

Original article

Protective potential of piroxicam on human peripheral blood mononuclear cells against suppressive capacity of U-87 MG cell line

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Table 1s: effect of dexamethasone and piroxicam on the proliferation of T cells in PBMCs

Dexamethasone Con. (μ M)	0	0.1	0.5	1
OD ₅₇₀	0.713	0.489	0.455	0.425
	0.699	0.508	0.434	0.424
	0.67	0.54	0.447	0.428

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
0 vs. 0.1	0.1817	0.1352 to 0.2281	Yes	****	<0.0001
0 vs. 0.5	0.2487	0.2022 to 0.2951	Yes	****	<0.0001
0 vs. 1	0.2683	0.2219 to 0.3148	Yes	****	<0.0001
0.1 vs. 0.5	0.06700	0.02056 to 0.1134	Yes	**	0.0074
0.1 vs. 1	0.08667	0.04022 to 0.1331	Yes	**	0.0015
0.5 vs. 1	0.01967	-0.02678 to 0.06611	No	ns	0.5568
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1
0 vs. 0.1	0.6940	0.5123	0.1817	0.01450	3
0 vs. 0.5	0.6940	0.4453	0.2487	0.01450	3
0 vs. 1	0.6940	0.4257	0.2683	0.01450	3
0.1 vs. 0.5	0.5123	0.4453	0.06700	0.01450	3
0.1 vs. 1	0.5123	0.4257	0.08667	0.01450	3
0.5 vs. 1	0.4453	0.4257	0.01967	0.01450	3

Piroxicam Con. (μ M)	0	3	14	30
OD ₅₇₀	0.713	0.677	0.696	0.737
	0.699	0.732	0.668	0.691
	0.67	0.759	0.677	0.712

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
0 vs. 3	-0.02867	-0.09265 to 0.03532	No	ns	0.4701
0 vs. 14	0.01367	-0.05032 to 0.07765	No	ns	0.8711
0 vs. 30	-0.01933	-0.08332 to 0.04465	No	ns	0.7252
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1
0 vs. 3	0.6940	0.7227	-0.02867	0.02222	3
0 vs. 14	0.6940	0.6803	0.01367	0.02222	3
0 vs. 30	0.6940	0.7133	-0.01933	0.02222	3

Table 2s: effect of dexamethasone and piroxicam on the proliferation of T cells in PBMCs in stimulated and non-stimulated conditions

Dexamethasone con. (μM)	Proliferation in unstimulated condition (OD₅₇₀)			Proliferation in stimulated condition (OD₅₇₀)		
0	0.424	0.461	0.401	0.758	0.703	0.723
0.1	0.315	0.317	0.367	0.486	0.483	0.443
1	0.319	0.369	0.344	0.447	0.44	0.462

Difference between column means	
Mean of Unstimulated	0.3686
Mean of Stimulated	0.5494
Difference between means	-0.1809
SE of difference	0.01200
95% CI of difference	-0.2070 to -0.1547

Piroxicam Con. (μM)	Proliferation in unstimulated condition (OD₅₇₀)			Proliferation in stimulated condition (OD₅₇₀)		
0	0.424	0.461	0.401	0.758	0.703	0.723
3	0.405	0.382	0.342	0.627	0.54	0.484
30	0.578	0.331	0.458	0.698	0.649	0.704

Difference between column means	
Mean of Unstimulated	0.4202
Mean of Stimulated	0.6540
Difference between means	-0.2338
SE of difference	0.02986
95% CI of difference	-0.2988 to -0.1687

Table 3s: the effect of drugs on the protection of T cells in PBMCs co-cultured with U-87 MG cell line

Drug concentration	Protection (%) after piroxicam treatment			Protection (%) after dexamethasone treatment		
0	47	-8	-53	47	-8	-53
1*	24.33333	34.83333	32.33333	-142.333	-144.833	-135.833
2**	-3.33333	8.166667	0.166667	-159.333	-154.333	-164.333
3***	-2.83333	9.666667	7.166667	-182.167	-176.167	-186.667

Protection (%)	Piroxicam	Dexamethasone
Mean	8.042	-121.7
Std. Deviation	15.47	79.75
Std. Error of Mean	7.735	39.88
Lower 95% CI	-16.57	-248.6
Upper 95% CI	32.66	5.238

*Con. 1: piroxicam 3, dexamethasone 0.1. (μM) **Con. 2: piroxicam 14, dexamethasone 0.5. (μM) *Con. 3: piroxicam 30, dexamethasone 1 (μM).

Table 4s: the effect of dexamethasone and piroxicam on the SOD3 activity of T cells in PBMCs

Group	Non-treated	Piroxicam Conc. (30µM)	Dexamethasone Conc. (1µM)
SOD3 activity (U/mL)	0.7	0.85	0.25
	0.9	0.4	0.75
	0.3	1.2	0.85

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Non-treated vs. Column B	-0.1833	-0.9903 to 0.6236	No	ns	0.7512
Non-treated vs. Column C	0.01667	-0.7903 to 0.8236	No	ns	0.9974
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1
Non-treated vs. Column B	0.6333	0.8167	-0.1833	0.2819	3
Non-treated vs. Column C	0.6333	0.6167	0.01667	0.2819	3

Table 5s: the effect of dexamethasone and piroxicam on the TAC levels of T cells in PBMCs

Group	Non-treated	Piroxicam Conc. (30µM)	Dexamethasone Conc. (1µM)
TAC levels (µmol/L)	0.7	0.85	0.25
	0.56	0.4	0.75
	0.87	1.2	0.85

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Non-treated vs. Column B	-0.1067	-0.8313 to 0.6180	No	ns	0.8819
Non-treated vs. Column C	0.09333	-0.6313 to 0.8180	No	ns	0.9075
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1
Non-treated vs. Column B	0.7100	0.8167	-0.1067	0.2531	3
Non-treated vs. Column C	0.7100	0.6167	0.09333	0.2531	3

Table 6s: the effect of drugs on the LDH activity of T cells in PBMCs

Group	Non-treated	Piroxicam Conc. (30µM)	Dexamethasone Conc. (1µM)
LDH activity (U/L)	31	34	25
	38	27	43
	29	32	22

Brown-Forsythe test					
F (DFn, DFd)	0.5104 (2, 6)				
P value	0.6242				
P value summary	ns				
Are SDs significantly different (P < 0.05)?	No				
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	10.89	2	5.444	F (2, 6) = 0.09939	P=0.9068
Residual (within columns)	328.7	6	54.78		
Total	339.6	8			

Table 7s: the effect of drugs on the LDH activity of U-87 MG cell line

Group	Non-treated	Piroxicam Conc. (30µM)	Dexamethasone Conc. (1µM)
LDH activity (U/L)	85	46	11
	72	37	18
	78	21	24

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Non-treated vs. Column B	43.67	22.54 to 64.79	Yes	**	0.0019
Non-treated vs. Column C	60.67	39.54 to 81.79	Yes	***	0.0003
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1
Non-treated vs. Column B	78.33	34.67	43.67	7.379	3
Non-treated vs. Column C	78.33	17.67	60.67	7.379	3

Table 8s: the effect of drugs on the LDH activity in the co-culture condition

Group	Non-treated	Piroxicam (30µM)	Dexamethasone (1µM)
LDH activity (U/L)	24	98	37
	27	165	17
	34	139	29

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Non-treated vs. Column B	-105.7	-153.7 to -57.59	Yes	**	0.0013
Non-treated vs. Column C	0.6667	-47.41 to 48.74	No	ns	0.9988
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1
Non-treated vs. Column B	28.33	134.0	-105.7	16.79	3
Non-treated vs. Column C	28.33	27.67	0.6667	16.79	3

Table 9s: the effect of drugs on the IFN- γ levels of T cells in PBMCs

Non-treated	Piroxicam Con. (μ M)	3	30	Dexamethasone Con. (μ M)	0.1	1
55.48	IFN- γ levels (pg/ml)	42.48	27.93	IFN- γ levels (pg/ml)	46.52	23.21
24.55		31.35	60.2		37.18	22.71
36.57		38.8	44.03		50.17	36.57
26.8		5	15.7		27.3	21.7
21.1		16.6	36.4		28.1	12.6

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
0 vs. 3	6.054	-16.98 to 29.08	No	ns	0.9268
0 vs. 30	-3.952	-26.98 to 19.08	No	ns	0.9869
0 vs. 0	0.000	-23.03 to 23.03	No	ns	>0.9999
0 vs. 0.1	-4.954	-27.98 to 18.08	No	ns	0.9662
0 vs. 1	9.542	-13.49 to 32.57	No	ns	0.6970
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1
0 vs. 3	32.90	26.85	6.054	8.544	5
0 vs. 30	32.90	36.85	-3.952	8.544	5
0 vs. 0	32.90	32.90	0.000	8.544	5
0 vs. 0.1	32.90	37.85	-4.954	8.544	5
0 vs. 1	32.90	23.36	9.542	8.544	5

Table 10s: the effect of drugs on the IFN- γ levels of T cells in co-culture condition

Non-treated	Piroxicam Con. (μ M)	3	30	Dexamethasone Con. (μ M)	0.1	1
60.48	IFN- γ levels (pg/ml)	50.29	89.9	IFN- γ levels (pg/ml)	60.63	56.38
31.14		58.79	82.89		60.3	34.57
58.5		60.1	38.2		28.6	29.4
29.9		18.1	30		17.6	18.14
26.9		20.3	73.7		32.8	15.5

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
0 vs. 3	-0.1320	-33.96 to 33.70	No	ns	>0.9999
0 vs. 30	-21.55	-55.38 to 12.28	No	ns	0.3189
0 vs. 0	0.000	-33.83 to 33.83	No	ns	>0.9999
0 vs. 0.1	1.398	-32.43 to 35.23	No	ns	0.9999
0 vs. 1	10.59	-23.24 to 44.42	No	ns	0.8658
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1
0 vs. 3	41.38	41.52	-0.1320	12.55	5
0 vs. 30	41.38	62.94	-21.55	12.55	5
0 vs. 0	41.38	41.38	0.000	12.55	5
0 vs. 0.1	41.38	39.99	1.398	12.55	5
0 vs. 1	41.38	30.80	10.59	12.55	5

Table 11s: the effect of drugs on the TGF- β levels of T cells in PBMCs

Non-treated	Piroxicam Con. (μ M)	3	30	Dexamethasone Con. (μ M)	0.1	1
97.4	TGF- β levels (pg/ml)	179.54	86.9	TGF- β levels (pg/ml)	50.94	87.77
56.35		111.74	151.34		33.74	99.4
23.1		96.5	36.5		68	95.9
112.28		110.8	80.6		37.9	76.7

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
0 vs. 3	-52.36	-120.2 to 15.45	No	ns	0.1650
0 vs. 30	-16.55	-84.36 to 51.26	No	ns	0.9382
0 vs. 0	0.000	-67.81 to 67.81	No	ns	>0.9999
0 vs. 0.1	24.64	-43.17 to 92.45	No	ns	0.7723
0 vs. 1	-17.66	-85.47 to 50.15	No	ns	0.9216
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1
0 vs. 3	72.28	124.6	-52.36	24.56	4
0 vs. 30	72.28	88.84	-16.55	24.56	4
0 vs. 0	72.28	72.28	0.000	24.56	4
0 vs. 0.1	72.28	47.65	24.64	24.56	4
0 vs. 1	72.28	89.94	-17.66	24.56	4

Table 12s: the effect of drugs on the TGF- β levels of U-87 MG cell line

Non-treated	Piroxicam Con. (μ M)	3	30	Dexamethasone Con. (μ M)	0.1	1
176.5	TGF- β levels (pg/ml)	33.6	23.3	TGF- β levels (pg/ml)	187	188
105.4		42.3	30.4		241	89.9
113.7		66.3	46.8		96.9	107.1

Holm-Sidak's multiple comparisons test	Mean Diff.	Significant?	Summary	Adjusted P Value
0 vs. 3	84.47	No	ns	0.1359
0 vs. 30	98.37	No	ns	0.0847
0 vs. 0	0.000	No	ns	>0.9999
0 vs. 0.1	-43.10	No	ns	0.5800
0 vs. 1	3.533	No	ns	0.9941
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.
0 vs. 3	131.9	47.40	84.47	35.74
0 vs. 30	131.9	33.50	98.37	35.74
0 vs. 0	131.9	131.9	0.000	35.74
0 vs. 0.1	131.9	175.0	-43.10	35.74
0 vs. 1	131.9	128.3	3.533	35.74

Table 13s: the effect of drugs on the TGF- β levels of T cells in co-culture condition

Non-treated	Piroxicam Con. (μ M)	3	30	Dexamethasone Con. (μ M)	0.1	1
153.44	TGF- β levels (pg/ml)	132.4	162.24	TGF- β levels (pg/ml)	74.33	246.14
133.44		69.8	178.9		75.64	129.84
70.9		76.4	160.6		146	88.5
120.4		24.2	95.8		120.6	163.3

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
0 vs. 3	43.85	-41.63 to 129.3	No	ns	0.4987
0 vs. 30	-29.84	-115.3 to 55.64	No	ns	0.7967
0 vs. 0	0.000	-85.48 to 85.48	No	ns	>0.9999
0 vs. 0.1	15.40	-70.08 to 100.9	No	ns	0.9816
0 vs. 1	-37.40	-122.9 to 48.08	No	ns	0.6366
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1
0 vs. 3	119.5	75.70	43.85	30.95	4
0 vs. 30	119.5	149.4	-29.84	30.95	4
0 vs. 0	119.5	119.5	0.000	30.95	4
0 vs. 0.1	119.5	104.1	15.40	30.95	4
0 vs. 1	119.5	156.9	-37.40	30.95	4