Preemptive Intraperitoneal Administration of Pentoxyphylline Ameliorates Cytokine Response to Intraperitoneal Injection of Acetic acid in a Dose-dependent Fashion in Albino Rats

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Abstract: The objective of this work was to evaluate the effect of preemptive administration of pentoxyphylline (PTX) on cytokine response to intraperitoneal (IP) administration of acetic acid. Thirty two male albino rats divided into 4 equal groups: Negative control group (C1 group) received IP injection of 2 ml saline, Groups PTX-1 and PTX-2 received IP injection of PTX in a dose of 0.8 and 1.6 mg/kg, respectively, in 2 ml injection volume and Positive control group (C2 group) received no preemptive medication. Thirty minutes after priming, IP injection of acetic acid was conducted for PTX-1, PTX-2 and C-2 group. Two venous blood samples, the 1st as baseline sample for all groups and the 2nd sample was obtained from PTX and C-2 groups 4-hours after IP injection of acetic acid for estimation of serum levels of interleukin (IL)-10, IL-6 and tumor necrosis factor (TNF)-α. Serum levels of all studied cytokines estimated at 4 hrs after IP injection of acetic acid was significantly higher in PTX-1, PTX-2 and C-2 groups compared to their baseline levels. Four-hours serum levels of TNF-α and IL-6 were significantly higher in group C-2 compared to PTX-1 and PTX-2 groups with significantly higher levels estimated in PTX-1 compared to PTX-2 group. However, 4 hrs serum levels of IL-10 were significantly lower in group C-2 compared to PTX-1 and PTX-2 groups with significantly lower levels estimated in PTX-1 compared to PTX-2 group. Definite dose-dependency was evident in the obtained study and was manifested as a negative significant correlation between serum TNF-α and IL-6 and injected dose of PTX, while serum IL-10 showed positive significant correlation with injected dose of PTX. It is concluded that preemptive administration of PTX attenuates release of IL-6 & TNF-α and stimulates the release of IL-10 in a dose-dependent fashion after direct exposure to extensive injury.

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1. Introduction:

Pain is an unpleasant sensation associated with varied consequences affecting the body systems and is usually associated with both changes in hormonal and immune milieu. Postoperative pain as a phenomena, caused by a flow of nociceptive information occurs in two phases: Phase I is directly related to nociceptive stimulation that accompanies tissue injury during the operation, whereas Phase II, emerging after the operation, is the result of inflammatory response in the injured tissues and is caused by Phase I-induced changes in nociceptive structures of the nervous system (**Bonnet and Marret, 2007; Vadivelu *et al.*, 2010**).

Numerous ion channels are expressed in sensory neurons. These channels are molecular sensors that detect adverse stimuli and when these channels are activated, portions of sensory neurons are depolarized and trigger the nociceptive neural signals. In pathologic conditions, various types of ion channels are activated with inflammatory signals which sensitize channels in sensory neurons and cause primary hyperalgesia, (**Lee *et al.*, 2005**).

The activation of nociceptors by tissue injury induces the synthesis and/or release of proinflammatory compounds which alter the sensitivity of voltage-gated ion channels in a manner that may result in hyperalgesia or allodynia. This altered sensitivity is mediated by the activation of intracellular signal transduction pathways, the activation of cAMP-protein kinase C pathway increase nociceptor sensitivity to chemical, thermal, or physical stimuli (**Piper and Docherty, 2000; Kidd and Urban, 2001**).

Tissue injury causes the release of proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin-1 beta (IL-1ß) which are involved in many aspects of inflammation, including cell migration, edema development, fever and hyperalgesia. Resident cells such as macrophages, mast cells and lymphocytes are able to release large amounts of TNF-α and IL-1ß after stimulation by exogenous inflammatory stimuli and/or endogenous mediators, such as lipopolysaccharide (LPS) and enterotoxins, (**Boraschi *et al.*, 1998; Dinarello, 2000; Oppenheim, 2001**).

The produced TNF-α triggers the release of a cascade of cytokines, which mediate the release of prostaglandins and sympathomimetic amines, the (final) mediators involved in the sensitization of nociceptors. Indeed, TNF-α stimulates the production of IL-1ß and IL-6, which in turn stimulate the production of cyclooxygenase products and IL-8/neutrophil chemoattractant-1 thereby enhancing the production of sympathomimetic amines (**Ferreira *et al.*, 1988; Lorenzetti *et al.*, 2002**). IL-6 is also able to promote the phenotypic responses and its actions can be classified as both pro- and anti-inflammatory. The local balance of IL-6 and IL-10 is an important determinant of subsequent immune responses. Th2 responses predominate in critically ill patients and after surgery (**Opree & Kress, 2000**).

Pentoxyphylline (PTX; 3,7-diethyl-1-(5-oxo-hexyl)-xanthine), a methyl-xanthine, inhibits TNF synthesis via the inhibition of phosphodiesterase and the increase of intracellular cyclic adenosine monophosphate (cAMP). It has also been shown to depress TNF production by macrophages at the transcription level (**Voisin *et al.*, 1998**). In sepsis, activation of inflammatory cells and excessive production of proinflammatory cytokines leads to tissue injury, multiple organ failure, and death. Activated neutrophils play a central role in the pathogenesis of ARDS and multiple organ failure. PTX was found to markedly decrease neutrophil activation (**Lall *et al.*, 2006**).

The current study aimed to evaluate the effect of preemptive administration of pentoxyphylline on cytokine response to intraperitoneal administration of acetic acid.

2. Material and Methods

Animals

The present study comprised 32 male albino rats with weight range of 250-300 grams. Rats were housed under conditions of optimum light, temperature and humidity (12 h light–dark cycle, 22±1.1^{o}C, 60–80% humidity) with access to water and food *ad libitum* until use. Rats were maintained on a balanced diet (bread, barely, carrots, lettuce, and milk) and fresh-water supply.

Rats were divided into 4 equal groups (n=8) according to preemptive medication: Negative control group (C1 group) received IP injection of 2 ml saline, Groups PTX-1 and PTX-2 received IP injection of PTX in a dose of 0.8 and 1.6 mg/kg, respectively, in 2 ml injection volume and Positive control group (C2 group) received no preemptive medication. The dose of PTX was calculated in relation to the human dose according to **Peiga & Barnes (1964)**. Thirty minutes after priming, acetic acid (0.1 ml/ 10 g body weight of a 0.6% solution, v/v) was injected intraperitoneally in animals of PTX-1, PTX-2 and C-2 group, (**Ribeiro *et al.*, 2000**)

Biochemical Evaluation

Two venous blood samples, withdrawn from the tail vein, were obtained. The 1st was obtained from all of the four groups prior to injection of saline or PTX. The 2nd sample was obtained from PTX and C-2 groups 4-hours after IP injection of acetic acid. Blood samples were allowed to clot and then centrifuged at 5000 rpm for 10 minutes and supernatant was separated, placed in pyrogen-free Eppendorf tubes and stored at -80°C until ELISA assayed (within one month) for estimation of serum levels of IL-10 (**Taga and Tosato, 1992**), IL-6, (**Engvall *et al.*, 1972**) and TNF-α, (**Beutler *et al.*, 1985**) using Quantikine ELISA kits from R & D Systems, Inc. (Minneapolis, MN).

Statistical analysis

Results were presented as mean±SD and were analysed by paired t-test using SPSS (Version 15, 2006); p value of <0.05 was considered statistically significant

3. Results

Baseline estimated serum levels of TNF-α showed non-significant (p>0.05) difference between studied groups. However, serum levels of TNF-α estimated at 4-hrs after IP injection of acetic acid were significantly higher in PTX-1, PTX-2 and C-2 groups compared to their baseline levels. Moreover, serum levels of TNF-α estimated at 4-hrs after IP injection of acetic acid were significantly higher in group C-2 compared to PTX-1 and PTX-2 groups with significantly higher levels estimated in PTX-1 compared to PTX-2 group (Table 1, Fig. 1).

Baseline estimated serum levels of IL-6 showed non-significant (p>0.05) difference between studied groups. However, serum levels of IL-6 estimated at 4-hrs after IP injection of acetic acid were significantly higher in PTX-1, PTX-2 and C-2 groups compared to their baseline levels. Moreover, serum levels of IL-6 estimated at 4-hr after IP injection of acetic acid were significantly higher in group C-2 compared to PTX-1 and PTX-2 groups with significantly higher levels estimated in PTX-1 compared to PTX-2 group (Table 2, Fig. 2).

Baseline estimated serum levels of IL-10 showed non-significant (p>0.05) difference between studied groups. However, serum levels of IL-10 estimated at 4-hrs after IP injection of acetic acid were significantly higher in PTX-1, PTX-2 and C-2 groups compared to their baseline levels. Moreover, serum levels of IL-10 estimated at 4-hrs after IP injection of acetic acid were significantly lower in

group C-2 compared to PTX-1 and PTX-2 groups with significantly lower levels estimated in PTX-1 compared to PTX-2 group (Table 3, Fig. 3).

Definite dose-dependency was evident in the obtained study and was manifested as a negative significant correlation between serum TNF-α (Fig. 4) and IL-6 (Fig. 5) and injected dose of PTX, while serum IL-10 showed positive significant correlation with injected dose of PTX (Table 4, Fig. 6).

Table (1): Mean serum levels of TNF-α estimated at baseline and 4-hours after IP injection of acetic acid(M±SD).

	C-1 group	PTX-1 group	PTX-2 group	C-2 group
Baseline	0.683±0.094	0.649±0.11	0.655±0.111	0.665±0.102
4-hrs level		1.831±0.24	1.275±0.267	2.463±0.46
Statistical analysis		t=1.081, p_1>0.05	t=0.832, p_1>0.05	t=0.390, p_1>0.05
		t=17.175, p_2<0.001	t=6.378, p_2<0.001	t=10.649, p_2<0.001
		t=3.871, p_3=0.006	t=7.223, p_3<0.001	
			t=4.870, p_4=0.002	

Data are presented as mean±SD

p_1: significance versus C-1 group p_1: significance versus C-2 group

p_3: significance versus 4-hr levels in C-2 group p_4: significance versus 4-hr levels in PTX-1 group

Table (2): Mean serum levels of IL-6 estimated at baseline and 4-hours after IP injection of acetic acid(M±SD).

	C-1 group	PTX-1 group	PTX-2 group	C-2 group
Baseline	54±14.2	56.5±12.6	53±15.2	55.5±16.3
4-hrs level		106.7±13.26	85.13±17.12	203.6±55.87
Statistical analysis		t=0.303, p_1>0.05	t=0.101, p_1>0.05	t=0.214, p_1>0.05
		t=6.070, p_2=0.001	t=3.697, p_2=0.008	t=5.437, p_2=0.001
		t=5.437, p_3=0.001	t=6.427, p_3<0.001	
			t=5.142, p_4=0.001	

Data are presented as mean±SD

p_1: significance versus C-1 group p_1: significance versus C-2 group

p_3: significance versus 4-hr levels in C-2 group p_4: significance versus 4-hr levels in PTX-1 group

Table (3): Mean serum levels of IL-10 estimated at baseline and 4-hours after IP injection of acetic acid(M±SD).

	C-1 group	PTX-1 group	PTX-2 group	C-2 group
Baseline	22.6±4.1	24±3.93	23.5±4.63	23±4.9
4-hrs level		38.63±7.48	54.75±13.24	30.75±4.77
Statistical analysis		t=1.494, p_1>0.05	t=0.929, p_1>0.05	t=0.391, p_1>0.05
		t=17.175, p_2<0.001	t=6.378, p_2<0.001	t=10.649, p_2<0.001
		t=6.047, p_3=0.001	t=4.858, p_3=0.002	
			t=6.810, p_4<0.001	

Data are presented as mean±SD

p_1: significance versus C-1 group p_1: significance versus C-2 group

p_3: significance versus 4-hr levels in C-2 group p_4: significance versus 4-hr levels in PTX-1 group

Table (4): Correlation between serum levels of studied cytokines estimated at 4-hr after IP injection and preemptive dose of PTX

	r	p
TNF-α (pg/ml)	-0.761	=0.001
IL-6 (pg/ml)	-0.601	=0.014
IL-10 (pg/ml)	0.575	=0.025

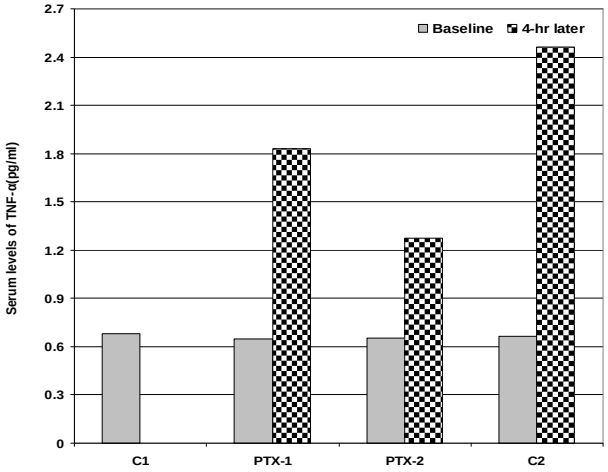

Fig. (1): Mean serum levels of TNF-α estimated at baseline and 4-hr later

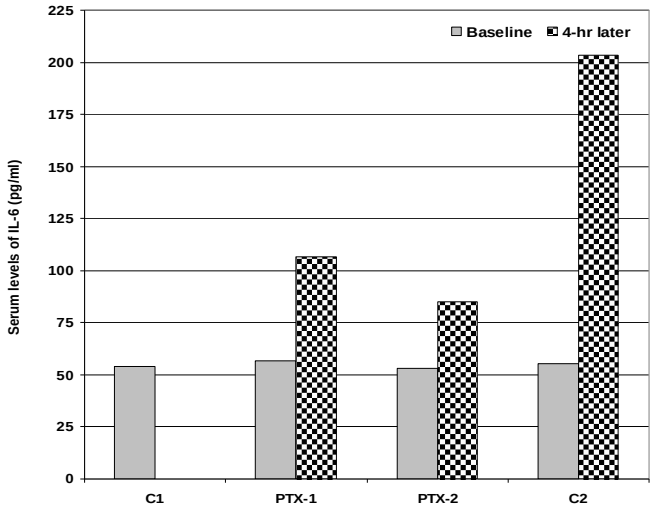

Fig. (2): Mean serum levels of IL-6 estimated at baseline and 4-hr later

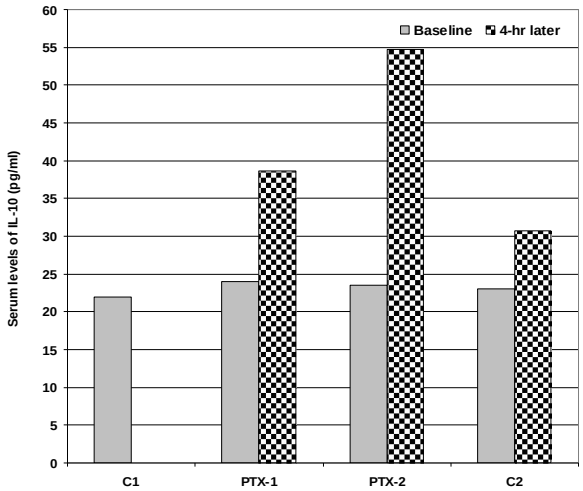

Fig. (3): Mean serum levels of IL-10 estimated at baseline and 4-hr later

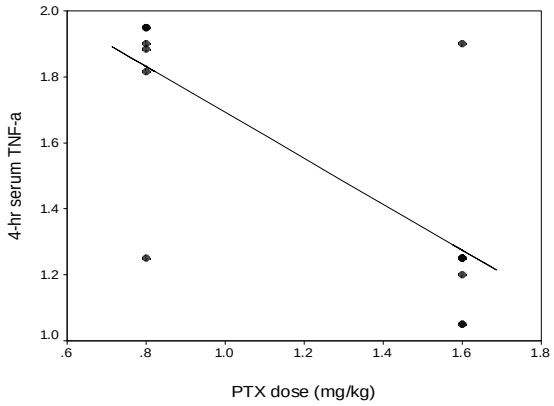

Fig. (4): Correlation between used PTX dose and serum TNF-α at 4-hr after IP injection of acetic acid

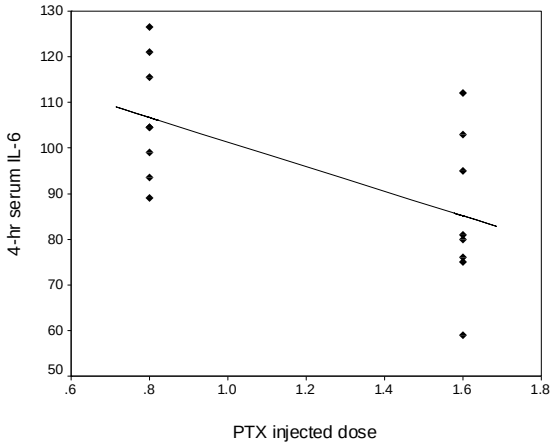

Fig. (5): Correlation between used PTX dose and serum IL-6 at 4-hr after IP injection of acetic acid

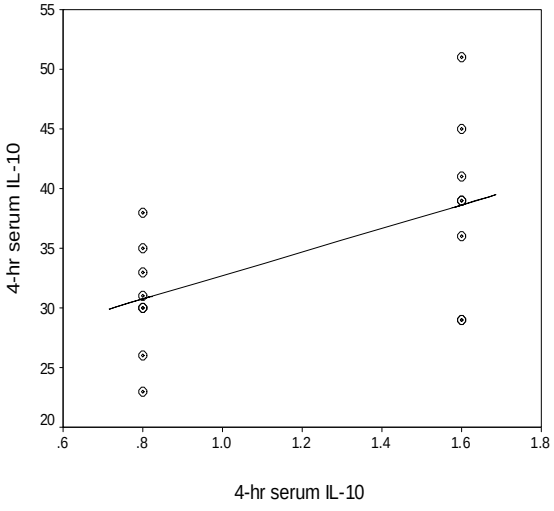

Fig. (6): Correlation between used PTX dose and serum IL-10 at 4-hr after IP injection of acetic acid

4. Discussion

At 4-hrs after IP injection of acetic acid, animals included in PTX groups and C-2 group showed significantly higher serum levels of studied cytokines compared to their own baseline levels. However, the response was accentuated in C-2 group which showed significantly higher serum IL-6 and TNF- **α** and significantly lower IL-10 serum levels compared to PTX groups. These data indicated an ameliorative effect of preemptive PTX injection prior to peritoneal insult on animals immunologic milieu with suppression of pro-inflammatory and accentuation of anti-inflammatory cytokines.

These data go in hand with **Ji *et al.* (2004)** who reported that PTX suppressed the production of proinflammatory cytokines such as TNF- **α** and IL-6 in rat intestine, and enhanced the production of IL-10 and that this suppressive effect of proinflammatory cytokines may act by inhibiting NF-κB activation, but not by induction of IL-10. **Coimbra *et al.*(2004)** reported that PTX when incubated with the whole blood leads to a significant decrease in polymorphonuclear leucocytes oxidative burst and inhibition of oxidative burst occurs distal to protein kinase-C and may be either due to direct inhibition of NADPH oxidase or inhibition of mitogen activated protein kinase phosphorylation, leading to decreased adhesion molecule expression and TNF- **α** synthesis.

Coimbra *et al.* (2005) reported that animals treated with PTX showed a significant reduction in liver injury score and neutrophil infiltration with significantly decreased TNF- **α** and IL-6 concentrations. In addition, a significant decrease in NF-κB-positive staining in hepatocytes and Kuppfer cells was observed in PTX-treated animals compared to the LPS group and concluded that PTX down regulates the inflammatory response significantly and decreases liver injury in acute endotoxemia. **Zhang *et al.*(2007)** found PTX significantly improved hepatopulmonary syndrome without altering portal or systemic hemodynamics and downregulated pulmonary microvascular endothelin B (ET_B) receptor levels and endothelial nitric oxide synthase (eNOS) expression and activation and that these changes were associated with a reduction in circulating TNF levels and NF-κB activation and complete inhibition of Akt activation.

Oliveira-Júnior *et al.* (2008), found pretreatment with PTX improves oxygenation, reduces TNF-α concentration and increases the concentration of corticosterone in bronchoalveolar lavage upon lung lesion induced by HCl in rats. **Nowak *et al.* (2008)** evaluated behavioral activity changes in response to acute and chronic nociceptive stimulus in rats and reported the effectiveness of PTX and concluded that the use of cytokine inhibitors might offer new strategies of drug-resistant chronic pain treatment.

Wei *et al.* (2009), reported that rat's tibial fracture chronically up-regulated TNF-α, IL-1ß and IL-6 mRNA and protein levels in hindpaw skin and PTX treatment significantly reduced the mRNA expression and cytokine protein levels for all these cytokines and concluded that these results suggest that pro-inflammatory cytokines contribute to the nociceptive and vascular sequelae of fracture and that PTX treatment can reverse these complex regional pain syndrome-like changes.

Costantini *et al.* (2009), administration of PTX immediately after 30% total body surface area full-thickness steam burn of male balb/c mice attenuated burn-induced intestinal permeability, decreased the burn-induced phosphorylation of p38 MAPK and decreased phosphorylation of extracellular signal-related kinase (1/2) at 2 and 24 hours after injury with decreased intestinal interleukin-6 levels and concluded that PTX attenuates burn-induced intestinal permeability and subsequent intestinal inflammation and was also associated with decreased acute lung injury; because of its compelling anti-inflammatory effects, PTX may be an ideal candidate for use as an immunomodulatory adjunct to resuscitation fluid

Ji *et al.* (2010), investigated whether a single dose of PTX given immediately following severe remote burn would protect the brain from the injurious effects in rats divided randomly into the sham burn group, burn placebo-treated group and burn PTX-treated group and found PTX substantially suppressed the burn-induced surge in the levels of TNF-α, IL-1ß, and IL-6 in the rat-brain tissues with reduced level of burn-induced apoptosis. PTX also significantly reduced the activation of nuclear transcription factor NF-κB and reduced the activation of glial cells in the brain tissue; **Ji *et al.* (2010)** concluded that an early, single dose of PTX dramatically reduced brain inflammation and apoptosis for up to 16h post-injury

Costantini *et al.* (2010), investigated lung inflammation in male Sprague-Dawley rats underwent 60 minutes of hemorrhagic shock down to a mean arterial blood pressure of 35 mmHg followed by resuscitation with lactated Ringer solution or hypertonic saline solution containing 25 mg/kg PTX and found PTX decreased lung inflammation significantly compared with lactated Ringer through attenuation of NF-κB signaling and increased cAMP response element-binding protein-DNA binding activity and concluded that hypertonic saline-PTX mixture may have therapeutic potential in the attenuation of ischemia-reperfusion injury observed after severe hemorrhagic shock

Through the present study there was a significant difference between animals received 0.8 mg/kg and those received 1.6 mg/kg in favor of PTX-2 group as regards the decreased release of inflammatory cytokines and the increased serum levels of IL-10. Thus, PTX inhibitory effect on cytokine release is dose dependent, this agreed with the experimental results of **Ji *et al.* (2004)** who reported that the inhibiting NF-κB activation is a dose-dependent activity of PTX. In support of this, there was positive significant correlation between used dose of PTX and serum IL-10 and negative significant correlation with serum TNF- **α** and IL-6. **Krakauer *et al.* (2007),** examined the ex vivo cytokine response of superantigen-stimulated whole-blood cells as a first step to therapeutic efficacy testing for bacterial superantigen-induced shock in NHP after oral dosing of pentoxifylline. Doses of 120mg/kg of pentoxifylline effectively attenuated staphylococcal enterotoxin B-induced TNF-α, interferon-γ and IL-2 in ex vivo culture of nonhuman primates whole-blood cells by 88%, 81%, and 76%, respectively, whereas lower doses of 48 or 72mg/kg had no inhibitory effect; thus cytokine release of stimulated peripheral blood cells provides a convenient biological measurement of the anti-inflammatory potency of pentoxifylline which was evident to be dose dependent with lower limit effective dose. **Toda *et al.* (2009)** using male Wistar rats given IP injections of 0.5 ml normal pig serum, which induced rat liver fibrosis in the absence of obvious hepatocyte injury, twice a week for 10 weeks with or without concomitant oral administration of PTX and found PTX prevented the development of fibrosis in this animal model and reduced the serum levels of IL-6 in a dose-dependent manner; in vitro, by the addition of PTX to the culture medium of the rat hepatic stellate cells its proliferation was significantly inhibited with reduced IL-6 in the culture supernatant.

It could be concluded that preemptive administration of pentoxyphylline attenuates release of pro-inflammatory cytokines (IL-6 & TNF- **α**) and stimulates the release of anti-inflammatory cytokines (IL-10) in a dose-dependent fashion after direct exposure to extensive injury and could be recommended as a priming drug prior to surgical interferences especially in immuno-compromised patients.

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References

1. Beutler B, Greenwald D, Hulmes JD, Chang M, Pan YCE, Mathison J, Ulevitch R and Cerami A.,1985. Identify of tumor necrosis factor and the macrophage secreted factor cachectin. Nature; 16: 552-4.
2. Bonnet F and Marret E., 2007. Postoperative pain management and outcome after surgery. Best Pract Res Clin Anaesthesiol.; 21:99-107.
3. Boraschi D, Cifone MG, Falk W, Flad HD, Tagliabue A and Martin MU., 1998. Cytokines in inflammation. Eur. Cytokine Netw., 9: 205–12.
4. Coimbra R, Loomis W, Melbostad H, Tobar M, Porcides RD and Hoyt DB., 2004. LPS-Stimulated PMN Activation and Proinflammatory Mediator Synthesis is Downregulated by Phosphodiesterase Inhibition: Role of Pentoxifylline. J Trauma; 57: 1157-63.
5. Coimbra R, Porcides RD, Melbostad H, Loomis W, Tobar M, Hoyt DB and Wolf P., 2005. Nonspecific phosphodiesterase inhibition attenuates liver injury in acute endotoxemia. Surg Infect.; 6: 75-85.
6. Costantini TW, Deree J, Martins JO, Putnam JG, de Campos T and Coimbra R., 2010. A novel fluid resuscitation strategy modulates pulmonary transcription factor activation in a murine model of hemorrhagic shock. Clinics (Sao Paulo); 65:621-8.
7. Costantini TW, Peterson CY, Kroll L, Loomis WH, Putnam JG, Wolf P, Eliceiri BP, Baird A, Bansal V and Coimbra R., 2009. Burns, inflammation, and intestinal injury: protective effects of an anti-inflammatory resuscitation strategy. J Trauma; 67:1162-8.
8. Dinarello CA: Pro-inflammatory cytokines. Chest 2000, 118: 503-8.
9. Engvall E and Perlmann P., 1972. Enzyme-linked immunosorbent assay. Quantitation of specific antibodies by enzyme-labeled anti-immunoglobulin in antigen-coated tubes. J Immunol.; 109: 129-35.
10. Ferreira SH, Lorenzetti BB, Bristow AF and Poole S., 1988. Interleukin-1 beta as a potent hyperalgesic agent antagonized by a tripeptide analogue. Nature; 334: 698–700.
11. Ji Q, Zhang L, Jia H and Xu J., 2004. Pentoxifylline inhibits endotoxin-induced NF-kappa B activation and associated production of proinflammatory cytokines. Ann Clin Lab Sci.; 34: 427-36.
12. Ji Q, Jia H, Dai H, Li W and Zhang L., 2010. Protective effects of pentoxifylline on the brain following remote burn injury. Burns. 2010 Jun 21. [Epub ahead of print]

13. Kidd BL and Urban LA., 2001. Mechanisms of inflammatory pain. Br J Anesth.; 87 : 3–11.
14. Krakauer T, Stephens J, Buckley M and Tate M., 2007. Superantigen-induced cytokine release from whole-blood cell culture as a functional measure of drug efficacy after oral dosing in nonhuman primates. Res Vet Sci.; 83:182-7.
15. Lee Y, Lee CH and Oh U., 2005. Painful channels in sensory Neurons. Mol Cells; 20: 315-24.
16. Lall RN, Loomis W, Melbostad H, Hoyt DB, Lane T and Coimbra R., 2006. Phosphodi-esterase inhibition attenuates stored blood-induced neutrophil activation: a novel adjunct to blood transfusion. J Am Coll Surg.; 202: 10-7.
17. Lorenzetti BB, Veiga FH, Canetti CA, Poole S, Cunha FQ and Ferreira SH., 2002. Cytokine-induced neutrophil chemoattractant 1 (CINC-1) mediates the sympathetic component of inflammatory mechanical hypersensitivity in rats. Eur. Cytokine. Netw; 13: 456–61.
18. Nowak Ł, Zurowski D, Garlicki J and Thor PJ., 2008. Role of pendoxifiline (PTX) in different and cute and chronic models of pain in rats. Folia Med Cracov.; 49:103-9.
19. de Oliveira-Júnior IS, Maganhin CC, Carbonel AA, Monteiro CM, Cavassani SS and Oliveira-Filho RM., 2008. Effects of Pentoxifylline on TNF-Alpha and Lung Histopathology in HCl-Induced Lung Injury. Clinics; 63: 77–84.
20. Oppenheim JJ., 2001. Cytokines: past, present and future. Int. J. Hematol., 74: 3–8.
21. Opree A and Kress M., 2000. Involvement of the proinflammatory cytokines tumor necrosis factor-a, IL-1b and IL-6 but not IL-8 in the development of heat hyperalgesia: effects on heat-evoked calcitonin gene-related peptide release from rat skin. J Neurosci; 20: 6289–93.
22. Peiga & Barnes, 1964. Evaluation of drug activities. Vol. I, Academic press, 1964.
23. Piper AS and Docherty RJ., 2000. One-way cross-desensitization between P2X purinoceptors and vanilloid receptors in adult rat dorsal root ganglion neurones. J Physiol.; 532.3: 685–96.
24. Ribeiro RA, Vale ML, Thomazzi SM, Paschoalato AB, Poole S, Ferreira SH and Cunha FQ.,2000. Involvement of resident macrophages and mast cells in the writhing nociceptive response induced by zymosan and acetic acid in mice. Eur. J. Pharmacol., 387: 111–8.
25. Taga K and Tosato G.,1992. IL10 inhibits human T cell proliferation and IL2 production. J Immunol.; 148: 1143-8.
26. Toda K, Kumagai N, Kaneko F, Tsunematsu S, Tsuchimoto K, Saito H and Hibi T., 2009. Pentoxifylline prevents pig serum-induced rat liver fibrosis by inhibiting interleukin-6 production. J Gastroenterol Hepatol.; 24:860-5.
27. Vadivelu N, Mitra S and Narayan D., 2010. Recent advances in postoperative pain management. Yale J Biol Med.; 83:11-25.
28. Voisin L, Breuillé D, Ruot B, Rallière C, Rambourdin F, Dalle M and Obled C., 1998. Cytokine modulation by PX differently affects specific acute phase proteins during sepsis in rats. Am J Physiol.; 275: R1412–R1419.
29. Wei T, Sabsovich I, Guo TZ, Shi X, Zhao R, Li W, Geis C, Sommer C, Kingery WS and Clark DJ., 2009. Pentoxifylline attenuates nociceptive sensitization and cytokine expression in a tibia fracture rat model of complex regional pain syndrome. Eur J Pain.; 13:253-62.
30. Zhang J, Ling Y, Tang L, Luo B, Chacko BK, Patel P and Fallon MB., 2007. Pentoxifylline attenuation of experimental hepatopulmonary syndrome. J Appl Physiol.,: 102: 949.

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