British Journal of Pharmacology (1998) 124, 1254–1260
http://www.stockton-press.co.uk/bjp

Effects of inhibitors of the activity of poly (ADP-ribose) synthetase on the liver injury caused by ischaemia-reperfusion: a comparison with radical scavengers

[1]Joanne Bowes & [1,2]Christoph Thiemermann

[1]William Harvey Research Institute, St Bartholomew's and the Royal London School of Medicine and Dentistry, Charterhouse Square, London, EC1M 6BQ

1 Poly (ADP-ribose) synthetase (PARS) is a nuclear enzyme activated by strand breaks in DNA which are caused by reactive oxygen species (ROS) and peroxynitrite. Excessive activation of PARS may contribute to the hepatocyte injury caused by ROS *in vitro* and inhibitors of PARS activity reduce the degree of reperfusion injury of the heart, skeletal muscle and brain *in vivo*. Here we compared the effects of various inhibitors of the activity of PARS with those of deferoxamine (an iron chelator which prevents the generation of hydroxyl radicals) and tiron (an intracellular scavenger of superoxide anion) on the degree of hepatic injury caused by ischaemia and reperfusion of the liver in the anaesthetized rat or rabbit.

2 In the rat, ischaemia (30 or 60 min) and reperfusion (120 min) of the liver resulted in significant increases in the serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) indicating the development of liver injury. Intravenous administration of the PARS inhibitors 3-aminobenzamide (3-AB, 10 mg kg^{-1} or 30 mg kg^{-1}), 1,5-dihydroxyisoquinoline (ISO, 1 mg kg^{-1}) or 4-amino-1,8-naphthalimide (4-AN, 3 mg kg^{-1}) before reperfusion did not reduce the degree of liver injury caused by ischaemia-reperfusion.

3 In contrast to the PARS inhibitors, deferoxamine (40 mg kg^{-1}) or tiron (300 mg kg^{-1}) significantly attenuated the rise in the serum levels of AST and ALT caused by ischaemia-reperfusion of the liver of the rat.

4 In the rabbit, the degree of liver injury caused by ischaemia (60 min) and reperfusion (120 min) was also not affected by 3-AB (10 mg kg^{-1}) or ISO (1 mg kg^{-1}).

5 These results support the view that the generation of oxygen-derived free radicals mediates the liver injury associated with reperfusion of the ischaemic liver by mechanism(s) which are independent of the activation of PARS.

Keywords: Liver; reperfusion injury; PARS inhibitors; deferoxamine; tiron; oxygen-derived free radicals

Introduction

Hepatic injury caused by ischaemia and reperfusion of the liver is an important clinical problem associated with e.g. liver transplantation. Hepatocyte injury may occur directly due to ischaemia, but there is substantial evidence that reperfusion itself leads to additional injury (Flaherty & Wesfeldt, 1988). Reactive oxygen species (ROS) generated upon reperfusion contribute to the pathophysiology of ischaemia-reperfusion injury of the liver and other organs (McCord, 1985; Gonzalez-Flecha *et al*., 1993). ROS have been directly demonstrated using electron paramagnetic resonance (e.p.r.) spectroscopy (Connor *et al*., 1992) and chemiluminescence (Nunes *et al*, 1995), and various strategies to reduce ROS levels protect the liver against ischaemia-reperfusion injury. These include antioxidant enzymes such as superoxide dismutase and catalase (Atalla *et al*., 1985; Koo *et al*., 1991), radical scavengers such as mannitol and α-tocopherol (Marubayashi *et al*., 1986), and agents which prevent the generation of radicals such as allopurinol (Marotto *et al*., 1989) and deferoxamine (Omar *et al*., 1989). ROS cause cell injury by peroxidation of membrane lipids, denaturation of proteins such as enzymes and ion channels, and DNA injury. Exposure of cultured cells to ROS including hydrogen peroxide (H_2O_2), peroxynitrite ($ONOO^-$) or superoxide anions (O_2^-) results in strand breakage of DNA and subsequent activation of the nuclear enzyme, poly (ADP-ribose) synthetase (PARS) (Berger, 1985; Szabo *et al*., 1996). Once activated, PARS catalyzes the transfer of poly (ADP-ribose) groups from nicotinamide adenine dinucleotide (NAD) to nuclear proteins with concomitant formation of nicotinamide. Under conditions of oxidant stress, DNA injury causes excessive activation of the PARS enzyme resulting in a fall in the intracellular levels of its substrate NAD (Schraufstatter *et al*., 1986a). As NAD is necessary for mitochondrial respiration, depletion of NAD leads to a fall in the intracellular levels of ATP. In addition, the nicotinamide formed when PARS is activated can be recycled to NAD in a reaction that consumes ATP (Carson *et al*., 1986). A decline in the intracellular levels of ATP results in severe cellular dysfunction and ultimately cell death (Schaufstatter *et al*., 1986a). Inhibition of the activity of PARS e.g. with 3-aminobenzamide, prevents the NAD and ATP depletion caused by oxidant stress, and improves cell survival (Schraufstatter *et al*., 1986a). However, the relative contribution of PARS in mediating cell injury appears to depend on the oxidant used and the cell type studied. There is substantial evidence that activation of PARS contributes to injury of lymphocytes (Schraufstatter *et al*., 1986a), P388D1 cells (a macrophage tumour cell line (Schraufstatter *et al*., 1986b), endothelial cells (Thies & Autor, 1991; Aalto &

[2]Author for correspondence.

Raivio, 1993), smooth muscle cells (Szabo *et al*., 1996), cardiac myoblasts (Bowes *et al*., 1998), fibroblasts (Yamamoto *et al*., 1993) and neurones (Zhang *et al*., 1994) exposed to oxidant stress.

We have discovered that inhibition of the activity of PARS during reperfusion reduces (i) the degree of necrosis caused by ischaemia and reperfusion of the heart and skeletal muscle of the rabbit and (ii) attenuates the contractile dysfunction caused by global ischaemia and reperfusion in the isolated, perfused heart of the rabbit (Thiemermann *et al*., 1997). Thus, we proposed that (i) activation of PARS contributes to reperfusion injury and (ii) that inhibition of PARS activity may be useful in the therapy of reperfusion injury of the heart and skeletal muscle, and (iii) may also be beneficial in reperfusion injury of other organs. Our proposal has recently been supported by a study carried out using a mouse model of ischaemia-reperfusion of the brain. Inhibitors of the activity of PARS reduce the degree of necrosis caused by cerebral ischaemia and reperfusion (Eliasson *et al*., 1997; Endres *et al*., 1997). In mice in which the gene encoding the PARS enzyme has been inactivated (PARS knockout mice) the degree of cerebral infarction caused by ischaemia-reperfusion was greatly reduced when compared with wild-type mice (Eliasson *et al*., 1997; Endres *et al*., 1997).

DNA injury has been demonstrated in rat reperfused liver *in vivo* (Loft *et al*., 1995), and studies with isolated hepatocytes have provided some evidence that activation of PARS plays a role in hepatic injury caused by oxidant stress (Stubberfield & Cohen, 1988). This study was designed to investigate whether activation of PARS during reperfusion, contributes to the hepatocellular injury caused by ischaemia and reperfusion in the anaesthetized rat or rabbit. Various inhibitors of the activity of PARS (3-aminobenzamide, 1,5-dihydroxyisoquinoline, 4-amino-1,8-naphthalimide) were used and their effects were compared with those of the iron-chelator, deferoxamine, or the intracellular superoxide anion scavenger, tiron.

Preliminary accounts of this study have been presented to the British Pharmacological Society (Bowes & Thiemermann, 1998).

Methods

This study was carried out on 107 male Wistar rats (Tuck, Rayleigh, Essex, U.K.) weighing 250 to 330 g, and 22 male New Zealand White rabbits (Foxfield, Petersfield, Hampshire, U.K.) weighing 2.5 to 3.0 kg. All animals received a standard diet and water *ad libitum*.

Ischaemia and reperfusion of the rat liver

Male Wistar rats (250–330 g) were anaesthetized with thiopentone sodium (Intraval, 120 mg kg^{-1}, i.p.). The trachea was cannulated to facilitate respiration. The left carotid artery was cannulated and connected to a pressure transducer (Sensnor 840, Horton, Norway) for the measurement of mean arterial pressure (MAP) and heart rate (HR) which were recorded on a MacLab 8 recording system (AD Instruments, London, U.K.). The left femoral vein was cannulated for the administration of drugs. Body temperature was maintained at $37 \pm 1^{\circ}C$ by means of a rectal probe thermometer attached to a homeothermic blanket control unit (Harvard Apparatus Ltd., Edenbridge, Kent, U.K.). A transverse laparotomy was performed and the liver was isolated by dividing the peritoneal attachments. The hepatic triad was located and the vessels (hepatic artery, hepatic portal vein and bile duct) supplying the left lobes of the liver were isolated and a marker thread placed around the vessels. The animals were then allowed to stabilize for 30 min. Subsequently, the hepatic vessels were occluded for 30, 45 or 60 min using an artery clip. Drugs were administered 1 min before reperfusion (0.2 ml bolus, i.v.) and reperfusion followed for 2 h by removal of the artery clip. Occlusion was verified by change of the hepatic lobes to a paler shade, and reperfusion by a blush. This model of partial hepatic ischaemia allows the interruption of blood flow to approximately 70% of the liver without obstructing the portal flow to the remaining lobes. This allows the avoidance of systemic haemodynamic changes often caused by portal stasis.

Ischaemia and reperfusion of the rabbit liver

Male New Zealand White rabbits (2.5–3.0 kg) were premedicated with Hypnorm (0.1 mg kg^{-1}, i.m.; containing 0.315 mg ml^{-1} fentanyl citrate and 10 mg ml^{-1} fluanisone; Janssen Pharmaceutical Ltd.). After 10 min, general anaesthesia was induced with sodium pentobarbitone (30 mg kg^{-1}, i.v.; Sagatal, May and Baker) via the marginal ear vein and maintained with supplementary doses of sodium pentobarbitone as required. A tracheotomy was performed under additional local anaesthetic (Xylocaine 2%, Astra Pharmaceuticals) and the rabbits were ventilated with room air by a Harvard ventilator (rate 36–40 strokes min^{-1}, tidal volume 18–20 ml). Body temperature was maintained at $38 \pm 1^{\circ}C$ by means of a rectal probe thermometer attached to a homeothermic blanket control unit (Harvard Apparatus Ltd, Edenbridge, Kent, U.K.). The right carotid artery was cannulated and connected to a pressure transducer (Spectramed P23XL) to monitor arterial blood pressure and heart rate, and the right marginal ear vein was cannulated for intravenous administration of drugs. A right subcostal incision was made parallel to the rib cage. The hepatic triad was located and the left hepatic artery and hepatic portal vein were separated from the bile duct and lymphatics. A marker thread was placed around the hepatic artery and portal vein. The animals were allowed to stabilize for 30 min. The hepatic vessels were occluded with an artery clip for 60 min. Drugs were administered 1 min before reperfusion (2 ml bolus, i.v.). This was followed by reperfusion for 2 h by removal of the artery clip.

Determination of the ischaemic area of the liver in the rat and in the rabbit

At the end of the experiment the vessels were re-occluded using the artery clip and Evans blue dye (4 ml of 2% w/v) was injected into the animal via the venous cannula. The Evans blue solution stained the perfused hepatic lobes, while the lobes normally supplied by the occluded vessels remained uncoloured. This allowed visual determination of the ischaemic and normally perfused areas of the liver.

Measurement of hepatic injury in the rat and in the rabbit

Blood samples (0.5 ml) were obtained at baseline (time 0) and 180 min (at the end of the reperfusion period). Blood was collected into a serum gel S/1.3 tube (Sarstedt, Germany) from the carotid artery cannula. The samples were centrifuged

(6000 r.p.m., for 3 min.) to prepare serum. Serum samples were analysed within 24 h by a contract research laboratory for veterinary and clinical chemistry (Vetlab Services, Sussex, U.K.). The following biochemical markers of liver injury were measured: alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

Experimental design

In the first study aimed at elucidating the effects of inhibitors of PARS activity in ischaemia-reperfusion injury of the rat liver, all animals were randomized into 13 groups (see Table 1). Different groups of animals were subjected to 30 or 60 min of liver ischaemia followed by 120 min of reperfusion and treated with one of the three, chemically distinct inhibitors of PARS activity. To ensure that the liver injury afforded by 30 or 60 min of ischaemia plus 120 min of reperfusion can be attenuated (in our hands) by an intervention (positive control), we have elucidated the effects of the iron-chelator, deferoxamine, on hepatocellular injury in our model. Please note that the dose of deferoxamine used here is known to reduce hepatic injury caused by ischaemia (30 min) and reperfusion of rat liver *in vivo* (Omar *et al*., 1989). Having demonstrated that deferoxamine caused a substantial reduction of the degree of liver injury caused by ischaemia-reperfusion, we also investigated the effects of tiron, an intracellular superoxide anion scavenger (Kengatharan *et al*., 1996).

In a separate set of experiments, we investigated whether the two PARS inhibitors 3-aminobenzamide ($n=6$) or 1,5-dihydroxyisoquinoline ($n=8$) affect the degree of liver injury caused by ischaemia (60 min) and reperfusion (120 min) in the rabbit (control, $n=8$).

Statistical analysis

All data are expressed as mean $\pm$ s.e.mean of n independent experiments. Statistical comparisons between groups were made by a one way ANOVA followed by a Bonferroni test. A P value of less than 0.05 was considered to be statistically significant.

Drugs and materials

Unless otherwise stated all compounds were obtained from Sigma Chemical Co. (Poole, Dorset). Thiopentone sodium (Intraval) was purchased from Rhone Merieux Ltd. (Harlow, Essex), sodium pentobarbitone (Sagatal) from May and Baker (Dagenham, U.K.), Hypnorm from Janssen Pharmaceutical Co. (Oxford, U.K.) and lignocaine (Xylocaine) from Astra Pharmaceuticals (Kings Langley, U.K.). 1,5-Dihydroxyisoquinoline and 4-amino-1,8-naphthalimide were obtained from Aldrich (Poole, Dorset) and dissolved in 10% dimethylsulphoxide (DMSO).

Table 1 Experimental design

Group	*Ishaemia*	*Treatment*	*Dose*	n
1	60 min	Vehicle (saline)		8
2	60 min	Vehicle (DMSO)	10%	6
3	60 min	3-Aminobenzamide	10 mg kg^{-1}	6
4	60 min	3-Aminobenzamide	30 mg kg^{-1}	6
5	60 min	1,5-Dihydroxyisoquinoline	1 mg kg^{-1}	6
6	60 min	4-Amino-1,8-naphthalimide	3 mg kg^{-1}	6
7	60 min	Tiron	300 mg kg^{-1}	6
8	60 min	Deferoxamine	40 mg kg^{-1}	6
9	30 min	Vehicle (saline)		8
10	30 min	Vehicle (DMSO)	10%	6
11	30 min	3-Aminobenzamide	10 mg kg^{-1}	6
12	30 min	1,5-Dihydroxyisoquinoline	1 mg kg^{-1}	6
13	30 min	Tiron	300 mg kg^{-1}	6
14	30 min	Deferoxamine	40 mg kg^{-1}	6

Results

Effect of duration of ischaemia on hepatocellular injury caused by hepatic ischaemia-reperfusion injury in the anaesthetized rat

Baseline values for AST were within the normal physiological range. Ischaemia for 30 min and reperfusion for 60 min resulted in a substantial rise in the serum levels of AST and ALT. Reperfusion for 120 min resulted in a further increase in serum levels of both transaminases (Figure 1). The rise in serum levels of AST and ALT caused by 60 min and 120 min reperfusion following 60 min ischaemia were greater than those following 30 min ischaemia and similar to those following 45 min ischaemia (Figure 1).

Effect of inhibition of PARS activity on hepatic injury following ischaemia-reperfusion in the anaesthetized rat

Baseline values for AST (224 ± 25 iu ml^{-1}) and ALT (155 ± 7 iu ml^{-1}) were not different between groups. Hepatic ischaemia (60 min) followed by reperfusion (120 min) caused significant increases in serum levels of AST and ALT. Administration of the PARS inhibitors 3-aminobenzamide (3-AB; 10 mg kg^{-1} or 30 mg kg^{-1}), 1,5-dihydroxyisoquinoline (ISO; 1 mg kg^{-1}) or 4-amino-1,8-naphthalimide (4-AN;

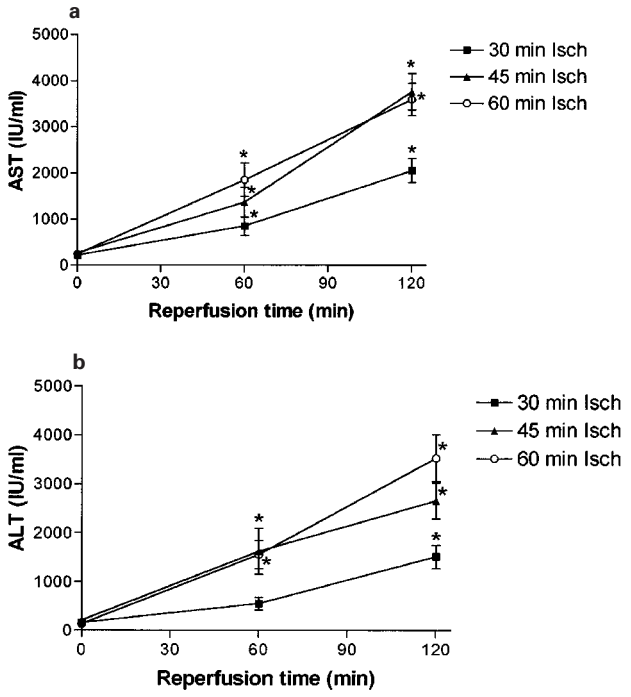

Figure 1 Time course of the increase in the serum levels of (a) AST and (b) ALT caused by ischaemia and reperfusion of the liver of the anaesthetized rat. Animals were subjected to 30 min ($n=8$), 45 min ($n=8$) or 60 min ($n=8$) of hepatic ischaemia followed by 120 min reperfusion. Results are expressed as mean and vertical lines show s.e.mean of n observations. *$P<0.05$ when compared to baseline (time 0).

3 mg kg^{-1}) 1 min before reperfusion, did not reduce the rise in the serum levels of AST and ALT caused by hepatic ischaemia-reperfusion (Figure 2). Similarly neither 3-aminobenzamide nor 1,5-dihydroxyisoquinoline reduced the lesser hepatic injury caused by 30 min ischaemia and 120 min reperfusion (Figure 3). Administration of the 10% DMSO vehicle also had no effect on AST and ALT levels (data not shown).

Effect of antioxidants on hepatic injury following ischaemia-reperfusion in the anaesthetized rat

Administration, before reperfusion after 30 or 60 min of hepatic ischaemia, of the intracellular superoxide anion scavenger, tiron (300 mg kg^{-1}) caused a significant reduction of the rise in serum AST and ALT. Similarly, the iron-chelator deferoxamine (40 mg kg^{-1}) reduced the rise in serum AST and ALT caused by 30 min or 60 min ischaemia and 120 min reperfusion (Figures 4 and 5).

Effect of inhibition of PARS activity on hepatic injury following ischaemia-reperfusion in the anaesthetized rabbit

Hepatic ischaemia (60 min) and reperfusion (120 min) in the anaesthetized rabbit caused a substantial increase in serum levels of AST and ALT at 120 min reperfusion. Administration of the inhibitors of PARS activity 3-aminobenzamide (10 mg kg^{-1}) or 1,5-dihydroxyisoquinoline (1 mg kg^{-1}) 1 min before reperfusion did not have any effect on the rise in AST and ALT (Figure 6).

Discussion

Having discovered that various, chemically distinct inhibitors of PARS activity reduce the degree of tissue necrosis associated with ischaemia-reperfusion of the heart and skeletal muscle (Thiemermann *et al*., 1997), we have proposed that (i) activation of PARS contributes to ischaemia-reperfusion injury and (ii) that inhibitors of PARS activity may be useful in the therapy of ischaemia-reperfusion injury of various organs. Our hypothesis that the activation of PARS contributes to ischaemia-reperfusion

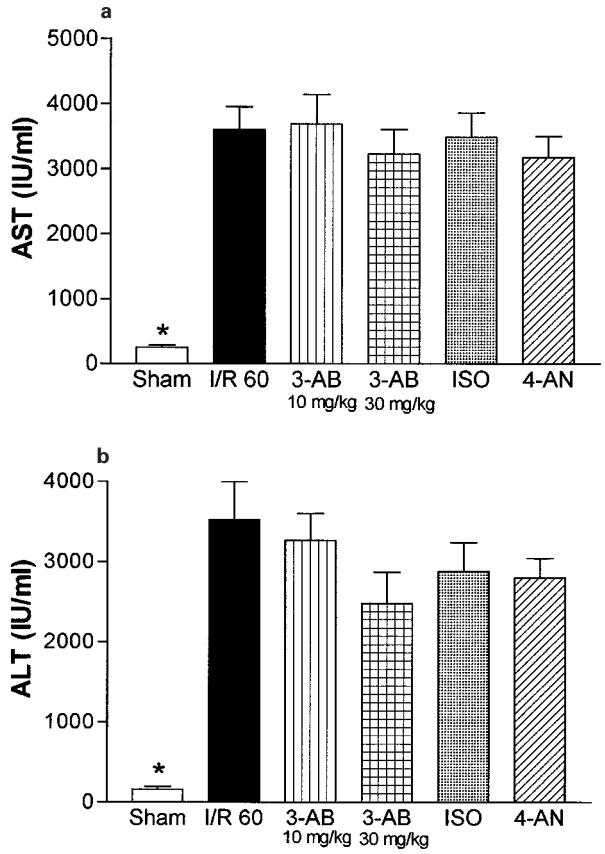

Figure 2 The effect of inhibitors of PARS activity on the rise in the serum levels of (a) AST and (b) ALT caused by ischaemia and reperfusion of the liver of the anaesthetized rat. When compared to sham-operated control rats (sham, $n=6$), ischaemia (60 min) and reperfusion of the liver (I/R 60, solid column, $n=8$) caused a significant rise in the serum levels of AST and ALT. Please note that administration before reperfusion of the PARS inhibitors 3-aminobenzamide (3-AB, 10 mg kg^{-1}, vertical striped column, $n=6$), 3-AB (30 mg kg^{-1}, squared column, $n=6$), 1,5-dihydroxyisoquinoline (ISO, 1 mg kg^{-1}, stippled column, $n=6$) or 4-amino,1-8, naphthalimide (4-AN, 3 mg kg^{-1}, diagonally striped column, $n=6$) did not reduce the rise in the serum levels of AST or ALT caused by I/R of the liver. Data are expressed as mean$\pm$s.e.mean of n observations. *$P<0.05$ when compared to control (I/R 60).

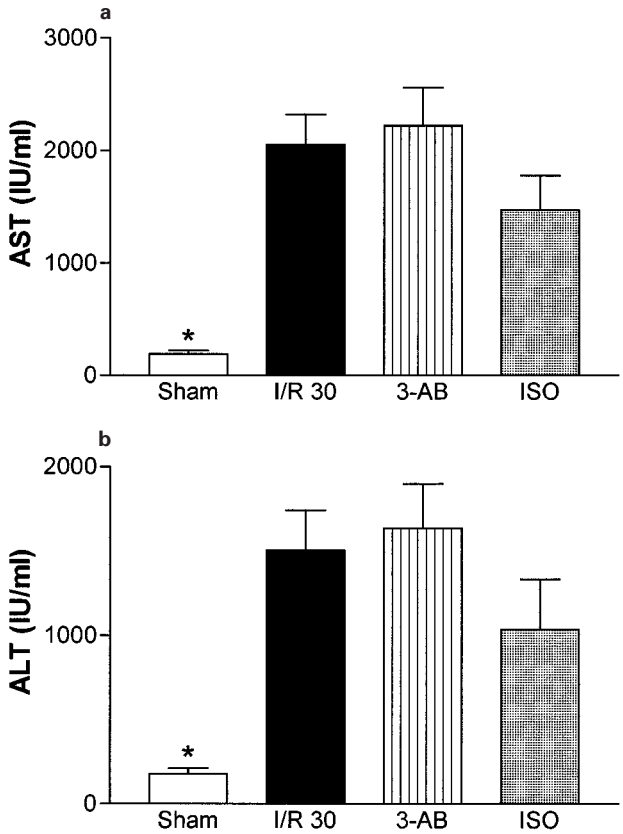

Figure 3 The effect of inhibitors of PARS activity on the rise in the serum levels of (a) AST and (b) ALT caused by ischaemia and reperfusion of the liver of the anaesthetized rat. When compared to sham-operated control rats (sham, $n=6$), ischaemia (30 min) and reperfusion of the liver (I/R 30, solid column, $n=8$) caused a significant rise in the serum levels of AST and ALT. Please note that administration before reperfusion of the PARS inhibitors 3-aminobenzamide (3-AB, 10 mg kg^{-1}, vertical striped column, $n=6$) or 1,5-dihydroxyisoquinoline (ISO, 1 mg kg^{-1}, stippled column, $n=6$) did not reduce the rise in the serum levels of AST or ALT caused by I/R of the liver. Data are expressed as mean$\pm$s.e.mean of n observations. *$P<0.05$ when compared to control (I/R 30).

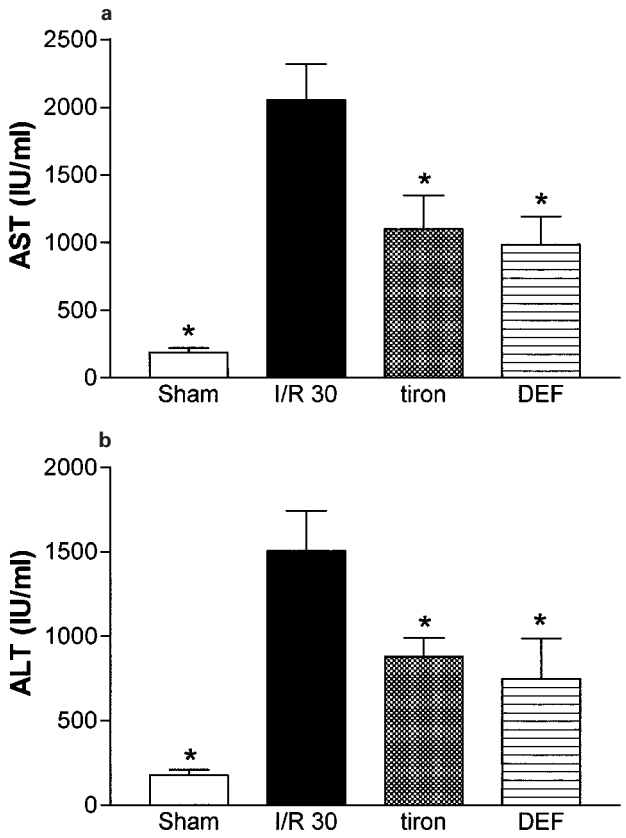

Figure 4 The effect of radical scavengers on the rise in the serum levels of (a) AST and (b) ALT caused by ischaemia and reperfusion of the liver of the anaesthetized rat. When compared to sham-operated control rats (sham, $n=6$), ischaemia (30 min) and reperfusion of the liver (I/R 30, solid column, $n=8$) caused a significant rise in the serum levels of AST and ALT. Please note that administration before reperfusion of the superoxide anion scavenger tiron (300 mg kg^{-1}, checked column, $n=6$) or the iron chelator deferoxamine (DEF, 40 mg kg^{-1}, horizontal striped column, $n=6$) reduced the rise in the serum levels of AST or ALT caused by I/R of the liver. Data are expressed as mean $\pm$ s.e.mean of n observations. $^*P<0.05$ when compared to control (I/R 30).

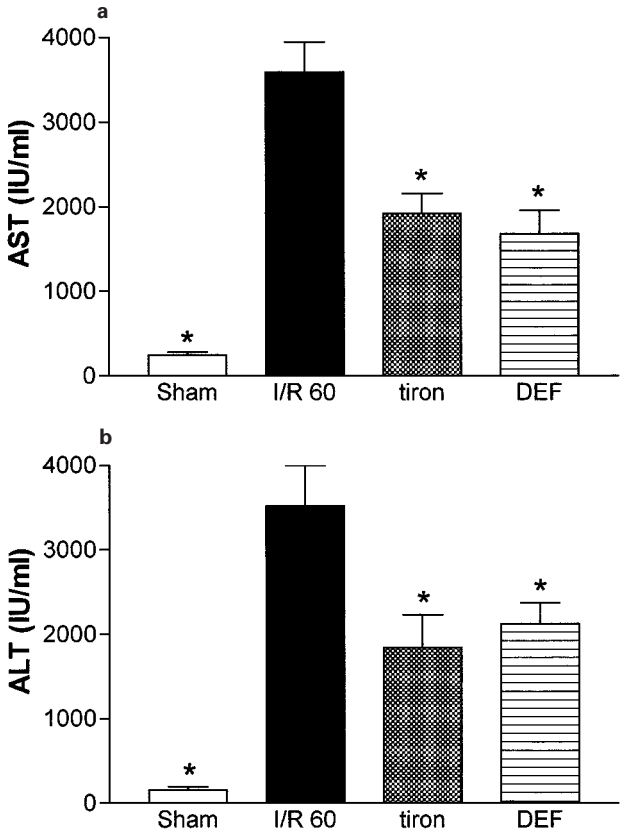

Figure 5 The effect of radical scavengers on the rise in the serum levels of (a) AST and (b) ALT caused by ischaemia and reperfusion of the liver of the anaesthetized rat. When compared to sham-operated control rats (sham, $n=6$), ischaemia (60 min) and reperfusion of the liver (I/R 60, solid column, $n=8$) caused a significant rise in the serum levels of AST and ALT. Please note that administration before reperfusion of the superoxide anion scavenger tiron (300 mg kg^{-1}, checked column, $n=6$) or the iron chelator deferoxamine (DEF, 40 mg kg^{-1}, horizontal striped column, $n=6$) reduced the rise in the serum levels of AST or ALT caused by I/R of the liver. Data are expressed as mean $\pm$ s.e.mean of n observations. $^*P<0.05$ when compared to control (I/R 60).

injury of the heart is supported by the recent findings that 3-aminobenzamide also reduces infarct size in rats (Zingarelli *et al*., 1986) and pigs (Ruetten, unpublished data) with ischaemia-reperfusion injury. The concept that the activation of PARS may contribute to ischaemia-reperfusion injury of other tissues, is supported by studies documenting that inhibition of PARS activity reduces the degree of injury/necrosis in rodent models of ischaemia-reperfusion of the gut (Cuzzocrea *et al*., 1997) and the brain (Eliasson *et al*., 1997; Endres *et al*., 1997). We demonstrate here that three chemically distinct inhibitors of PARS activity are unable to reduce the degree of liver injury caused by ischaemia (30 or 60 min) followed by reperfusion (2 h) of the rat liver. One could argue that the inability of the PARS inhibitors to protect the liver against ischaemia-reperfusion injury is due to either the doses of the compounds or the species chosen for our study. However, it is not likely that a difference in species and/or dose regimen accounts for the lack of effect of PARS inhibitors in ischaemia-reperfusion injury of the liver for the following reasons: (i) it is well documented that 3-AB, ISO and 4-AN are potent inhibitors of PARS activity in many cultured cells of the rat including hepatocytes. (ii) In the rat, PARS inhibitors including 3-AB (at doses similar to the ones used here) reduce the degree of ischaemia-reperfusion injury of the heart (Zingarelli *et al*., 1986), gut (Cuzzocrea *et al*., 1997) and brain (Eliasson *et al*., 1997). (iii) In the rabbit, all of the PARS inhibitors used in this study (at the same doses) reduce ischaemia-reperfusion injury of the heart and skeletal muscle (Thiemermann *et al*., 1997), but not the liver. Thus, it appears that an increase in PARS activity does not contribute to the tissue injury associated with ischaemia-reperfusion of the liver of the rat or rabbit. It could also be argued that the degree of liver injury caused by ischaemia and reperfusion in this model is too severe to allow the demonstration of a protective effect of PARS inhibitors. However, this is not the case, as the PARS inhibitors failed to reduce the degree of liver injury caused by relatively short durations of ischaemia (e.g. 30 min, this study), while the antioxidants deferoxamine and tiron caused a significant reduction in the injury caused by ischaemia-reperfusion of the rat liver.

The role of PARS in the injury associated with oxidant stress in cultured hepatocytes is still controversial. For example, exposure of isolated hepatocytes to hydrogen peroxide produces a rapid, concentration-dependent depletion of NAD which precedes the loss of ATP and cell viability.

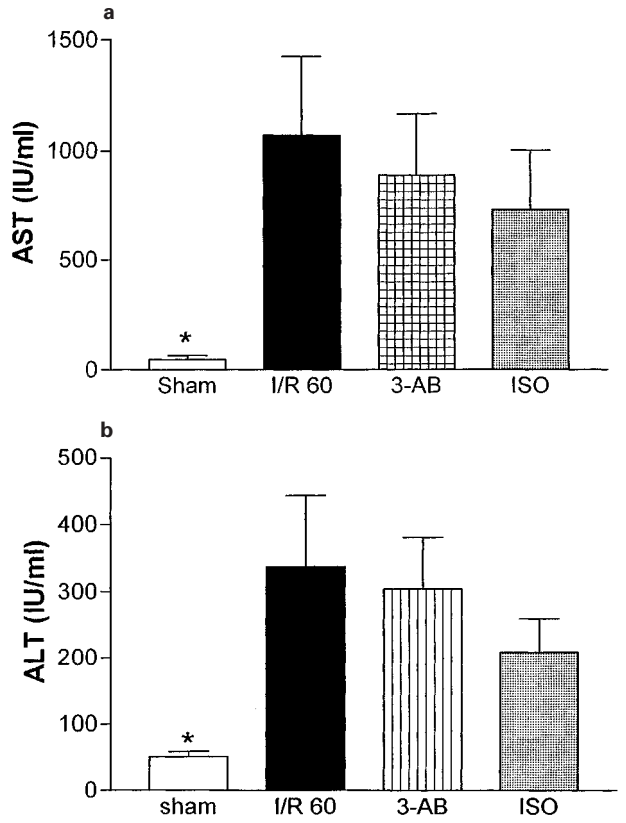

Figure 6 The effect of inhibitors of PARS activity on the rise in the serum levels of (a) AST and (b) ALT caused by ischaemia and reperfusion of the liver of the anaesthetized rabbit. When compared to sham-operated control rabbits (sham, $n=6$), ischaemia (60 min) and reperfusion of the liver (I/R 60, solid column, $n=8$) caused a significant rise in the serum levels of AST and ALT. Please note that administration before reperfusion of the PARS inhibitors 3-aminobenzamide (3-AB, 10 mg kg^{-1}, vertical striped column, $n=6$) or 1,5-dihydroxyisoquinoline (ISO, 1 mg^{-1}, stippled column, $n=8$) did not reduce the rise in the serum levels of AST or ALT caused by I/R of the liver. Data are expressed as mean$\pm$s.e.mean of n observations. *$P<0.05$ when compared to control (I/R 60).

Inhibitors of PARS activity including 3-AB, nicotinamide and theophylline protected against the NAD depletion and the cytotoxic effects of hydrogen peroxide in these cells (Stubberfield & Cohen, 1988). In rat hepatocytes, hydrogen peroxide causes (i) single strand breaks in DNA, (ii) depletion of NAD, (iii) an increase in PARS activity, and (iv) cell death. Inhibition of PARS activity with 3-AB or benzamide did not affect either the fall in NAD or the death of hepatocytes caused by oxidant stress, suggesting that the activation of PARS does not play a role in the injury of hepatocytes caused by hydrogen peroxide (Yamamoto *et al*., 1993). However, there is good evidence that (i) ischaemia-reperfusion of the liver is associated with the generation of oxygen-derived free radicals which results in DNA damage (Loft *et al*., 1995) and (ii) radical scavengers and antioxidants reduce the degree of liver injury caused by ischaemia-reperfusion of the rat (Marotto *et al*., 1989; Omar *et al*., 1989). Indeed, our study confirms that reactive oxygen species, probably the superoxide anion and hydroxyl radical, do play a role in ischaemia-reperfusion injury of the liver. Administration of the intracellular superoxide anion scavenger, tiron, or the iron chelator, deferoxamine reduced the rise in serum AST and ALT following ischaemia-reperfusion of the rat liver. Chelation of iron would prevent the generation of the highly toxic hydroxyl radical by preventing the Fenton reaction which is catalyzed by iron.

In conclusion, this study demonstrates that deferoxamine and tiron, but not the PARS inhibitors 3-aminobenzamide, 1,5-dihydroxyisoquinoline or 4-amino-1,8-naphthalimide, attenuate the degree of injury associated with ischaemia-reperfusion of the liver of the rat and rabbit. We therefore propose that the generation of oxygen-derived free radicals mediates the liver injury associated with reperfusion of the ischaemic liver by mechanism(s) which are independent of the activation of PARS. In contrast, the activation of PARS plays an important role in reperfusion injury of the heart, skeletal muscle, brain and gut.

J.B. is the recipient of a British Heart Foundation (BHF) PhD studentship (FS 96/015). C.T. is a Senior Research Fellow of the BHF (FS 96/018).

References

AALTO, T.K. & RAIVIO, K.O. (1993). Nucleotide depletion due to reactive oxygen metabolites in endothelial cells: effects of antioxidants. *Pediatric Res.*, **34,** 572–576.

ATALLA, S.L.L.H., PEREYRA, L/H., MACKENZIE, G.H. & CEDERNA, J.P. (1985). Influence of oxygen-derived free radical scavengers on ischaemic livers. *Transplantation,* **40,** 584–589.

BERGER, N.A. (1985). Symposium; cellular response to DNA damage: the role of poly (ADP-ribose). *Radiat. Res.*, **101,** 4–15.

BOWES, J., PIPER, J. & THIEMERMANN, C. (1998). Inhibition of the activity of poly (ADP-ribose) synthetase reduces cell injury caused by hydrogen peroxide in rat cardiac myoblasts. *Br. J. Pharmacol. Proc.*, **123,** 93P.

BOWES, J. & THIEMERMANN, C. (1998). Comparison of the effects of inhibitors of poly (ADP-ribose) synthetase and radical scavengers in a rat model of hepatic ischaemia and reperfusion. *Br. J. Pharmacol. Proc.*, **123,** 282P.

CARSON, D.A., SET, S., WASSON, B. & CARRERA, C.J. (1986). DNA strand breaks, NAD metabolism and programmed cell death. *Exp. Cell. Res.*, **164,** 273–281.

CONNOR, H.D., GAO, W., NUKINA, S., LEMASTERS, J.J., MASON, R.P. & THURMAN, R.G. (1992). Evidence that free radicals are involved in graft failure following orthotopic liver transplantation in the rat — an electron paramagnetic resonance spin trapping study. *Transplantation,* **54,** 199–204.

CUZZOCREA, S., ZINGARELLI, B., COSTANTINO, G., SZABO, A., SALZMAN, A.L., CAPUTI, A.P. & SZABO, C. (1997). Beneficial effects of 3-aminobenzamide, an inhibitor of poly (ADP-ribose) synthetase in a rat model of splanchnic artery occlusion and reperfusion. *Br. J. Pharmacol.*, **121,** 1065–1074.

ELIASSON, M.J.L., SAMPEI, K., MANDIR, A.S., HURN, P.D., TRAYSTMAN, R.J., BAO, J., PIEPER, A., WANG, Z., DAWSON, T.M., SNYDER, S.H. & DAWSON, V.L. (1997). Poly (ADP-ribose) polymerase gene disruption renders mice resistance to cerebral ischaemia. *Nature Med.*, **3,** 1089–1095.

ENDRES, M., WANG, Z.Q., NAMURA, S., WAEBER, C. & MOSCOWITZ, M.A. (1997). Ischaemic brain injury is mediated by the activation of poly (ADP-ribose) polymerase *J. Cereb. Blood Flow Metab.*, **17,** 1143–1151.

FLAHERTY, J.L. & WESFELDT, M.L. (1988). Reperfusion injury. *Free Radic. Biol. Med.,* **5,** 409–419.

GONZALEZ-FLECHA, B., CUTRIN, J.C. & BOVERIS, A. (1993). Time course and mechanism of oxidative stress and tissue damage in rat liver subjected to in vivo ischaemia-reperfusion. *J. Clin. Invest.,* **91,** 456–464.

KENGATHARAN, M., DEKIMPE, S.J., WILLIS, D., THIEMERMANN, C. & VANE, J.R. (1996). L-Arginine or tiron attenuate the induction of heme-oxygenase-II in lungs and the in vivo oxidation of dihydrorhodamine in a model of gram-positive shock. *Br. J. Pharmacol.,* **119,** P244.

KOO, A., KOMATSU, H., TAO, G., INOUE, M., GUTH, P.H. & KAPLOWITZ, N. (1991). Contribution of no-reflow phenomenon to hepatic injury after ischaemia-reperfusion: evidence for a role for superoxide anion. *Hepatology,* **15,** 507–514.

LOFT, S., LARSEN, P.N., RASMUSSEN, A., FISCHER-NIELSEN, A., BONDESEN, S., KIRKEGAARD, P., RASMUSSEN, L.S., EJLERSEN, E., TORNØE, K., BERGHOLDT, R. & POULSEN, H.E. (1995). Oxidative DNA damage after transplantation of the liver and small intestine in pigs. *Transplantation,* **59,** 16–20.

MAROTTO, M.E., THURMAN, R.G. & LEMASTERS, J.J. (1989). Early midzonal cell death during low flow hypoxia in the isolated, perfused rat liver: protection by allopurinol. *Hepatology,* **8,** 585–590.

MARUBAYASHI, S.K., DOHI, K., OCHI, K. & KAWASAKI, T. (1986). Role of free radicals in ischemic rat liver cell injury: prevention of damage by α-tocopherol administration. *Surgery,* **99,** 184–192.

MCCORD, J.M. (1985). Oxygen-derived free radicals in post-ischaemic tissue injury. *N. Engl. J. Med.,* **312,** 159–163.

MIZUMOTO, K., GLASCOTT, J.R. & FARBER, J.L. (1993). Roles for oxidative stress and poly (ADP-ribosyl) ation in the killing of cultured hepatocytes by methyl methanesulfonate. *Biochem. Pharmacol.,* **46,** 1811–1818.

NUNES, F.A., KUMAR, C., CHANCE, B. & BRASS, C.A. (1995). Chemiluminescent measurement of increased free radical formation after ischaemia-reperfusion. *Dig. Dis. Sci.,* **40,** 1045–1053.

OMAR, R., NOMIKOS, I., PICCORELLI, G., SAVINO, J. & AGARWAL, N. (1989). Prevention of postischaemic lipid peroxidation and liver cell injury by iron chelation. *Gut,* **30,** 510–514.

SCHRAUFSTATTER, I.U., HYSLOP, P.A., HINSHAW, D.B., SPRAGG, R.G., SKLAR, L.A. & COCHRANE, C.G. (1986a). Hydrogen peroxide induced injury and its prevention by inhibitors of poly (ADP-ribose) polymerase. *Proc. Natl. Acad. Sci. U.S.A.,* **83,** 4908–4912.

SCHRAUFSTATTER, I.U., HINSHAW, D.B., HYSLOP, P.A., SPRAGG, R.G. & COCHRANE, C.G. (1986b). DNA strand breaks activate poly adenosine diphosphate-ribose polymerase and lead to depletion of nicotinamide adenine dinucleotide. *J. Clin. Invest.,* **77,** 1312–1320.

STUBBERFIELD, C.R. & COHEN, G.M. (1988). NAD depletion and cytotoxicity in isolated hepatocytes. *Biochem. Pharmacol.,* **37,** 3967–3974.

SZABO, C., ZINGARELLI, B. & SALZMAN, A.L. (1996). Role of poly-ADP ribosyltransferase activation in the vascular contractile and energetic failure elicited by exogenous and endogenous nitric oxide and peroxynitrite. *Circ. Res.,* **78,** 1051–1063.

THIEMERMANN, C., BOWES, J., MYINT, F. & VANE, J.R. (1997). Inhibition of the activity of poly (ADP-ribose) synthetase reduces ischemia-reperfusion injury in the heart and skeletal muscle. *Proc. Natl. Acad. Sci. U.S.A.,* **94,** 679–683.

THIES, R.L. & AUTOR, A.P. (1991). Reactive oxygen injury to cultured pulmonary artery endothelial cells: Mediation by poly (ADP-ribose) polymerase activation causing NAD depletion and altered energy balance. *Arch. Biochem. Biophys.,* **286,** 353–363.

YAMAMOTO, K., TSUKIDATE, K. & FARBER, J. (1993). Differing effects of the inhibitors of poly (ADP-ribose) polymerase on the course of oxidative cell injury in hepatocytes and fibroblasts. *Biochem. Pharmacol.,* **46,** 483–491.

ZHANG, J., DAWSON, V.L., DAWSON, T.M. & SNYDER, S.H. (1994). Nitric oxide activation of poly (ADP-ribose) synthetase in neurotoxicity. *Science,* **263,** 687–689.

ZINGARELLI, B., CUZZOCREA, S., ZSENGELLER, Z., SALZMAN, A.L. & SZABO, C. (1986). Protection against myocardial ischaemia and reperfusion injury by 3-aminobenzamide, an inhibitor of poly (ADP-ribose) synthetase. *Cardiovasc. Res.,* **36,** 205–215.

(Received February 4, 1998
Revised March 16, 1998
Accepted April 6, 1998)