Pretreatment with pentoxifylline attenuates lung injury induced by intestinal ischemia/reperfusion in rats

Pré-tratamento com pentoxifilina atenua a lesão pulmonar induzida por isquemia/reperfusão intestinal em ratos


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ABSTRACT

PURPOSE: To investigate the protective effect of pentoxifylline against the lung injury observed after intestinal ischemia (I) followed by a period of reperfusion (R).

METHODS: Twenty-eight male Wistar rats were equally divided into 4 experimental groups and operated under ketamine-xylazine anesthesia. (1) Sham: falsely-operated animals; (2) SS+IR: intestinal ischemia was accomplished by clipping the superior mesenteric artery during 60 minutes, with an administration of a standard volume of saline solution (SS) 5 min before the end of the ischemia period; the clip was then released for a 120-min period of reperfusion; (3) I+PTX+R: ischemia as above, PTX was administered (25 mg/kg) and the gut reperfused as above; (4) PTX+I+PTX+R: Five minutes before arterial occlusion PTX was administered; the superior mesenteric artery was then clipped for 60 minutes. After 55-min ischemia, an additional dose of PTX was administered; the clip was removed for reperfusion as above. At the 60th min of reperfusion a third dose of PTX was administered.

RESULTS: PTX markedly attenuated lung injury as manifested by significant decreases (all P<0.001 as compared with the SS+IR group) of pulmonary wet/dry tissue weight ratio, total protein content, myeloperoxidase activity and tumor necrosis factor-alpha. Moreover, it was apparent that in the group PTX+I+PTX+R the improvements have been even more significant.

CONCLUSION: PTX exerted a protective effect on the lung from the injuries caused by intestinal ischemia/reperfusion.


RESUMO

OBJETIVO: Avaliar os efeitos protetores da pentoxifilina (PTX) na lesão pulmonar observada após isquemia (I) seguida de reperfusão (R) intestinal.

MÉTODOS: Vinte e oito ratos machos foram divididos aleatoriamente em quatro grupos experimentais e operados sobre anestesia quetamina-xilazina. (1) Sham: animais falsamente operados; (2) SS+IR: isquemia intestinal realizada pelo clampeamento da artéria...
mesentérica superior durante 60 minutos, com a administração de solução salina (SS) 5 minutos antes do período de isquemia, após a retirada do clamp houve a reperfusão por mais 120 minutos; (3) I+PTX+R: isquemia como mencionado anteriormente seguida da administração de PTX (25 mg/Kg) 5 minutos antes do final da isquemia (60 minutos) seguida de reperfusão por mais 120 minutos; (4) PTX+I+PTX+R: 5 minutos antes da isquemia foi administrado PTX, após 55 minutos de isquemia foi administrado outra dose de PTX e a reperfusão mantida por mais 120 minutos, sendo que aos 60 minutos da reperfusão outra dose de PTX foi administrada.

RESULTADOS: A pentoxifilina reduziu os marcadores de lesão pulmonar (proteínas totais, malondialdeído, atividade da mieloperoxidase e fator de necrose tumoral) quando comparada com o grupo não tratado (P<0.001), contudo esta redução foi mais significante no grupo PTX+I+PTX+R.

CONCLUSÃO: A pentoxifilina exerce efeito protetor no pulmão no trauma causado por isquemia/reperfusão intestinal.

reperfusion. Group I+PTX+R: ischemia procedure as above and, 5 minutes before reperfusion, PTX was administered (25 mg/kg in 0.1 mL). Group PTX+I+PTX+R: pre-treatment with PTX 5 minutes before ischemia, then another PTX dose 5 minutes before the end of ischemia and a final dose of PTX at the 60th minute of the reperfusion period. Body temperature was maintained to 37°C (37 ± 0.6°C) with a heating pad.

At the end of experiment the animals were euthanized (T-61 Euthanasia Solution®, Schering-Plough, SP, Brazil).

**Bronchoalveolar lavage (BAL) fluid for protein assay**

Upon thoracotomy, bronchoalveolar lavages (BALs) were taken by flushing three times the right lung with sterile saline via an intratracheal cannula, using a Miniplus 3® peristaltic pump (Gilson, WI, USA). The recovery rate was >85%. The collected BALs were centrifuged at 300 x g for 4 min at 4°C and the supernatant was used for protein determination by the method of Lowry et al.27.

The upper lobe of right lungs was removed and homogenized (Thorton, INPC, SP, Brazil) in PBS for further myeloperoxidase (MPO) activity, thiobarbituric reactive substances-malondialdehyde (TBARS-MDA) and tumor necrosis factor-alpha (TNF-α) measurements.

**Determination of lipid derived oxidation products**

The thiobarbituric acid reactive substance-malondialdehyde (TBARS-MDA) content of lung tissue was determined using the method described by Ohkawa et al.28.

**Myeloperoxidase (MPO) activity**

Homogenates were centrifuged at 15,000 x g for 10 min at 4°C. A 100 mL-aliquot of supernatant was mixed with 900 mL of 50 mmol·L⁻¹ phosphate buffer (pH= 6.0) containing 0.167 mg·mL⁻¹ of o-dianisidine dihydrochloride and 0.0005% hydrogen peroxide. One unit of peroxidase activity is taken as the amount of enzyme decomposing 1 mmol of hydrogen peroxide per minute at 25°C. Decomposition of hydrogen peroxide was calculated from the oxidation of o-dianisidine by using an absorption coefficient of 11.3 mmol·L⁻¹·cm⁻¹ at 460 nm29.

**Cytokine assay**

Tissue TNF-α content was measured in lung homogenates using commercially available, rat-specific enzyme immunoassay (ELISA) kits (Quantikine, R&D Systems, MN, USA). Results were expressed as pg/mL. Absorbance was determined at 450 nm using a microplate reader (Bio-Tek Instruments, VT, USA).

**Morphology**

The middle lobe of the right lung was fixed in buffered formalin. After embedding in paraffin, 4-μm sections of the tissues were stained with hematoxylin-eosin (HE) for light microscopy coupled to a video camera (Axiolab Standart 2.0 and AxionCam, Zeiss, Jena, Germany, respectively). The slides were evaluated blindly by an independent consultant histopathologist.

**Wet to dry tissue weight ratio**

The lower lobe of the right lung was isolated and immediately weighed (wet weight) before being dried for 48 h at 80°C and weighed again (dry weight). The wet/dry weight ratio was then calculated.

**Statistical analysis**

Statistical analyses were carried out with a SPSS 11.0 statistical software (SPSS Inc. Software, IL, USA). Data were analyzed by 1-way analysis of variance (ANOVA) with a LSD post-hoc test to determine comparisons and differences between groups, respectively. All values are expressed as mean ± standard deviation with P<0.05 being considered significant.

**Results**

No deaths were recorded throughout the experiment. The protein content in BALs (Figure 1) increased in the group SS+IR (62.71 ± 4.61 mg/mL), and decreased in the groups treated with PTX (I+PTX+R: 50.14 ± 3.93 mg/mL; PTX+I+PTX+R: 36.28 ± 3.35 mg/mL).
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The lipid peroxidation (TBARS-MDA; Figure 2) was significantly higher in the control IR group (SS+IR: $62.14 \pm 4.22$ nmol/mg protein) as compared with the I+PTX+R ($52.28 \pm 2.63$ nmol/mg protein) and the PTX+I+PTX+R ($43.42 \pm 1.27$ nmol/mg protein) groups.

Myeloperoxidase activity (MPO; Figure 3) was lower in the group PTX+I+PTX+R ($30.71 \pm 2.81$ U/mg protein) when compared with SS+IR ($47.43 \pm 2.15$ U/mg protein) and I+PTX+R ($40.43 \pm 3.21$ U/mg protein).

The levels of TNF-alpha (Figure 4) in lung homogenates were higher in all groups when compared with those of the Sham group. The has been an increase of TNF-alpha levels in the group SS+IR ($67.14 \pm 6.82$ pg/mL), and a reduction in PTX-treated groups, I+PTX+R ($55.86 \pm 2.27$ pg/mL) and PTX+I+PTX+R ($47.57 \pm 2.64$ pg/mL).

Figure 5 shows typical lung histological features from...
both normal and sham-operated [Sham] rats. Lungs from the group saline solution plus ischemia-reperfusion [SS+IR] showed severe changes. As seen, there was a marked leukocyte (neutrophil) infiltration; the alveolar, perivascular, and capillary dilation lead to alveolar capillaries wall thickening. These parameters in the groups I+PTX+R and PTX+I+PTX+R were all significantly improved.

Lung weights (W/D ratios) in Sham group (3.62 ± 0.31) were not altered after the 120-min experimental period. The SS+IR group had a significantly higher ratio (6.16 ± 0.26) than that in the Sham group. This parameter significantly decreased in the groups I+PTX+R (5.34 ± 0.25) and PTX+I+PTX+R (4.5 ± 0.35) when compared with SS+IR.

Discussion

Our current study showed that I/R caused acute lung injury, as reflected by increases in protein concentration, MDA levels and MPO activity in the lung homogenates, W/D ratio, and TNF-alpha levels in lung homogenate and gene expression in lung tissue.

Multiple factors contribute to I/R-induced inflammatory responses, including complement activation, reactive oxygen species (ROS), cytokines, and chemokines. The pulmonary vascular endothelium is a barrier to prevent fluids and proteins into

FIGURE 5 - Histological lung sections of lungs from sham-operated rats [Sham] show a normal pulmonary architecture. In the group SS+IR it can be seen massive alveolar congestion and neutrophil infiltration. In the groups treated with pentoxifylline, (I+PTX+R) and (PTX+I+PTX+R) the interstitial congestion was minimal, and the leukocyte (neutrophil) infiltration was strongly reduced. Hematoxylin and cosin staining. Scale bar: 20 µm. (H.E - 200X).

Intestinal I/R injury causes local damage as well as remote organ injury and dysfunction. The pathophysiology of I/R injury is complex, the induction of a systemic proinflammatory cytokine response is believed to play a major role, and TNF-alpha is among the most important contributors. In a elegant study conducted by Câmara-Lemarroy et al., showed that intestinal I/R-induced lung injury was associated with elevated levels of circulating serum TNF-alpha, and the treatment of one dose of PTX reduced the intensity of injury and the tissue edema. Lung cytokine levels are actually determined by the amount of cytokines released from a variety of cell populations, including alveolar macrophages, endothelial cells, and epithelial cells, and these multiple sources mean that reduced neutrophil infiltration of the lungs may not directly translate to a decreased level of cytokines in the lung.

Our studies corroborated with these authors and expanded of the result. In our study, the TNF-alpha levels in homogenate lung tissue were higher in the saline-treated group (SS+IR) and reduced in the group I+PTX+R, but the decreased was more significant in the group treated with three doses of pentoxifylline (PTX+I+PTX+R).

Morphological examination indicate the severe impact in the lung tissue submitted to I/R non-treated, by other hand the treatment with PTX reduced the lung damage, especially in the group treated with three doses of PTX.

Our study demonstrated that pentoxifylline had preventive effects and therapeutic potential in I/R-induced lung injury, but our results must be considered carefully. The clinical relevance of this manuscript refers to the previous use of pentoxifylline in situations requiring procedures ischemia with reperfusion to reduce or prevent distant organs damage. Therefore, the long-term effect of pentoxifylline warrants further investigation.

In conclusion, our study showed that pentoxifylline decreased lung edema, ameliorated lung histological change, reduced the production of inflammatory mediators, and neutrophils in intestinal I/R-induced lung injury. Pentoxifylline has been given
safely in previous clinical studies. Therefore, the administration of pentoxifylline may be a useful prophylactic or adjunct drug therapy for intestinal I/R-induced lung injury.

References


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Received: May 11, 2011
Review: July 12, 2011
Accepted: August 15, 2011
Conflict of interest: none
Financial source: FAPESP

1Research performed at Department of Surgery, Department of Morphology and Genetics, Federal University of Sao Paulo (UNIFESP), Brazil. Part of PhD degree thesis. Mentor: Itamar Souza Oliveira-Júnior.