STUDY OF THE EFFECT OF PENTOXIFYLLINE IN HEPATIC ISCHEMIA-REPERFUSION INJURY IN ALBINO RATS

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ABSTRACT
Hepatic ischemia reperfusion injury (IRI) is a common pathological process of traumatic surgical disease in the liver, liver transplantation, shock and infection. Inflammatory mediators are implicated in the pathogenesis of IRI. Pentoxifylline (PTX) is a derivative of methylxanthines, acts as a phosphodiesterase inhibitor and thereby elevates the levels of cAMP. Interest in PTX has been recently reawakened because of its reported suppressive action on immune functions, particularly on cytokine production. It has been shown to be beneficial in organ transplantation. Pentoxifylline probably acts primarily by inhibiting tumor necrosis factor-α (TNF-α). We hypothesized that PTX treatment would attenuate hypoxic ischemic liver injury.

Thirty-six male albino rats were used throughout this experiment. Animals were divided into 2 main groups; each comprised 18 rats (sham-operated & IRI groups). Group (1): sham-operated (exposed to anesthesia & laparotomy), this group is subdivided into 3 equal subgroups. Subgroup 1A: Sham-operated received daily intra-gastric saline, subgroup 1B: sham-operated +PTX (8mg/kg/day) for 6 successive weeks before exposure to anesthesia& laparotomy, subgroup 1C: as 1B but received PTX (16mg/kg/day). Group (II): IRI group, divided into 3 equal subgroups, subgroup II A, received intra-gastric saline for 6 weeks before the induction of IRI, subgroup II B, received 8mg/kg/day PTX intra-gastrically for 6 weeks before induction of IRI, subgroup II C,
be beneficial in the treatment of endotoxaemia (8), tumor induced cachexia (9), inflammatory bowel disease (9) and AIDs (10), as well as organ transplantation (11&12). Pentoxifylline probably acts primarily by inhibiting tumor necrosis factor-α (7&13), but other cytokines, such as IL-1B, IL-2, IL-8, IL-10 and transforming growth factor are also implicated (14,15,16,17). This agent additionally reduces leucocytosis and neutrophilia and inhibits the phagocytic activities of monocytes, macrophages and neutrophils (9 &18) as well as degranulation in the later (5). It also modulates fibrinolytic activity, both in vitro and in vivo (19). PTX has been shown in human and animal studies to have a variety of physiological effects at cellular and vascular levels. PTX may either up or down regulates circulating adhesion molecules (20 &21). Interestingly, low doses of methyl -xanthines have been associated with suppression of neutrophil function such as chemotaxis, superoxide anion production, hydrogen peroxide production, deformability, phagocytosis and degranulation (22). Therefore these numerous potential beneficial effects make PTX an interesting modality in treatment of hepatic IRI. Thus we explored the role which PTX may play as an anticytokine therapy in alleviation of hepatic IRI in male albino rats with experimentally induced IRI.

MATERIALS & METHODS
This experiment was carried on 36 male albino rats, weighing 200-240 grams/rat. Animals were having free access to water and food. They were exposed to similar housing conditions of light, heat and humidity.

Drugs used:
Pentoxifylline (Sigma) dissolved in sterile isotonic saline.

Animal grouping:
Animals were divided into 2 groups, each comprised 18 rats (sham-operated & IRI groups). Then each of the two groups subdivided into 3 equal subgroups each consisted of 6 rats as the following:

I- Sham-operated groups:
- Sub-group IA : received intra-gastric saline 0.5ml/day for 6 weeks before anesthesia & laparotomy.
- Subgroup IB : received PTX, intra-gastric in a dose of 8mg/kg/day for
air acetylene flame. Its value was expressed in mg/gram of hepatic tissue.

Statistics:
Statistical analysis of the results was carried out by using the computer system SPSS (Statistical package for social science program; version 10). One-way analysis of variance (ANOVA) was done to compare between the studied groups, followed by Student’s "t" test according to Pipkins (28) to compare between each two means. The quantitative data were presented in the form of Mean ± standard error of means. P value < 0.05 is considered to be significant.

RESULTS
There was non-significant change of all the parameters studied in between sham groups (treated or non-treated by PTX). Experimental induction of hepatic IRI produced a significant increase in the specific liver enzyme ALT and in hepatic calcium contents. Furthermore, plasma TNF-α and plasma MDA showed significant elevation as compared to control (sham-operated) groups. When induction of IRI was preceded by intragastric administration of PTX in the 2 different doses (8 & 16 mg/kg/day) for 6 weeks, the previously mentioned parameters showed non-significant change as compared to sham-operated groups, and significantly decreased as compared to IRI group (table 1 & Fig. 1). However, when induction of IRI was preceded by intragastric administration of PTX in the 2 different doses (8 & 16 mg/kg/day) for 6 weeks, ALT showed significant increase as compared to sham groups and significant decrease as compared to IRI control group (tab.1 & Fig. 2)
Fig (1): Effect of Pentoxifylline (PTX) (8mg & 16/kg/day) on Plasma alanine aminotransferase (IU/L) in Sham and IRI groups. Mean ± SEMs. P< 0.05 , (N=6/group)

**Significant difference between IRI and corresponding sham groups.

*Significant difference between (IRI + PTX treated) and (IRI + saline) group.

Fig (2): Effect of Pentoxifylline (PTX) (8mg & 16/kg/day) on plasma malondialdehyde (MDA), Tumor necrosis factor (TNF-α) and hepatic calcium content in Sham and IRI groups. Mean ± SEMs. P< 0.05 , (N=6/group)

**Significant difference between IRI and corresponding sham groups.

*Significant difference between (IRI + PTX treated) and (IRI + saline) group.
ter reperfusion in a rat model of hepatic ischemia, and the levels correlated with the duration of ischemia. Further studies have shown that TNF-α mediates remote organ injury after prolonged ischemia injury to the liver (42&43). The present study demonstrated that PTX effectively inhibited the production of TNF-α in rats with induced hepatic IRI. These findings are consistent with Raetsch et al. (3) as he reported that inhibition of these cytokines by PTX evidently occurs at transcriptional level and can last for 5 days after the final PTX dose. In addition, Van Furth et al. (13), reported that PTX decreased bacterial-stimulated production of TNF-α by human leukocytes. Moreover, PTX blocks nuclear factor kappa B (NFKB) activation in stimulated kupffer cells which explains its activity in suppressing NFKB-dependent synthesis and release of tumor necrosis factor alpha (42&43).

In the present study, induction of hepatic IRI produced a significant increase in hepatic tissue calcium content, plasma MDA (index of lipid peroxidation), these findings are in agreement with previous studies as they proved that alteration of calcium homeostasis play a major role in cell necrosis (1). It has been demonstrated that increased intracellular concentration of calcium causes damage to hepatocytes (44). Furthermore, from experimental and clinical studies, it has become apparent that oxidative stress induced from increased free radical production plays a role in the pathogenesis of ischemic tissue injury (29). Among the underlying mechanisms through which increased calcium causes damage is the enhanced production and accumulation of toxic free radicals (33, 39). In the present study PTX pretreatment before induction of IRI prevent the increase in MDA. These findings were supported by previous studies (45, 46). They reported that low dose of methylxanthines have been associated with suppression of neutrophils function such as chemotaxis, superoxide anion production, hydrogen peroxide production, deformability, phagocytosis and degranulation. In addition, it has been documented that with the use of hepatitis A or B running a moderate course, PTX produced a positive action on lipid peroxidation; liver size and Jaundice


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دراسة تأثير إعطاء دواء البنتوكسيفين على الإصابة التي تحدث في كبد الفئران نتيجة تعرضها لقصور في الدورة الدموية بالكبد ثم إعادة ضخ الدم

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يعتبر قصور الدورة الدموية بالكبد عامل مشترك في العديد من الحالات التي تسبب الكبد. تلعب وسائط الالتهابات مثل معامل تحلل الأورام التاثير الشقوق الحرة وكذلك زيادة معدل الكالسيوم في خلايا الكبد دوراً هاماً في الإصابة الناتجة عن قصور الدورة الدموية الكبدية.

لقد أجريت هذا البحث لدراسة احتمال وجود تأثير وقائي لعقار البنتوكسيفين على الإصابة المحدثة عملياً في كبد الفئران البيضاء بسبب قصور الدورة الدموية به ثم استعادة وصول الدم بعد فترة.

تم إجراء هذا البحث على عدد 32 فأراً أبيضًا من الذكور يتراوح وزنها بين 200-240 جرام، قسمت إلى مجموعتين أساسيتين تتكون كل منها من 16 فأراً، الأولى قياسية غير معرضة إلى قصور في الدورة الدموية الكبدية، والثانية عرضت لقصور بالدورة الدموية لمدة 10 دقائق ثم استعادة وصول الدم لمدة 30 دقيقة.

تم تقسيم كلاً من هاتين المجموعتين إلى ثلاث تحت مجموعات كل منها يتكون من 6 فأراً، الأولى غير معالجة دوائياً وعولجت بمحلول متماثل، والثانية معالجة بالبنتوكسيفين بجرعة 8 مجم/كجم يومياً كما عولجت المجموعة الثالثة بجرعة أكبر من البنتوكسيفين 16 مجم/كجم يومياً وذلك عن طريق الفم لمدة 6 أسابيع متتالية.

أحذت القصور الدموية الكبدية في الفئران المخدرة بفلك الحزمة الوعائية الدموية الداخلية للكبد لمدة 90 دقيقة متبوعة بفترة 20 دقيقة استعادة للدورة الدموية وذلك برفع الفلك عن الحزمة.

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