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Rapid communication

Endogenous ligands of PPAR-γ reduce the liver injury in haemorrhagic shock

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Abstract

We demonstrate here for the first time that the novel, potent peroxisome proliferator-activated receptor (PPAR)-γ antagonist GW9662 (2-chloro-5-nitrobenzanilide) augments the degree of liver injury associated with haemorrhagic (haemorrhage for 90 min and resuscitation for 4 h), but not endotoxic (6 mg/kg *E. coli* endotoxin i.v. for 6 h) shock in the anaesthetised rat. Thus, endogenous ligands for PPAR-γ are released in haemorrhagic, but not endotoxic, shock in sufficient amounts to protect against injury.

Keywords: Shock; PPAR (peroxisome proliferator-activated receptor); GW9662

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor superfamily of ligand-activated transcription factors that are related to retinoid, steroid, and thyroid hormone receptors. The PPAR-γ receptor subtype seems to play a pivotal role in the regulation of cellular proliferation and inflammation. Recent evidence also suggests that the cyclopentenone prostaglandin 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2, which is a metabolite of prostaglandin D_2, functions as an endogenous ligand for PPAR-γ (Ricote et al., 1998). We have recently discovered that 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 reduces the tissue injury caused by ischaemia–reperfusion (Wayman et al., 2002; Chatterjee et al., in press), septic shock (Collin and Thiemermann, 2003) and inflammation (Cuzzocrea et al., 2002). This raises the questions as to whether endogenous ligands of PPAR-γ are produced in pathological states in sufficient amounts to protect our tissues and organs against further injury. Until very recently, it was not possible to investigate this question, as potent and selective antagonists for PPAR-γ were not available.

Here we report for the first time that novel, potent PPAR-γ antagonist GW9662 (2-chloro-5-nitrobenzanilide) (Leesnitzer et al., 2002) augments the degree of liver injury associated with haemorrhagic, but not endotoxic, shock in the anaesthetised rat.

Male Wistar rats were anaesthetised with thiopentone sodium (120 mg/kg i.p.) and subjected to either an injection of endotoxin (6 mg/kg i.v., *E. coli* endotoxin serotype 0127:B8) or haemorrhage (blood withdrawal from the carotid artery to reduce blood pressure to 45 mm Hg for 90 min) and resuscitation (with shed blood for 4 h). All rats were instrumented for the measurement of systemic haemodynamics. A blood sample was taken at the end of the respective experiments in order to determine the serum levels of the aspartate transaminase and alanine transaminase. Rats were treated with either GW9662 (1 mg/kg i.v. at 45 min prior to endotoxin or onset of haemorrhage) or its vehicle (10% dimethyl sulphoxide). Data were analysed by one-way (or two-way) analysis of variance (ANOVA) followed by a Dunnett's post hoc test for multiple comparisons.

Injection of endotoxin caused a fall in mean arterial blood pressure from baseline of 133 ± 6 to 87 ± 5 mm Hg at 6 h ($P < 0.05$). Endotoxaemia for 6 h also resulted in a significant increase in the serum levels of aspartate transaminase and alanine transaminase indicating the development of liver injury (Fig. 1B). Pretreatment of rats with the PPAR-γ antagonist GW9662 did not affect the liver injury (Fig. 1B) or the hypotension (from baseline of 135 ± 4 to 89 ± 9 mm Hg at 6 h), caused by endotoxin in the rat.

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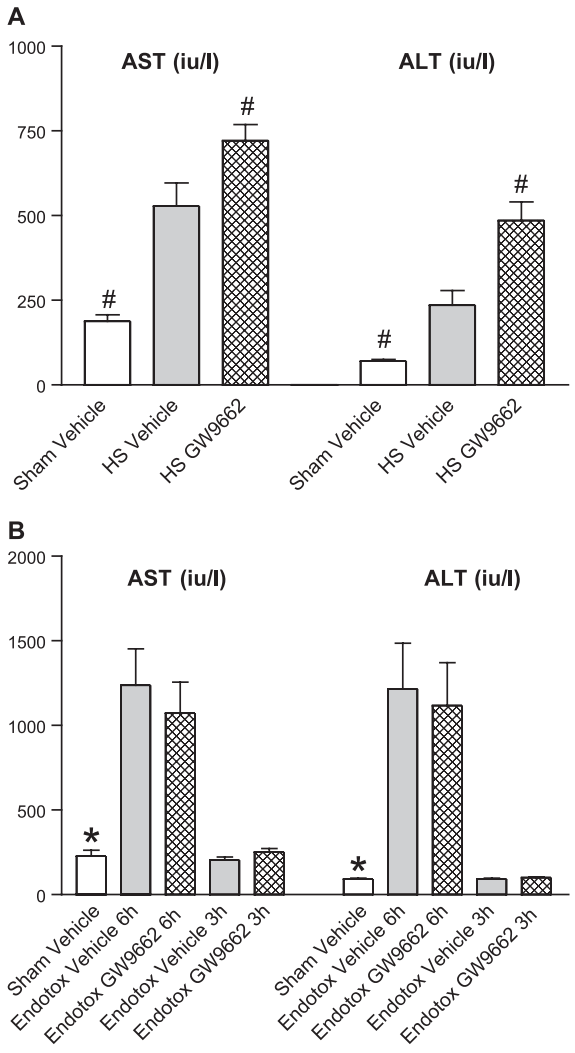

Fig. 1. Serum levels of aspartate transaminase (AST) and alanine transaminase (ALT) in rats subjected to either (A) haemorrhage and resuscitation with shed blood (haemorrhagic shock, HS) or (B) endotoxin (6 mg/kg i.v.) for either 6 or 3 h. The sham-operated animals were subjected to the surgical procedure alone and treated with saline (Sham HS Vehicle ($n=6$) and Sham Endotox Vehicle ($n=10$), 1 ml/kg i.v.). Control rats were treated (45 min prior to haemorrhage or endotoxin) with vehicle (HS Vehicle ($n=5$) or Endotox Vehicle (for 6 h $n=10$, for 3 h $n=6$), 10% dimethyl sulphoxide, 1 ml/kg i.v.) or GW9662 (HS GW9662 ($n=7$) or Endotox GW9662 (for 6 h $n=10$, for 3 h $n=6$), 1 mg/kg i.v.). $^{\#}P<0.05$ when compared with HS Vehicle by ANOVA followed by Dunnett's post hoc test or $^{*}P<0.05$ when compared with Endotox Vehicle by ANOVA followed by Dunnett's post hoc test.

Haemorrhage and resuscitation also resulted in a delayed fall in blood pressure (from baseline of 129 ± 2 to 65 ± 6 mm Hg at 4 h resuscitation, $P<0.05$) as well as liver injury (increases in the serum levels of aspartate transaminase and alanine transaminase) (Fig. 1A). Most notably, pretreatment of rats with the PPAR-γ antagonist GW9662 significantly enhanced the degree of liver injury (Fig. 1A), but not the circulatory failure associated with haemorrhagic shock (from baseline of 132 ± 2 to 73 ± 4 mm Hg at 4 h resuscitation). In sham-operated animals, GW9662 had no effect on aspartate transaminase and alanine transaminase or blood pressure (data not shown).

Thus, this study demonstrates for the first time that the novel, potent PPAR-γ antagonist GW9662 augments the degree of liver injury associated with haemorrhagic shock in the anaesthetised rat. This finding, therefore, indicates that haemorrhage and resuscitation results in the release of endogenous ligands of PPAR-γ. Moreover, the amounts of these ligands released are sufficient to protect the liver against the organ injury associated with haemorrhagic shock. We also report that in severe endotoxaemia endogenous PPAR-γ ligands are not released in amounts that are sufficient to protect the liver against further injury. One could argue that the degree of liver injury caused by endotoxic shock is too large to observe a further amplification by the PPAR-γ receptor antagonist. However, we also show that GW9662 does not increase the degree of liver injury (rise in transaminases) caused by 3 h of endotoxemia, which only caused a very small (if any) rise in aspartate transaminase and alanine transaminase (Fig. 1B). Thus, we believe that it is unlikely, but not impossible, that endogenous ligands of PPAR-γ reduce the liver injury associated with endotoxic shock.

The pathophysiology of haemorrhage and resuscitation (unlike the one of endotoxic shock) has a strong element of 'ischaemia–reperfusion' type injury and it is known that arachidonic acid is released and, hence, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 is produced, during ischaemia and reperfusion (Hercule and Oyekan, 2002). Whether prostaglandin D_2 or its active metabolite 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 are the endogenous PPAR-γ ligands, which contribute to the observed protection of the liver in haemorrhagic shock, also warrants further investigation.

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