patients were treated with carrimycin, and the main adverse reactions were mild. During the whole trial, only 1 patient withdrew from the study due to nausea and vomiting after the administration. No serious adverse events were reported throughout the clinical trial.

Chemical and physical property information

1. Drug name: Generic name: Carrimycin

English name: Carrimycin

Trade name: Bite

Previous name: Bette spiramycin, biotechamicin

Agent type: tablet (film coated tablet)

2. Chemical and physical properties

1). Sexual characteristics

Carrimycin is white powder, odorless, slightly bitter taste.

2). Solubility

Carrimycin is easily soluble in chloroform, acetone, anhydrous ethanol, slightly soluble in 0.1mol/L hydrochloric acid solution, almost insoluble in water and 0.1mol/L sodium hydroxide solution.

3). Humidification

The experimental results showed that: Carrimycin slightly moistureability.

4). Alkali degree

Carrimycin has an alkalinity between 8.5-9.0.

3. Stability

The preliminary test shows that this product has good stability, but has certain instability to heat and light. Therefore, it should be stored in a cool and dark condition.

Related Research Data

1. Pharmacodynamic research data

1.1. Anti-inflammatory mechanism of carrimycin

Carrimycin is a 16-member-ring macrolide antibiotic. Its mechanism of action is to bind to bacterial ribosomes and inhibit their protein synthesis. In vitro and clinical trials have shown that it has antibacterial activity against Gram-positive bacteria, mycoplasma, chlamydia, some gram-negative bacteria, and has antibacterial activity and tissue permeability against toxoplasmosis, Legionella, etc.

1.1.1. The PI3K-AKT-mTOR pathway can be inhibited by carrimycin

Mammalian target of rapamycin (mTOR) is a downstream molecule of PI3K/Akt/mTOR and other signaling pathways. It plays an important role in cell proliferation, differentiation, metastasis and survival, and has become an important target for cancer therapy.

A549 cells were cultured in vitro and treated with different concentrations of carrimycin for 48 h. mTOR protein and its upstream and downstream proteins in the cytoplasm were investigated by Western Blot method. The results showed that the expression of mTOR and p-mTOR was inhibited by carrimycin , while the expression of P70S6K1 (ribosomal protein S6 kinase) and p-P70S6K1 downstream of mTOR was inhibited by carrimycin (Figure 1).

PI3K is a kind of intracellular phosphatidylinositol kinase. The PI3Ks protein family is involved in the regulation of cell proliferation, differentiation, apoptosis and glucose transport and other cellular functions.Increased PI3K activity is often associated with a variety of cancers.AKT (protein kinase B) is the primary downstream effector of PI3K. AKT activates mTOR by phosphorylating mTOR directly or by inactivating TSC2 (nodular sclerosis complex 2).Treatment of A549 cells with carrimycin for 48 h had no significant effect on PI3K, but significantly down-regulated the levels of p-PI3K and downstream p-AKT protein (Figure 1).

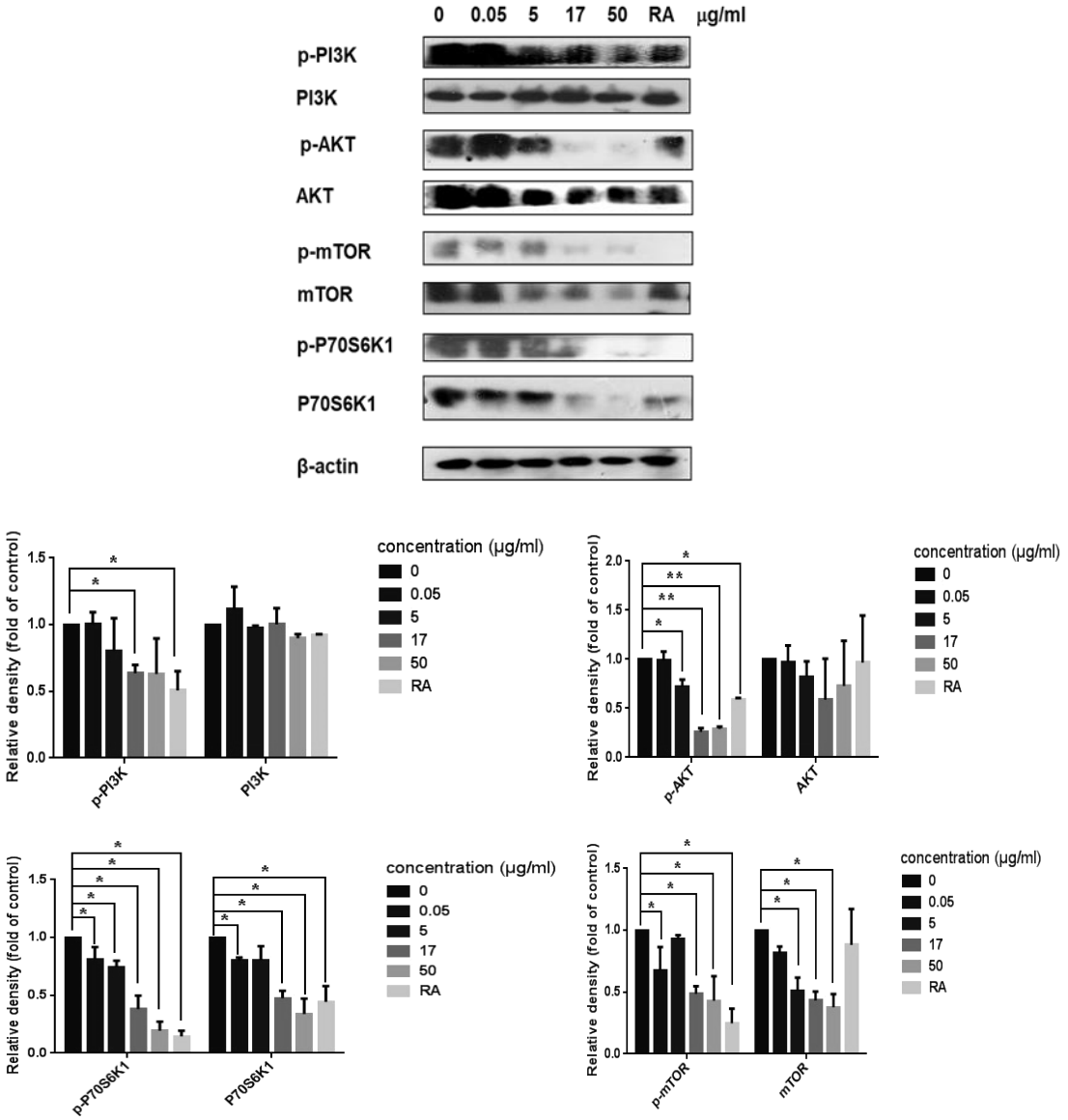

Figure 1: Carrimycin inhibited the PI3K-AKT-mTOR pathway. Western Blot assay was used to detect the expression of p-PI3K, PI3K, p-AKT, AKT, p-mTOR, mTOR, p-P70S6K1, and P70S6K1 proteins. β-actin was used as control.

*p< 0.05 compared with the model group; ** p< 0.01 compared with the model group

1.1.2. Carrimycin inhibited PD-L1 expression

PD-L1 molecule plays an important role in inducing tumor-specific T cell apoptosis and tumor immune escape. The brown-yellow particles or clumps of PD-L1 molecules were positively expressed in the membrane of PD-L1 lung cancer cells. The expression of PD-L1 on the cell membrane of A549 was significantly reduced by carrimycin (0.05, 5μg/ml) (Figure 2).

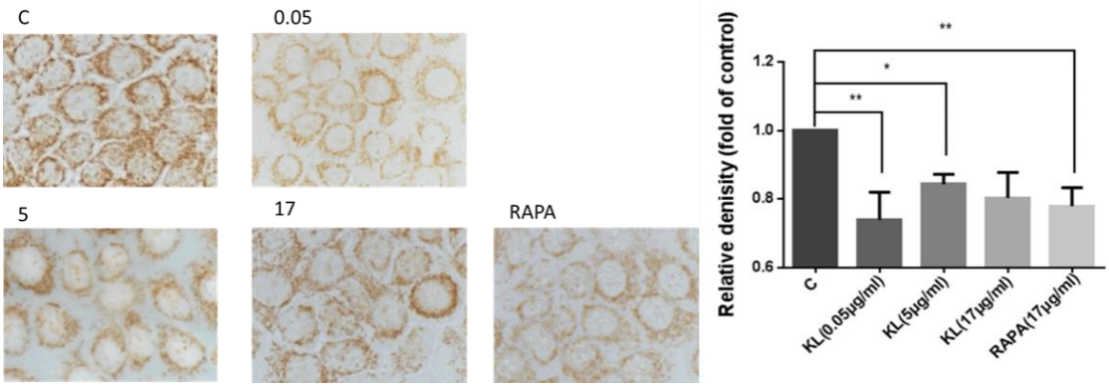

Figure 2: The expression of PD-L1 protein was detected by immunohistochemistry. Brown-yellow particles or clumps of PD-L1 molecules were positively expressed in the membrane of A549 lung cancer cells.

The above study showed that the mTOR protein and its upstream and downstream proteins in the cytoplasm were investigated by Western Blot after being treated with different concentrations of carrimycin for 48 h. The results showed that the expression of mTOR and p-mTOR could be inhibited by carrimycin, and the expression of P70S6K1 (ribosomal protein S6 kinase) and p-P70S6K1 downstream of mTOR could be inhibited by carrimycin. These results suggest that carrimycin can inhibit the mTOR pathway, especially the expression of S6K1 downstream, which may result in the inflammatory factor TNF-α, decreased secretion of IL-6, and enhanced secretion of anti-inflammatory factor IL-10.

1.1.3. Carrimycin can reduce the levels of IL-1β and IL-4

The infection model of mice was established and the effects of carrimycin on inflammatory factors in various tissues and organs of mice were tested.Kunming mice were randomly divided into 5 groups: normal group, model group, low group (30 mg/kg), medium group (60 mg/kg), and high group (120 mg/kg) after establishing Staphylococcus aureus infection model.Each group was divided into 8 time points: 0 h, 0.5 h, 2.5 h, 4.5 h, 12 h, 24 h, 48 h and 72 h.There were 3 mice at each time point.Mice in the normal group were given no drug or injection of bacteria, and mice in the model group were given no injection of bacteria. All mice were given carrimycin by intragastric administration (500μl), and the dose was doubled on the first day, followed by normal daily administration, and the model group was given the same volume of solvent. The results showed that the IL4 factor was significantly decreased in the lung, kidney, liver and spleen, and the effect was more significant in the liver and spleen. Carrimycin significantly reduced IL-1β in small intestine, lung, spleen, liver and kidney, especially in small intestine and lung.

1.2. Study on the immune regulatory mechanism of carrimycin

1.2.1 Effects of carrimycin on phagocytosis of macrophages

Itraconazole was used as positive control. The normal saline group was the placebo control group. The positive control group was itraconazole: 50mg/kg, p.O. The experimental group was given carrimycin: 50mg/kg, po. for five days.

The results showed that compared with the placebo group (NC), the Keli and itraconazole groups (Yiqu) enhanced the ability of macrophages to phagocytic chicken erythrocytes to some extent.

1.2.2. The effect of Keli on the function of neutrophils was detected

In mice, carrimycin and itraconazole could significantly promote the migration of neutrophils to the site of inflammation, especially in individual mice. No further enhancement was found after three days of treatment compared with seven days of treatment. In mice, carrimycin and itraconazole significantly increased the number of total T cells (CD3 positive cells), including both CD4 and CD8 positive cells.

1.2.3 Study on the transdifferentiation of differentiated macrophages induced by carrimycin

The results showed that the expression levels of TNF-α and iNos in M2-type macrophages were significantly higher than those induced by LPS+INF-γ, better than itraconazole, and the expression levels of Arg-1 in M2-type macrophages were significantly inhibited.

A series of studies carried out by Hu Yiguo et al in West China Hospital suggest that riomycin can strongly promote the transformation of M2-type macrophages to M1-type macrophages and improve the function of M1-type macrophages, which is mainly manifested in the following three aspects:

Cultured RAW246.7 cell line in vitro, first add drug for 1h, then add cytokines to induce its differentiation, detect the corresponding RNA level, it was found that carrimycin can increase the expression of TNF-α and iNos, inhibit the expression of Argue, suggesting that carrimycin may promote the differentiation and function of M1 macrophages.

Cultured RAW246.7 cell lines in vitro, the RAW cells were first induced to differentiate into M1 type macrophages by adding cytokines, and then the corresponding drugs were added to detect the corresponding RNA level. It was found that the expression of TNF-α and iNos induced by LPS+INF-γ was not further

enhanced by carrimycin. However, it could significantly inhibit the expression of Arg1 in M1-type macrophages, suggesting that carrimycin could potentially enhance the function of M1-type macrophages.

RAW246.7 cell lines were cultured in vitro. Cytokines were added to induce RAW cells to differentiate into M1 macrophages, and then corresponding drugs were added to induce cells to transform into M2-type macrophages. The corresponding RNA levels were detected, and it was found that the expression levels of TNF-α and iNos in M2-type macrophages were significantly increased by carrimycin. The expression levels of TNF-α and iNos induced by LPS+INF-γ were significantly higher than those induced by LPS+INF-γ, and the expression level of Arg1 in M2-type macrophages was significantly inhibited.

2. Pharmacological research data

Mice were given carrimycin 60mg/kg and 180mg/kg orally once, respectively. The free activity of mice was similar to that of the control group, but no significant effect was observed.After the oral administration of 30mg/kg, 60mg/kg and 180mg/kg of carrimycin, the mice were intritoneally injected with 25 mg/kg of pentobarbital sodium, which was similar to the control group, and no obvious synergistic effect was observed.The systolic blood pressure and diastolic blood pressure of the anesthetized dogs were decreased slightly with the extension of time, while the heart rate, electrocardiogram and respiration showed no significant effects compared with before administration.

3. Data from toxicological studies

3.1 Hematological toxicity

The experimental group was carrimycin group: 50mg/kg, po. for five consecutive days.

The blood routine results of normal mice after administration showed no significant changes in the total number of white blood cells, neutrophils, lymphocytes and so on.

	Absolute number / relative ratio		**UNITS**	**control-1**	**control-2**	**Colimycin -1**	**Cremycin -2**
Total white blood cell count		WBC	10^9/L	5.15	5.63	4.53	4.35
Neutrophils	#	NEUT	10^9/L	1.65	1.49	0.99	1.51
Lymphocytes	#	LYMPH	10^9/L	3.29	3.92	3.3	2.59
Monocytes	#	MONO	10^9/L	0.05	0.06	0.09	0.04
Eosinophils	#	EOS	10^9/L	0.1	0.07	0.11	0.09
Basophils	#	BASO	10^9/L	0.01	0.01	0.01	0.02
Large volume of unstained cells	#	LUC	10^9/L	0.05	0.08	0.03	0.1
Neutrophils	%	NEUT	%	32.1	26.5	21.8	34.8
Lymphocytes	%	LYMPH	%	63.8	69.7	73	59.5
Monocytes	%	MONO	%	1	1	1.9	1
Eosinophils	%	EOS	%	2	1.2	2.5	2.1
Basophils	%	BASO	%	0.3	0.2	0.2	0.4
Large volume of unstained cells	%	LUC	%	0.9	1.4	0.6	2.3
Red Blood cells		RBC	10^12/L	10.22	10.74	10.07	10.28
Hemoglobin concentration		HGB	g/L	164	170	160	163
Hematocrit		HCT	%	51.1	52.6	49	49.1
Red blood cell volume		MCV	fL	50	49	48.7	47.8

Average amount of hemoglobin in red blood cells	MCH	pg	16	15.8	15.9	15.9
Average red blood cell hemoglobin	MCHC	g/L	320	323	326	333
Red blood cell distribution width	RDW	%	13.4	13.8	13	12.7
Platelets	PLT	10^9/L	892	635	1044	805
Average platelet volume	MPV	fL	9.4	9.1	9.5	9.1

The experiment confirmed that no obvious hematological toxicity was observed under the condition of continuous administration of carrimycin for five days.

3.2 Mutagenesis

Carrimycin Ames test has shown that carrimycin is less likely to cause gene mutation in vitro.

Using Chinese hamster lung cells (CHL) to test the subject drug with or without a metabolic activator (S9), it was shown that the likelihood of carrimycin causing chromosomal aberrations in vitro was small.

The possibility of mutagenicity of carrimycin in mammalian cells was tested in mature male NIH mice. The results indicated that carrimycin did not affect normal mitosis of cells and had no inhibitory effect on bone marrow under the tested conditions. In conclusion, the possibility of gene mutation, chromosomal aberration or mutagenesis caused by carrimycin is small.

3.3 Acute toxicity

The toxicity, death and median lethal dose of carrimycin in mice, rats and dogs were determined. The results showed that the mortality rate of mice and rats was 35% and 15% when the dose was 4000 mg/kg, LD_{50}Greater than 4000 mg/kg; Dogs only saw gastrointestinal irritation and vomiting at doses of 2000mg/kg, 3000mg/kg and 4500 mg/kg, and were generally in good condition after vomiting, with no death, LD_{50}Ld > 4500 mg/kg.

3.4 Long-term toxicity

The rats were given oral administration for 60 consecutive days, and the results showed that 20mg/kg and 200mg/kg were generally in good condition, the hematology,

various biochemical indexes and routine urine examination were normal, and no abnormality was found in pathological examination, so it was a safe dose.The animals in the 500mg/kg group showed vertical hair, increased water intake and increased liver coefficient during administration, and recovered 20 days after withdrawal of the drug. No abnormalities were found in hematology and serum biochemical indexes, urine routine and pathological examination.

3.5 General reproductive toxicity

The results of perinatal toxicity test and teratogenic susceptibility toxicity test in mice indicated low reproductive toxicity of carrimycin. At 60 days before copulation in male mice, 14 days before copulation in female mice to the formative stage of most organs after copulation, continuous administration of 20 mg/kg and 500mg/kg of this product showed no other reproductive toxicity except for a slight increase in the absorption rate. The weight, conception rate, stillbirth rate, number of live births and live birth weight of pregnant mice in the administration group were no different from those in the negative control group. No teratogenic effects were observed in the administration group.

3.6 Effect on P450 isozyme (EROD) activity of rat liver microsomes

Oral administration of 500mg/kg, 125mg/kg and 30mg/kg for 5 consecutive days showed that there was no significant effect on liver EROD activity of rats, and there was no significant effect on liver weight and liver/body ratio of rats. No pathological morphological changes were observed in the carrimycin group.

4. Pharmacokinetic study

Pharmacokinetic parameters of single clinical administration:

The pharmacokinetic process of ISV-SPM-III, ISV-SPM-II, ISV-SPM-I and their 3 active metabolites in healthy subjects was consistent with the two-compartment open model of primary elimination.The absorption was rapid and the serum concentration reached the peak between 1.79 and 4.54 h, C_{max}0.0071-0.0300 μg/ml, respectively; The Vz/F ranges from 6559.60 to 11504.96 L.The protodrug was rapidly transformed into active metabolites.The protodrug is eliminated more slowly, $T_{1/2\beta}$Between 23 and 27 h, CL/F was 194.03 to 293.58L/h;Rapid elimination of metabolites, $T_{1/2\beta}$Between 4.54 and 14.89h.

Linear pharmacokinetic characteristics:

In the dose range of 200 to 500mg, the dose per unit body weight of each component was compared with C_{max} and AUC_{last}($r>0.599$, $P<0.01$) and AUC_{max} With

$T_{1/2}$(r<0.267, P>0.05) and dose per unit of body weight with $T_{1/2\beta}$Was not significant (r<0.13, P>0.05), indicating that $T_{1/2\beta}$It was not affected by dose and peak concentration. The results show that the in vivo disposal of each component has no nonlinear dynamic characteristics. C after medication$_{max}$ And AUC_{last} Increases with dose, in the range of 200 to 500mg, and AUC when dose is increased by 100mg$_{0\text{-}24h}$ The scaling factor of increase is greater than 1.0.

Pharmacokinetic characteristics of multiple administration:

The first dose was 0.4g/ time, and the dosage was 0.2g/ time for 2-7 days. The high blood concentration was obtained in a short time. The stable state was reached after 7 days of continuous administration, and the peak concentration of each component was similar to that on the first day of administration. Microbial method was used to determine the C of the total active components and active metabolites of carrimycin $_{max}$Was 118.98~331.02ng/ml, much higher than the total blood concentration of the original drug and active metabolites obtained by LC-MS-MS assay. Because of the multi-component mixture of the drug, The components of isvalyl spiramycin (ISV-SPM-I, ISV-SPM-II, ISV-SPM-III) determined by LC-MS-MS accounted for 65% of the active substances. The microbial assay method could better reflect the pharmacokinetic behavior of the total active components and active metabolites of Kolythromycin tablets in human body. The mean elimination half-life of the determination was 26.78h, which supported the dosing regimen of once a day.

Accumulation of multiple dosing:

There is a certain accumulation of drugs in the body during the continuous 7 days of administration of carrimycin tablets. The accumulation factors of ISV-SPM-III, ISV-SPM-II, ISV-SPM-I, SPM-III, SPM-II and SPM-I were 2.78, 2.57, 2.73, 1.53, 1.80 and 1.18, respectively. The fluctuation coefficients (%) were 110.59, 127.01, 204.07, 273.89, 340.43 and 397.01, respectively.It indicated that there was a certain degree of accumulation in the body after multiple dosing intervals of 24 h.Compared with the single dose group, the T of each component after multiple doses of 200 mg orally administered Carrimycin tablets$_{max}$There were no significant changes (P>0.05), $AUC_{0\text{-}last}$Were significantly increased (P<0.05), $T_{1/2\beta}$Both were slightly prolonged.C of the three primitive components$_{max}$ And the C of the other components$_{max}$ There is no significant change.

Effects of food:

The data of this study showed that there was no significant difference in the

pharmacokinetics of each active component after oral administration of 400 mg single dose of carrimycin tablets on fasting and after meals, that is, no influence of food on the pharmacokinetics of carrimycin tablets was observed.

Gender differences:

After single and multiple doses of 200 mg carrimycin tablets taken orally after meals, there were no significant gender differences in the three primary drugs and the three active metabolites in male and female subjects.

Absorption:

ISV-SPM-III, ISV-SPM-II, ISV-SPM-I and their 3 active metabolites in healthy subjects after 200mg oral administration of carrimycin tablets conform to the two-compartment open model of primary elimination. The absorption was rapid, and the serum concentration reached the peak at 1.79~4.54 h, C_{max}Was 0.0071-0.0300 μg/ml;

At 300, 400 and 500 mg doses, T of the three primitive components were calculated by the non-atrioventricular model method$_{max}$The dosages were 4.54~5.13 h, 4.17~5.29 h and 2.21~2.75h, respectively.C_{max}0.0211~0.0386, 0.0248~0.0407, 0.0158~0.0253μg/ml, respectively; T of three metabolites$_{max}$They are 2.83 to 3.50, 2.50 to 2.96, 2.96 to 3.50h, C_{max}Were 0.0435~0.0701, 0.05~0.072, 0.0045~0.0070μg/ml, respectively;Plasma drug elimination half-life $T_{1/2\beta}$They were 28.05-29.92 h, 21.76-25.80 h, 17.15-18.04 h and 20.19-27.68 h, 16.70-20.28 h, 9.83-18.53 h, respectively.

Distribution:

Tests in rats showed the highest concentration of carrimycin in liver tissues, followed by spleen, kidney, lung and intestine, and did not enter the brain and testes.After the introduction of carrimycin into rats, the oral bioavailability is high, the absorption of the drug in the gastrointestinal tract is fast, the peak time is about 1.0-3.5 hours, the drug enters the tissues quickly after entering the animal body, the concentration in plasma is low, most of it is rapidly transformed into the active metabolic product spiramycin in the body, but some of it still maintains the isovalentylation prototype. Isovalyl spiramycin can be detected in most tissues. After oral administration of equal-dose of carrimycin and spiramycin, the concentration of isovalyl spiramycin and spiramycin combined in most tissues of rats was higher than that after oral administration of equal-dose spiramycin, and the concentration of active

substance distributed in the tissues of the former was about twice of that of the latter.Tissue/plasma concentrations were also higher than those after equal doses of spiramycin, indicating a higher tissue affinity for carrimycin compared to spiramycin. The characteristics of this product are that the tissue concentration is higher than the blood concentration, the tissue concentration is maintained for a long time, and the concentration still reaches the MIC above 48 hours, which is another characteristic of this product. Moreover, the prototype drug and its main metabolites have antibacterial activity, making it play a therapeutic role in tissues, which is another characteristic of this product. The plasma protein binding rates of isovalyl spiramycin I, II and III were 34.8%, 51.3% and 65.1% by liquid chromatography-mass spectrometry (LC-MS).

The Vz/F of this product in healthy subjects was 6559.60~11504.96L, suggesting its wide distribution in vivo. At present, the detailed data of its distribution in human body are lacking.

Metabolism:

The metabolites of carrimycin in urine, feces and bile of rats after intragastric administration were determined by liquid chromatography-mass spectrometry (LC-MS). It was concluded that the metabolites of carrimycin in vivo mainly include:

(1) The removal of forloaminosaccharide (its products also have biological activity)

(2) Removal of the 4 "-isopentyl side chain (metabolite is spiramycin with biological activity)

(3) Reduction of aldehyde group

(4) Hydrolysis reaction occurs when lactone ring is opened

(5) The aldehyde group binds to cysteine

(6) The carbamose attached to the side chain is removed

At present, there is a lack of research data on human metabolic pathways.

The discharge

The excretion rates of urine and bile in rats were 2.183% and 4.71%, respectively. The excretion of carrimycin tablets (single dose 80 mg/kg and multiple dose 80 mg/kg for 6 days) in rats was determined by liquid chromatography-mass spectrometry. The results showed that the cumulative excretion of isopentyl spiramycin and spiramycin in urine and feces accounted for 37.42% of the dose in the single dose group.In the multi-dose group, the total cumulative excretion of isopentylspiramycin and

spiramycin in urine and feces accounted for 45.41% of the dose. Carrimycin tablets were mainly excreted in feces. Human trials have shown that less than 3% of this product is excreted in urine.

Detailed data on human excretion are lacking.

5. Phase I, II and III clinical trials

5.1 Results of Phase I safety and tolerability study of carrimycin tablets

Usage and dosage: Stage A subjects took orally orally 100mg, 200mg, 400mg and 600mg orally on an empty stomach, with 5 subjects in each group; Carrimycin 800mg was taken orally after a single meal (all were taken with 200ml warm boiling water), which was a placebo-controlled test. A total of 6 subjects were enrolled; Stage B subjects took 400mg of carrimycin orally after each meal, once in the morning and once in the evening on the first day, and once every day for 2-7 days. For 7 days in total, they were all taken with 200ml of warm boiled water. A total of 6 subjects were included.

Conclusions: The results of single dose administration in stage A and continuous administration in stage B showed that this product can cause mild gastrointestinal irritation when taken on an empty stomach, and can alleviate or avoid gastrointestinal discomfort if given after meals. No abnormality of routine hemuria and renal function was found in the 100mg~800mg oral dose or 400mg multi-dose continuous administration, no obvious effect on blood pressure and electrocardiogram, no rash and other allergic reactions were found.This indicated that the product was safe and well tolerated in the above dosage range, but the 800mg group may have transient mild elevation of ALT and AST in liver function.The potential effects of this product on liver function should be further investigated in Phase II clinical trials.

5.2 Results of Phase I pharmacokinetic study of carrimycin tablets

Pharmacological Center of Peking Union Medical College Hospital conducted a phase I clinical pharmacokinetic study of carrimycin tablets to investigate the absorption, distribution, metabolism and excretion process of carrimycin tablets in healthy subjects, and to investigate the linear kinetic characteristics of carrimycin tablets within the study dose range, the influence of food on absorption, and whether there are gender differences, so as to provide reference information for phase II clinical trials.

Usage and dosage: Single dose fasting oral carrimycin pharmacokinetics

first pre-test single dose fasting oral carrimycin 200mg, 200ml warm water to take, 2 healthy subjects were enrolled; For the second pre-test, a single dose of fasting oral carrimycin 600mg and 200ml warm boiled water were administered, and 4 healthy subjects were enrolled;In the third pre-test, a single dose of 200mg of fasting oral carrimycin and 200ml of warm boiled water were administered, and 3 healthy male subjects were enrolled.A formal study on the pharmacokinetics of single dose of fasting oral carrimycin (300mg, 400mg, 500mg, 200ml warm water) was conducted after meals, respectively. 12 healthy subjects were enrolled, 50/50 male and female, and 2 males were added in the medium dose group. Eight healthy male subjects were enrolled to study the effects of food on the pharmacokinetics of carrimycin, which was administered 400mg orally and 200ml hot water orally during fasting and after meals. Study on the pharmacokinetics of single and multiple doses of 200mg of carrimycin orally: 200mg of carrimycin orally after breakfast, on the first day, once in the morning; From the 8th to the 14th day, once a day in the morning, 12 healthy subjects were enrolled, half male and half female.

Conclusion: The in vivo process of carrimycin tablets after oral administration conforms to the two-compartment open model of primary elimination. The drug was rapidly absorbed and widely distributed in the body. The original drug was rapidly transformed into active metabolites. The proto drug is eliminated slowly, and the T of each component in a single dose$_{1/2\beta}$ Between 23 and 27h.There is some accumulation in the body when multiple doses are administered. No nonlinear pharmacokinetic features were observed in the dosage range of 200 to 500mg. No effects of food on drug absorption and metabolism were observed.No sex difference was observed in pharmacokinetic parameters.It is recommended that the dosage of 200 to 400mg should be given once a day for 5 to 7 days, not more than 10 days, after meals for mild and moderate infections.

5.3 Results of Phase II and III clinical trials of carrimycin tablets

The phase II and III trials were double-blind, randomized, double-simulated positive control trials in 18 clinical research centers.A total of 227 randomized patients with respiratory tract infection were enrolled in the phase II clinical trial of carrimycin , and 211 cases were actually completed.The phase II clinical trial of carrimycin showed no significant differences in clinical efficacy, bacterial clearance rate, comprehensive efficacy and safety between carrimycin group and azithromycin group.

A total of 273 patients with single-disease acute suppurative tonsillitis were enrolled in the phase III clinical trial of carrimycin , with 246 cases actually completed.263 patients with single disease acute bacterial tracheal bronchitis, 216 cases actually completed;And 178 patients with single disease bacterial sinusitis, with 129 cases actually completed.The results showed that there were no significant differences in clinical efficacy, bacteriological efficacy and comprehensive efficacy between the carrimycin and azithromycin groups, and there were no significant differences in safety.

5.4 Safety evaluation of Phase II and III clinical trials of carrimycin tablets

According to the completed Phase II and Phase III clinical trials of this product, a total of 649 patients were treated with carrimycin, and the main adverse reactions were mild. During the entire trial, only one patient dropped out of the study due to nausea and vomiting.No serious adverse events were reported throughout the clinical trial.

According to the summary of the Phase II and III clinical trials that have been completed, common adverse events (their drug-related degree is divided into five levels: Related to the drug, likely to be related to the drug, likely to be related to the drug, likely to be unrelated to the drug and not related to the drug, where related to the drug, likely to be related to the drug, likely to be related to the drug and likely to be related to the drug are counted as adverse reactions), the list of adverse reactions according to the occurrence of cases is as follows:

Adverse reactions	Carrimycin group (N=649)	
	cases	Percentage of occurrence
Clinical adverse reactions		
Digestive system abnormalities		
Mild nausea	12	1.85
vomiting	1	0.15
Burning sensation in the stomach	3	0.46
Gastrointestinal discomfort	4	0.62
Increased bowel movements	1	0.15
diarrhea	2	0.31

Neurological abnormalities		
dizzy	1	0.15
sleepy	2	0.31
drowsiness	2	0.31
Abnormal laboratory tests		
Elevated alanine aminotransferase (ALT)	14	2.16
Elevated aspartate aminotransferase (AST)	10	1.54
Elevated total bilirubin (TBIL)	5	0.77
Reduced white blood cell count	1	0.15
Neutrophils decreased	1	0.15
Positive urine protein	1	0.15

The total number of adverse reactions of this product in Phase II and Phase III clinical trials was 51, and the total adverse reaction rate was 7.86%. The main adverse reactions occurred as follows:

Common adverse reactions (incidence ≥1% <10%):

Digestive system: mild nausea;

Hepatobiliary system: elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

Rare adverse reactions (incidence ≥0.1% <1%):

Digestive system: mainly gastrointestinal reactions, including gastrointestinal discomfort, stomach burning, vomiting, increased frequency of defecation, diarrhea;

Hepatobiliary system: increased total bilirubin (TBIL);

Nervous system: dizziness, drowsiness, drowsiness;

Blood system: lower white blood cell count, lower neutrophils;

Urinary system: urine protein positive.

In addition to the adverse reactions observed in clinical trials, drug fever, rash, urticaria, eosinophilia and other allergic reactions may occasionally occur when using this product. Long-term use of the product can cause bacterial imbalance, cause double infection, candida infection, pseudomembranous enteritis and so on.

Drug Use Information

From 2002 to February 2004, the phase I clinical study of carrimycin was conducted to study the safety and tolerability of carrimycin as well as the pharmacokinetics.

From August 2004 to March 2005 and from May 2005 to April 2006, 54 phase II clinical trials and 227 phase II clinical trials of carrimycin were conducted.

From May 2006 to May 2007, 273 cases of the phase III phase A clinical trial of carrimycin were studied.

From October 2007 to December 2009, 263 cases of the phase III Phase B clinical trial of carrimycin (acute bacterial tracheobronchitis) were studied.

From October 2007 to February 2010, 263 cases of currimycin in phase III B clinical trial (bacterial sinusitis) were studied.

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