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Brief communication (Original)

Fenofibrate protects the intestine against ischemia/ reperfusion injury

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Background: Ischemia/reperfusion (I/R) injury is a potentially devastating condition, associated with a systemic inflammatory response. It occurs during shock, transplant procedures, or vascular surgery.

Objective: We evaluated the protective effects of Fenofibrate (FEN) over intestinal I/R injury.

Materials and methods: Intestinal I/R was induced in male Wistar rats by clamping the superior mesenteric artery for 60 minutes, followed by 60 minutes of reperfusion. Rats either received saline or FEN (100 mg/k, via gavage) daily, for three days before inducing I/R. Sham operated rats were used as normal controls. At the end of the procedure, tissue and blood samples were obtained. Serum concentrations of AST, ALT, LDH, tumor necrosis factor-alpha (TNF-alpha), malonaldehyde (MDA), and total antioxidant capacity (TAC) were determined. A histopathological analysis was also performed.

Results: After I/R, there was evident tissue injury, as well as serum elevations of AST, ALT, and LDH concentrations. These alterations were reduced by FEN treatment. TNF-alpha concentrations were increased in saline treated animals when compared with FEN treated group (2.26±1 ng/ml vs. 0.23±0.41 ng/ml, respectively, $p < 0.05$). A similar pattern was observed in MDA levels (7.42±1.72 μM/ml vs. 1.72±0.61 □M/ml, respectively, $p < 0.05$). TAC was reduced in saline treated animals (2.05±0.36 Trolox-Equivalents), but preserved in the FEN treated group (3.08±0.36 Trolox-Equivalents, $p < 0.05$).

Conclusion: FEN reduced intestinal I/R injury, probably due to anti-inflammatory and antioxidant properties. Its usefulness as a treatment for I/R should be studied.

Keywords: Fenofibrate, injury, intestine, ischemia/ reperfusion, rat

Ischemia/reperfusion (I/R) injury is a potentially serious consequence of transplant surgeries, shock or vessel occlusion. I/R injury is mediated by multiple mechanisms such as production of reactive oxygen species, inflammatory cell infiltration, and cytokine production [1, 2]. It has been recognized that local organ I/R also induces a systemic inflammatory response that can adversely alter the clinical course [3]. Rodent models of intestinal I/R are useful in order to study the physiopathological mechanisms of I/R injury as well as in the search for much needed novel protective strategies [1].

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Peroxisome proliferator-activated receptors (PPARs) are a group of nuclear receptors known to regulate the expression of inflammatory mediator genes, and the modulation of PPARs has been proposed as a strategy to reduce I/R injury [4]. Fenofibrate (FEN) is a drug of the fibrate class, used to treat dyslipidemia and know to be a PPAR-alpha activator. PPAR-alpha activation, using pharmacological ligands, has been shown to reduce I/R injury in the liver, heart, and kidney in experimental models [5-7]. In a recent study, FEN was shown to reduce I/R-induced renal injury, inflammation, and dysfunction [8]. To the best of our knowledge, this is the first study to investigate the effects of FEN over intestinal I/R.

Materials and methods

Animal procedures were performed in accordance with the proper use and care of laboratory animals, approved by the ethics committee of our institution. Experiments were performed on 15 male Wistar rats weighing 200 to 250 g. Animals were maintained under standard conditions, such as stable room temperature (24±3^{O}C), a 12 hours light/12 hours dark cycle, and access to commercial rat pellets and water *ad libitum.* Briefly, after pentobarbital sodium anesthesia (Anestesal, Pfizer Inc, Mexico) (35 mg/kg, i.p), midline laparotomy was performed. Animals were placed under heating lamp in order to preserve core body temperature at (37^{O}C). Ischemia was induced by cross-clamping the superior mesenteric artery for 60 minutes (ischemia), and removed to allow 60 minutes of reperfusion.

Rats were divided into three groups (n = 5). 1) a sham-operated group (Sham), where laparotomy was performed but intestines were only manipulated but not made ischemic. 2) a control (IR) group was subjected to I/R as described above, and 3) a FEN group, that received 100 mg/kg of FEN daily, via gavage, for three days before I/R.

Tissue examination

Immediately after concluding the reperfusion period, rats were sacrificed by exsanguination from the aorta and intestinal tissue samples were obtained. The Samples were then fixed in 10% neutral buffered formalin overnight, embedded in paraffin, and 4 µm-thick sections were stained with hematoxylin and eosin (H/E) and examined under light microscope by a blinded pathologist. The Chiu scale (9) of mucosal injury was used to evaluate the degree of histological alteration on 10 sections of 1 mm each to complete 1cm per animal. The scale consists of values from of 0 to 5, where 0 normal mucosa; 1 development of sub epithelial (Gruenhagen's) spaces, 2 extension of the sub epithelial space with moderate epithelial lifting from the lamina propria, 3 extensive epithelial lifting with occasional denuded villi tips, 4 denuded villi with exposed lamina propria and dilated capillaries, and 5 disintegration of the lamina propria, hemorrhage, and ulceration.

Serum analysis

Blood samples were used to determine serum levels of AST, ALT, and LDH by standard biochemical automated methods, using commercially available kits and DT6011 and DTSC11 analyzers (System Vitros Chemical, Johnson and Johnson, USA). Serum concentrations of TNF-alpha were determined using a rat ELISA kit (PeproTech, Mexico). Lipid peroxidation, expressed as malonaldehyde (MDA) levels, was assessed by the TBARS method using a commercially available colorimetric assay kit (Cayman chemicals, USA). Total serum antioxidant capacity (TAC) was assessed using a commercially available colorimetric assay kit (Cayman chemicals, USA). TAC values are expressed as Trolox Equivalents (TEs).

Statistical analysis

SPSS 11.0 statistical software (SPSS Inc. Software, Chicago, Illinois, USA) was used to analyze data using one-way analysis of variance (ANOVA) and with LSD post-hoc test so as to determine comparison between groups, and differences between groups, respectively. All values are expressed as mean±SD and p <0.05 was considered statistically significant.

Results

ALT, AST and LDH

AST values were significantly elevated after I/R in group IR compared to sham group (339.2±78.11 U/L vs. 145.25±12.1 U/L, p <0.05). FEN pretreatment maintained AST levels within sham group values (199.2±20.37 U/L) as shown in **Table 1**, significantly lower than in IR group (p <0.05). LDH levels in group IR were significantly increased compared to sham group (10309.40±2240.16 U/L vs. 6080.75±1042.55 U/L, p <0.05. Group FEN had 76774±1077.76 U/L, not different from sham group. A similar trend was observed in ALT values. After I/R, ALT values were increased in IR group compared to sham group (192±82.1 U/L vs. 52.5±11 U/L, p <0.05). FEN also reduced serum I/R-induced ALT elevations (113.8±2086 U/L, p <0.05).

Morphological examination

In sham group, normal intestinal mucosa was observed in all samples. In IR group severe mucosal injury was evident after I/R, reaching a Chiu score of 4.2±0.44. In FEN group there was some injury but it was significantly less than in IR group (Chiu score: 2±1, p <0.05).

Table 1. Chiu score and serum biochemical parameters: AST, LDH, and ALT levels.

Group	Chiu score	AST (U/L)	LDH (U/L)	ALT (U/L)
Sham	1±0	145.25±12.1	6080.75±1042.55	52.5±11
IR	4.2±0.44*	339.2±78.11*	10309.40±2240.16*	192±82.1*
FEN	2±1**	199.2±20.37**	76774±1077.76**	113.8±2086**

* = p <0.05 vs. Sham, ** = p <0.05 vs. IR

Serum TNF-alpha

TNF-alpha levels remained undetectable in sham group. In IR group TNF-alpha levels reached 2.26±1 ng/ml. FEN group had lower values (0.23±0.41 ng/ml, p <0.05).

MDA and TAC

Sham group had MDA concentrations of 2.56±0.83 μM/ml. In IR group, MDA levels were significantly increased (7.42±1.72 μM/ml, p <0.05). FEN (1.72±0.61 μM/ml) significantly reduced MDA levels compared to IR group after I/R (p <0.05) as shown in **Figure 1**. Sham group serum TAC was 2.75±0.19 TEs. In IR group, serum antioxidants were significantly depleted (2.05±0.36 TEs, p <0.05). FEN (3.08±0.36 TEs) prevented the depletion of serum TAC compared to IR group after I/R (p <0.05).

Discussion

Intestinal I/R caused severe injury to the mucosa, as evidenced by morphological alterations and elevations in serum markers of tissue injury such as AST and ALT. We also found evidence of concomitant liver damage, as evidenced by elevations of ALT. FEN was able to reduce these alterations, as well as the resulting elevations in TNF-alpha, an essential participant in the physiopathology of I/R injury [10]. FEN has been shown to inhibit the production of TNF-alpha *in vitro,* in cultured lipopolysaccharide-stimulated cells, through PPAR-alpha modulation [11]. Fenofibrate and other PPAR-alpha activators also reduce TNF-alpha levels in models of airway inflammation and hepatic I/R *in vivo* [5, 12]. PPAR-alpha inhibition with fibrates also reduces nuclear factor kappaB activation in cultured endothelial cells, a transcription factor that induces the production of TNF-alpha and other inflammatory cytokines [13]. In human metabolic-syndrome studies, FEN has also been proven to reduce plasma levels of TNF-alpha, among other inflammatory markers [14]. PPAR-alpha modulation of inflammatory cytokine genes probably explains the reduced levels of TNF-alpha we observed in the FEN treated group in our study.

Intestinal I/R induces oxidative stress and a decrease in serum TAC [1, 2, 15]. FEN treatment was also associated with a preservation of serum antioxidant capacity and reduction in serum MDA levels in our study. This suggests that FEN acts also

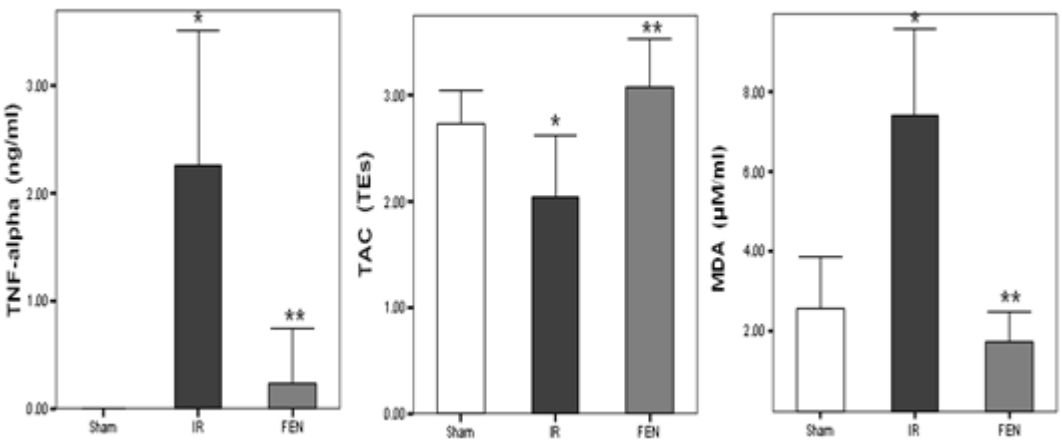

Figure 1. Serum concentrations of TNF-alpha, TAC, and MDA. * = p <0.05 vs. Sham, ** = p <0.05 vs. IR.

through anti-oxidant pathways. PPAR-alpha plays an important role in the physiological and pathological responses involving oxidative stress and antioxidant enzymes [16]. Indeed, FEN treatment in intact rats reduces lipid peroxidation, MDA production, and favorably modulates the oxidant-antioxidant balance [17]. In rodent models of acute injury (traumatic brain injury, as well as hepatic I/R injury), FEN also reduced the serum markers of oxidative stress [5, 18]. The antioxidant properties of FEN have also been studied in human patients with dyslipidemia, where reduced levels of lipid peroxidation and increases in antioxidants have been found in serum [19].

Conclusion

We have shown that FEN protects against I/R injury probably in virtue of its anti-inflammatory and antioxidant properties. The possibility of using fibrates in the treatment of pathologies involving I/R warrants consideration.

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