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European Journal of Pharmacology 406 (2000) 281–292

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S-15176 reduces the hepatic injury in rats subjected to experimental ischemia and reperfusion ☆

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Received 27 April 2000; received in revised form 31 July 2000; accepted 4 August 2000

Abstract

The protective effect of *N*-[(3,5-di-tertiobutyl-4-hydroxy-1-thiophenyl)]-3-propyl-*N*′-(2,3,4-trimethoxybenzyl)piperazine (S-15176) on liver injury induced by warm ischemia–reperfusion was investigated using a rat model. Animals were subjected to 2 h of ischemia followed by different reperfusion times. Hepatocyte integrity was assessed by measuring plasma alanine and aspartate aminotransferase activities, and by determining reduced and oxidized glutathione in plasma and bile. Hepatocyte function was quantitated by determining bile flow and liver ATP content. Ischemia–reperfusion resulted in severe hepatic injury involving a huge increase in alanine and aspartate aminotransferase activities, a drop in ATP content, and a decrease in bile flow. Plasma and bile reduced (GSH) and oxidized (GSSG) glutathione concentrations were inversely related: plasma levels increased when biliary levels decreased. This was associated with a decrease in animal survival (−34%). S-15176 pretreatment (1.25, 2.5, 5 or 10 mg kg^{-1} day^{-1}) improved the survival rate and limited tissue damages in a dose-dependent manner. The pretreatment also reduced the aminotransferase leakage from hepatocytes and the increase in plasma glutathione levels. In addition, normalization of the plasma GSSG/GSH ratio, a good index of an oxidative stress, was observed in groups treated with the higher dosage, suggesting that the antioxidant properties demonstrated for the compound in vitro (IC_{50} = 0.3 μM towards lipid peroxidation) could play a role in its protective effect. S-15176 pretreatment also protected the organ from the drop in ATP levels. At the higher dose, ATP content was maintained at a level almost 86% of the sham-operated group after 60 min of reperfusion. This was associated with a restoration of the biliary flow. These data suggest that S-15176 may be a useful drug in liver surgery to prevent ischemia–reperfusion injury.

Keywords: Liver, rat; Ischemia–reperfusion; Protective effect; ATP; Bile flow; Glutathione; Antioxidant

1. Introduction

Hepatic ischemia followed by reperfusion is a major clinical problem occurring during transplantation, liver resection and shock, all circumstances leading to the reduction or the interruption of hepatic blood flow. Ischemia–reperfusion results in severe injuries for the organ, leading to cell death. This death is generally thought to occur by necrosis (Majno and Joris, 1995), but recent studies demonstrate the coexistence of an apoptotic process (Choi, 1996; Kohli et al., 1999). Ischemia is characterized by a loss of cellular homeostasis involving an increase of cytosolic H^+ and Ca^{2+} concentrations and a rapid impairment of mitochondrial respiration, which induces a decrease in ATP synthesis. During the reperfusion phase, the mitochondrial repolarization resulted in a Ca^{2+} overload and an excess production of reactive oxygen species. This may increase the mitochondrial membrane permeability due to the formation of a complex channel, the mitochondrial permeability transition pore (Zoratti and Szabo', 1995; Bernardi et al., 1998; Crompton, 1999). This channel appears to be involved in the release of the apoptosis-inducing factors and the activation of caspases (Green and Reed, 1998; Kroemer et al., 1998).

☆ All animal procedures used in this study are in strict accordance with the French Agency's policies on animal experimentation.

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PII: S0014-2999(00)00599-9

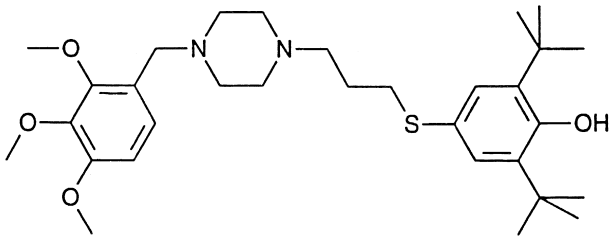

Fig. 1. Chemical structure of S-15176.

Recently, we have shown in an experimental model of ischemia–reperfusion that trimetazidine pretreatment limited the extent of cellular injuries (Settaf et al., 1999). This protective effect was due to the preservation of mitochondrial functions (Elimadi et al., 1998). However, trimetazidine did not show any antioxidant effect in vitro, an interesting property for a protective drug since ischemia–reperfusion injury in vivo is closely related to an excess of reactive oxygen species production (Jaeschke and Farhood, 1991; Zini et al., 1992; Crompton, 1999). Therefore, the goal of this work was to find a more active drug than trimetazidine.

To this end, we selected *N*-[(3,5-di-tertiobutyl-4-hydroxy-1-thiophenyl)]-3-propyl-*N′*-(2,3,4-trimethoxybenzyl)piperazine (S-15176) (Fig. 1) from a series of trimetazidine derivatives, on the basis of its strong radical oxygen species scavenging properties. The purpose of this study was to investigate its protective effect against hepatic ischemia and reperfusion injuries. We used an experimental model associating normothermic ischemia then reperfusion of rat liver in situ. Rats were pretreated or not with increasing doses of S-15176 and the hepatocyte integrity was assessed by analyzing the release of liver enzymes alanine and aspartate aminotransferase in the plasma and reduced (GSH) and oxidized (GSSG) glutathione levels in plasma and bile. The overall liver function was quantified by measuring bile flow and liver ATP content.

2. Materials and methods

2.1. Animals and drug administration

Male Wistar rats (250–260 g at the beginning of the experiments) were used. All animals were housed in small groups on a 12/12 h light/dark cycle and given food and water ad libitum. The animals fasted for 12 h before the operations, but water was available ad libitum. Animals were assigned to one of six different experimental groups (15 rats each): the sham-operated group, animals subjected to surgery but not to ischemia; the control group, rats injected intramuscular (i.m.) with 0.5 ml water/polyethylene glycol (50:50) solution before induction of the ischemia–reperfusion process; and the pretreated groups, animals which received S-15176 at 1.25, 2.5, 5 and 10 mg kg^{-1} day^{-1} i.m. for 5 days before ischemia–reperfusion. The last injection was performed 30 min before the induction of the ischemia. For the survival study, each experimental group included 35 animals. S-15176 was provided by Servier Laboratories. It was freshly prepared in a water/polyethylene glycol (50:50) solution and warmed to body temperature before injection.

2.2. Liver ischemia–reperfusion

2.2.1. Experimental protocol

Three different experiments were performed. The first one was designed to study survival and evolution of plasma liver enzymes and glutathione. The second analysed bile flow variations and bile glutathione concentrations. The third experiment measured hepatic ATP level changes before and after 120 min ischemia followed by reperfusion.

2.2.2. Surgery

The surgical procedure was performed 30 min after the last drug or water/polyethylene glycol administration. Throughout the experiment, body temperature was recorded (Odam Physiogard) and maintained at 36–38°C with the aid of a heating pad. The abdomen was opened through a midline incision under ether anaesthesia. After section of the liver ligaments, the hilum of the median and left lateral lobes were exposed by everting them upwards, and ischemia was induced by occluding the blood vessels and the bile ducts of these lobes using a small vascular clamp (Nauta et al., 1989). The liver was returned to its proper position and the abdomen was closed. Since blood flow to the right lateral and caudate lobes was kept intact, portal stasis was avoided during ischemia. During the period of ischemia, 0.5 ml of saline was given through the dorsal vein of the penis every 30 min to maintain hemodynamic stability. After 120 min of ischemia, reperfusion was induced by removing the vascular clamp and the non-ischemic lobes were resected. At different times, livers were quickly removed, weighed, washed with a cold isotonic saline solution and stored at −196°C until analysis. The reperfusion was checked by the recoloration of the liver and the absence of congestion in the intestine. Survival time was monitored every 6 h. Animals alive 15 days after the operation were considered permanent survivors. An autopsy was performed on all animals to verify the absence of surgically related complications.

To study bile flow, the common bile duct was catheterised with a polyethylene tubing. Bile flow was measured by collecting the bile gravimetrically for 15 min five times throughout the experimental procedure (once before clamping as a control and four times [30, 60, 90 and 120 min] after declamping). The bile ducts of the caudate and right lateral lobes (not subjected to the ischemia) were ligated after declamping to measure bile production from only the ischemic lobes.

2.3. Histology

Sections of liver exposed to ischemia reperfusion were fixed using routine procedures, stained with hematoxylin–eosine safran and examined by light microscopy.

2.4. Liver function tests

Bile and blood samples were collected between 11 am and 12 noon to avoid variation due to circadian rhythms. Blood samples were obtained from retro-orbital sinuses by means of a heparinized Pasteur pipette. Plasma samples were immediately stored at −40°C until required for analysis.

Alanine and aspartate aminotransferase activities were determined using standard laboratory methods and Biomerieux diagnostic kits (Biomerieux France). GSH and GSSG concentrations were determined by enzymatic procedures according to the method of Akerboom (1981).

Measurement of ATP content in the liver was performed using the method of Jaworec et al. (1974). A weighed portion of the frozen liver was ground in liquid nitrogen in five volumes of precooled 4% perchloric acid (w/v), and the mixture was thoroughly homogenised using a precooled polytron homogenizer (Beckman Instruments). The sample was centrifuged at 3000 rpm for 15 min at 4°C. The supernatant was removed and adjusted to pH 6.5 by addition of Na_2CO_3 and centrifuged at 3000 rpm for 5 min at 4°C. The hepatic ATP content was determined using a Sigma diagnostic kit (Sigma, Paris, France).

2.5. Assay of lipid peroxidation

Lipid peroxidation was assayed as the generation of thiobarbituric acid-reactive substances according to Braughler et al. (1986). Briefly, after preparation of liver membranes by means of a standard procedure, samples of 0.5 ml (0.5 mg protein) were preincubated in phosphate buffer with or without increasing concentrations of S-15176 for 10 min at 37°C. Then, lipid peroxidation was initiated by the addition of a mixture of Fe^{2+}/Fe^{3+}(50:150 μM). After 10 min of incubation, the reaction was stopped with 1 ml of 30% trichloroacetic acid, and 0.5 ml of desferrioxamine and 1.5 ml of 0.67% thiobarbituric acid were added. The mixture was then heated to 100°C for 1 h and re-cooled on ice for 10 min before centrifugation in an Eppendorf centrifuge. Results were expressed as μM thiobarbituric acid-reactive substances/mg of membrane protein.

2.6. Statistical analysis

Data are reported as mean values ± S.E.M. They were compared by one-way analysis of variance (ANOVA); a statistically significant difference was accepted if $P < 0.05$.

3. Results

3.1. In vitro antioxidant effect of S-15176

The effect of S-15176 on liver membranes from sham-operated animals was studied. Lipid peroxidation was induced by the addition of a mixture of Fe^{2+}/Fe^{3+} in the absence or presence of increasing concentrations of S-15176. S-15176 inhibited lipid peroxidation in a concentration-dependent manner with an IC_{50} value of 0.3 μM (Fig. 2). An identical result was found with rat brain synaptosomes (not shown). In this test, trimetazidine was inactive, confirming previous results (Elimadi et al., 1997).

3.2. Effects of S-15176 on rats survival and liver morphology

Only 65.71 ± 8.02% of rats ($n = 35$) survived when they were subjected to 120 min of normothermic ischemia followed by reperfusion. This survival rate was significantly improved in the treated groups ($Chi^2 = 13.28$, 4 d.o.f., $P = 0.01$). It increased to 91.42 ± 4.74% and 94.28 ± 3.92% when rats were treated with 5 and 10 mg kg^{-1} day^{-1} of S-15176, respectively. The effects of S-15176 seemed to be dose-dependent. At doses of 1.25 and 2.5 mg kg^{-1} day^{-1}, the percentages of surviving rats were 77.14 ± 7.09% and 85.70 ± 5.91%, respectively.

Examination of hematoxylin–eosine safran biopsy specimens from non-treated animals showed normal architectural hepatic paremshyma. However, sections from these livers displayed sinusoidal dilatations with moderate de-

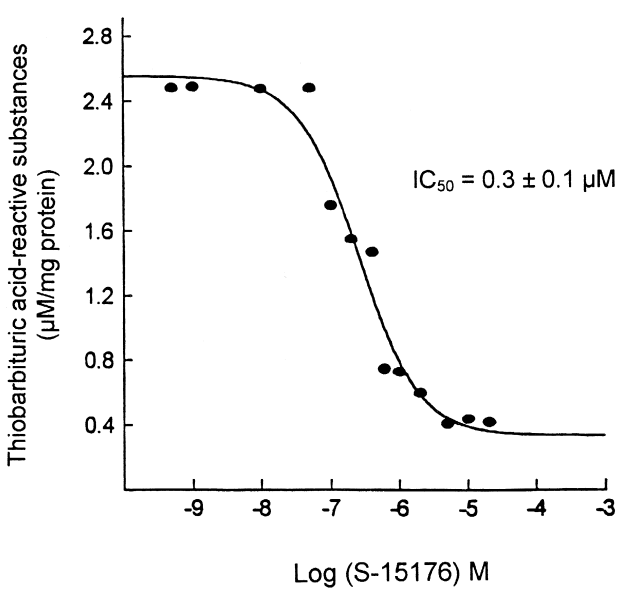

Fig. 2. Effect of S-15176 on lipid peroxidation of liver membranes. Lipid peroxidation is assessed as thiobarbituric acid-reactive substance generation.

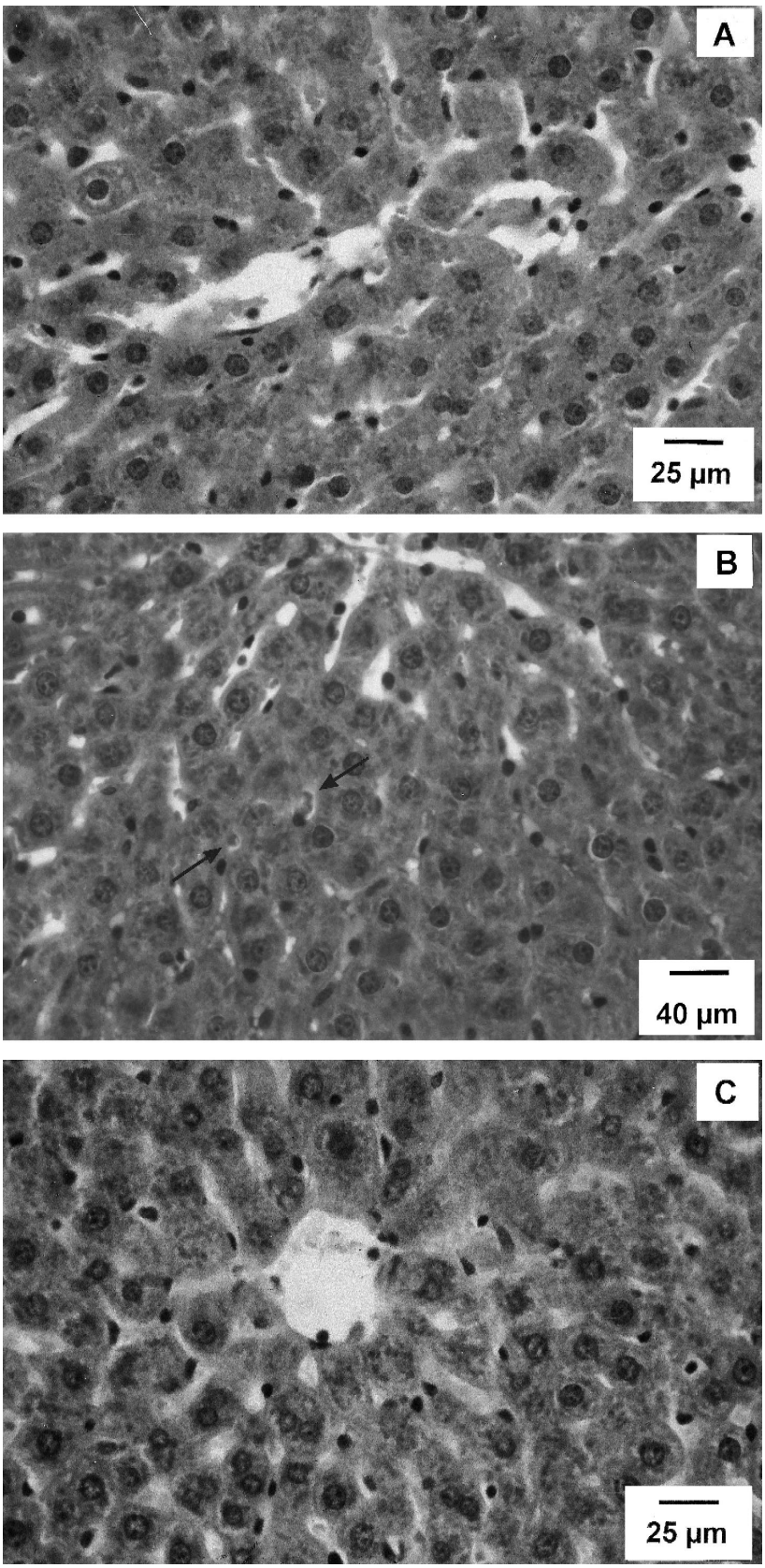

Fig. 3. Histopathological evaluation of rat livers after 120 min ischemia and 30 min reperfusion. Liver sections were stained with hematoxylin–eosine safran. Sections from non-treated animals showed sinusoidal (A) and centrolobular vein dilatations (B) with necrotic cells (arrows). S-15176 treatment reduced the lesions (C).

gree of peliosis (Fig. 3A). In addition, some necrotic cells were observed around dilated centrolobular veins (Fig. 3B). When rats were treated with S-15176, these lesions existed but were clearly less prominent. A typical section of a liver treated with 10 mg kg^{-1} S-15176 is shown in Fig. 3C.

3.3. Evolution of liver enzymes and glutathione in the plasma

Ischemia–reperfusion induced a dramatic increase in serum alanine and aspartate aminotransferase activities (Table 1). This reflects the membrane damage to the cells, which causes the cellular enzymes to leak. This increase was observed immediately after the reperfusion period and then decreased slowly. Ten days after the surgery, the level of the enzymes was not normalized, remaining 44% and 101% higher than the preoperative values (Table 1). Increasing doses of S-15176 reduced the plasma activities of both enzymes. This effect was already observed just after the reperfusion period at the lower dose of S-15176 (1.25 mg kg^{-1} day^{-1}) and the amelioration was faster than in the control non-treated group. In addition, at the higher S-15176 doses, i.e. 5 and 10 mg kg^{-1} day^{-1}, the recovery was complete 7 and 10 days after the induction of the ischemia for aspartate and alanine aminotransferase, respectively.

Numerous studies have suggested that oxidative stress is an important factor in inhibition of liver functions after ischemia–reperfusion (McCord, 1985; Marubayashi et al., 1994; Mathews et al., 1994). Liver is the major source of the hydrogen peroxide scavenger GSH and biliary and plasma GSH and GSSG are considered to be important markers of oxidative stress. Thus, we studied the evolution of GSH and GSSG in plasma and bile.

The alterations of the cell membrane after ischemia–reperfusion were confirmed by the appearance of high concentrations of oxidized and reduced glutathione in the plasma (Fig. 4). The release of GSSG (19-fold increase relative to the sham-operated group after 30 min reperfusion) was more pronounced than that of GSH (4.9-fold increase) regardless of the perfusion period. Consequently, the GSSG/GSH ratio increased (Fig. 5). S-15176 counteracted these increases in a dose-dependent manner at least for the GSSG and the GSSG/GSH ratio. It should be noted that for a dosage of 10 mg kg^{-1} day^{-1}, and for a 120-min reperfusion time, the GSSG/GSH ratio almost returned to the sham-operated value (Fig. 5).

At the biliary level, ischemia–reperfusion induced a drop in GSH (−76%) and GSSG (−56%) concentrations (Fig. 6). S-15176 dose-dependently enhanced GSSG level whereas the increase in GSH level remained constant with the dose (Fig. 6). In parallel, the GSSG/GSH ratio, which is a good index of oxidative stress (Lauterburg et al., 1984; Fujikawa et al., 1994), increased; this ratio was significantly greater for the higher dosages of S-15176 (Fig. 5).

3.4. Hepatic ATP concentration and bile flow

The evolution of liver ATP content after ischemia–reperfusion is shown in Fig. 7. ATP decreased in both the

Table 1
Plasma alanine and aspartate aminotransferase evolution after 120 min of warm ischemia followed by reperfusion

	Preoperative	Days after reperfusion			
		0	3	7	10
Plasma alanine aminotransferase					
Sham	33.9 ± 6.8 (15)	39.6 ± 6.2 (15)	39.3 ± 6.9 (15)	37.5 ± 6.6 (15)	36.9 ± 8.4 (15)
Non-treated	32.1 ± 0.7 (15)	1081 ± 224* (14)	212 ± 41* (11)	93.5 ± 14.0* (10)	46.2 ± 8.4* (10)
S-15176 (mg kg^{-1} day^{-1})					
1.25	38.0 ± 5.2 (15)	885 ± 90*,*** (14)	133 ± 21* (12)	66.2 ± 7.7* (12)	61.6 ± 7.9* (12)
2.5	35.7 ± 6.2 (15)	779 ± 102*,**** (14)	123 ± 16* (13)	62.2 ± 7.8* (13)	44.1 ± 6.8**** (13)
5	38.5 ± 4.1 (15)	707 ± 120*,**** (15)	91.1 ± 20.7* (14)	66.4 ± 7.1* (14)	36.5 ± 3.8 (14)
10	36.1 ± 5.5 (15)	730 ± 135*,***(15)	72.8 ± 17.1* (14)	49.8 ± 10.4* (14)	39.4 ± 7.6 (14)
Plasma aspartate aminotransferase					
Sham	41.2 ± 7.5 (15)	50.5 ± 9.2 (15)	48.5 ± 7.8 (15)	46.3 ± 5.0 (15)	42.7 ± 8.1 (15)
Non-treated	39.3 ± 4.6 (15)	1140 ± 191* (14)	260 ± 67* (11)	103 ± 12* (10)	78.8 ± 9.6* (10)
S-15176 (mg kg^{-1} day^{-1})					
1.25	38.5 ± 5.9 (15)	884 ± 148*,**** (14)	146 ± 26* (12)	78.6 ± 18.1* (12)	56.7 ± 10.3* (12)
2.5	32.9 ± 12.6 (15)	788 ± 112*,**** (14)	130 ± 27* (13)	99.8 ± 22.6* (13)	61.8 ± 7.0* (13)
5	30.2 ± 10.3 (15)	705 ± 37*,**** (14)	87.1 ± 25* (14)	43.4 ± 10.4**** (14)	35.1 ± 6.9 (14)
10	36.9 ± 4.9 (15)	794 ± 157*,**** (15)	122 ± 27* (14)	47.9 ± 10.6**** (14)	39.3 ± 6.9 (14)

Plasma alanine and aspartate aminotransferase activities (IU/l) were determined just after the end of 30 min reperfusion (0) and 3, 7, or 10 days after. The number of rats is indicated in parentheses.

*ANOVA, $P < 0.001$ vs. preoperative non-treated rats.

**ANOVA, $P < 0.01$, vs. non-treated rats at day 0.

***ANOVA, $P < 0.001$, vs. non-treated rats at day 0.

****ANOVA, $P < 0.005$, vs. preoperative non-treated rats.

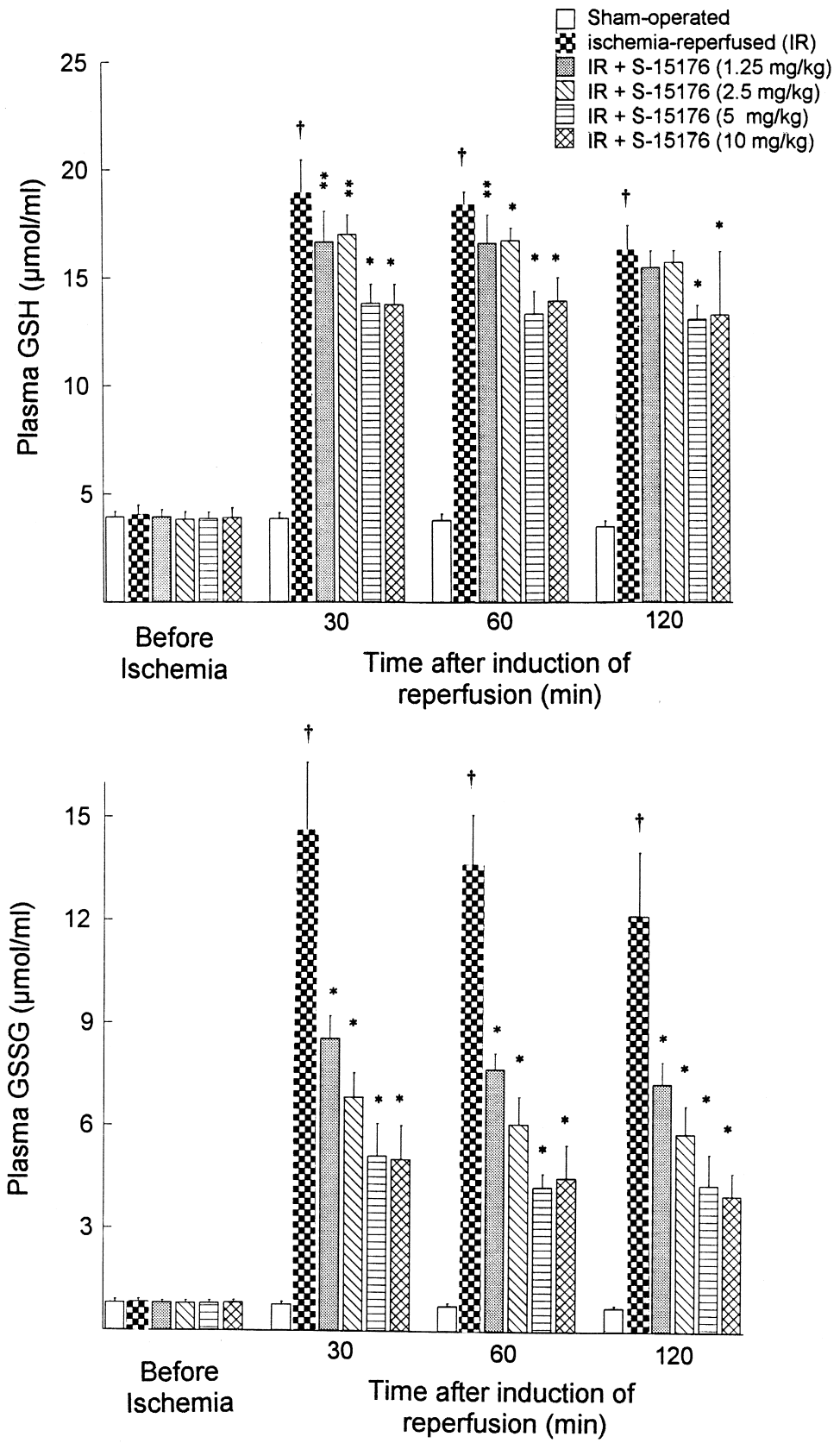

Fig. 4. Effect of increasing dosage of S-15176 on plasma glutathione concentrations measured after 120 min ischemia and different reperfusion times. $^{\dagger}P < 0.001$ vs. sham-operated. $^{*}P < 0.001$, $^{**}P < 0.005$ vs. control ischemia-reperfused rats.

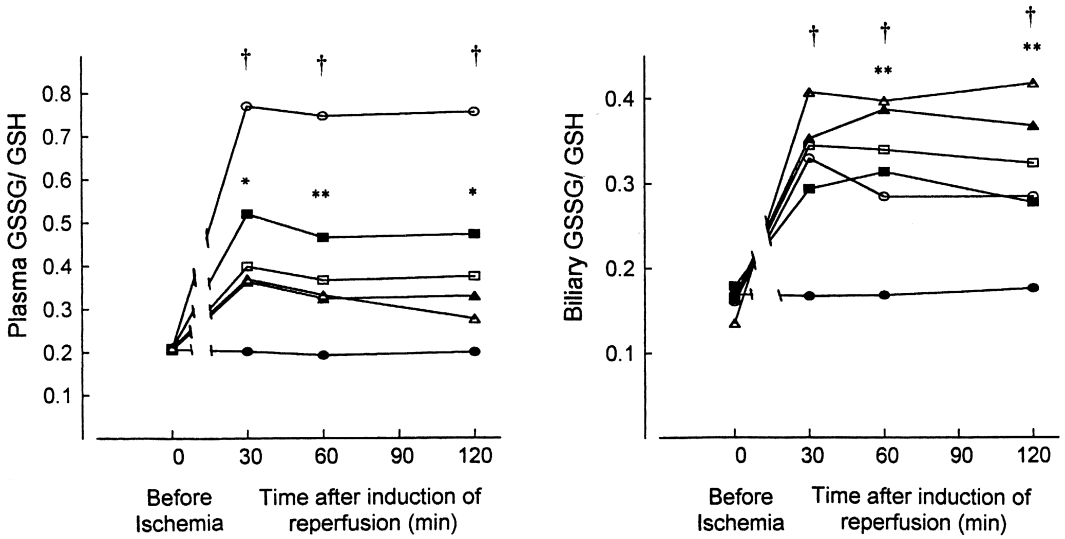

Fig. 5. GSSG/GSH ratio in bile and plasma after 120 min ischemia followed by different reperfusion times in the presence of increasing dosage of S-15176. $^{\dagger}P < 0.001$: ischemia-reperfused (○) vs. sham-operated rats (●). $^{*}P < 0.002$: rats pretreated with 1.25 (■), 2.5 (□), 5 (▲) or 10 mg kg^{-1} day^{-1} (△) vs. control ischemia-reperfused rats (○). $^{**}P < 0.05$: rats pretreated with 5 (▲) or 10 mg kg^{-1} day^{-1} (△) vs. control ischemia-reperfused rats (○).

non-treated (−67% and −56% at 30 and 60 min, respectively) and the treated groups. S-15176 pretreatment maintained a higher hepatic ATP level and this effect was observed at each dosage of the drug. It was especially marked after 60 min reperfusion at the higher S-15176 dose. Under these conditions, ATP content was maintained at a level almost 86% of the sham-operated group.

This protective effect was also observed when bile flow was studied (Fig. 8). S-15176 improved bile flow but a daily dosage of at least 2.5 mg kg^{-1} day^{-1} was necessary to obtain this effect. In addition, in this particular test, 5 mg kg^{-1} day^{-1} appeared slightly more effective than the highest dose.

4. Discussion

Protection of the liver is a major problem after transplantation or resection of the organ. Different pharmacological approaches have been used to prevent ischemia–reperfusion injury associated with hepatic surgery (Sakr et al., 1991; Karwinski et al., 1996; Nishizawa et al., 1997).

Recently, the anti-ischemic agent trimetazidine was shown to protect liver and renal functions from the effects of cold and warm ischemia (Hauet et al., 1997; Settaf et al., 1999). In the present study, we demonstrate that S-15176, a novel derivative of trimetazidine, provides significant protection against ischemia–reperfusion induced liver injuries.

We used a partial ischemic model since lobes 6 and 7 remained perfused during ischemia to avoid portal stasis but they were excised just before reperfusion and so only ischemic lobes supported the survival of the animals. Thirty-four percent of non-treated animals died 7 days after reperfusion. This is in agreement with the results of studies using the same experimental protocol (Nauta et al., 1989). The survival rates of animals treated with S-15176 were higher than that of the control group and increased gradually with increasing dosage. In the rats receiving 5 and 10 mg kg^{-1} day^{-1}, this rate increased to 91.42% and 94.28%, respectively.

This model is very harsh, since the ischemic lobes were dark; we observed many congested areas which have been shown to correspond to large areas of necrosis (Camargo et al., 1997). This is in agreement with other studies which indicated that this protocol of ischemia–reperfusion led to extensive damage of hepatocytes (Okuaki et al., 1996; Yamamoto et al., 1996), especially destruction of mitochondrial functions (Elimadi et al., 1998).

S-15176 prevents hepatic structural damage as measured by the release of alanine and aspartate aminotransferases into the circulation, which is a good indicator of liver injury (Nishimura et al., 1986; Cassidy and Reynolds, 1994). This effect was observed at the lower dosage and was particularly marked after the beginning (30 min) of reperfusion.

Ischemia–reperfusion also caused a massive blood release of GSSG and GSH. This is in agreement with the results of Denno et al. (1995) who concluded that the total amount of GSSG in the plasma is directly correlated with cellular oxidative stress. This confirms that hepatocellular membranes were permeabilized during ischemia–reperfusion and that the liver may have responded to the cellular oxidant stress by consuming its glutathione pool since the plasma GSSG/GSH ratio increased. Identical glutathione responses were found either in similar models of is-

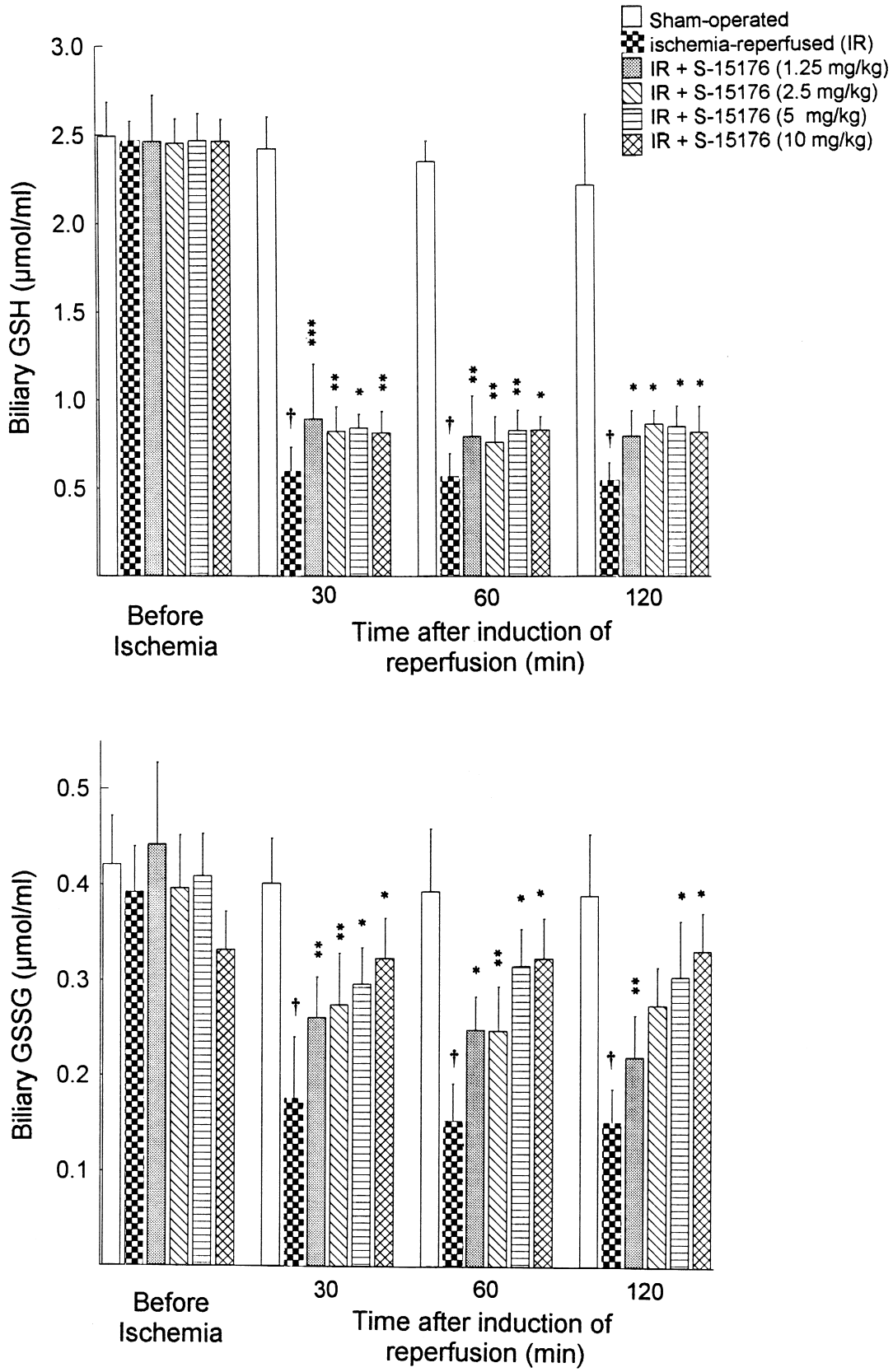

Fig. 6. Effect of increasing dosage of S-15176 on biliary glutathione concentrations measured after 120 min ischemia and different reperfusion times. $^{\dagger}P < 0.001$vs. sham-operated. $^{*}P < 0.001$, $^{**}P < 0.005$, $^{***}P < 0.01$ vs. control ischemia-reperfused rats.

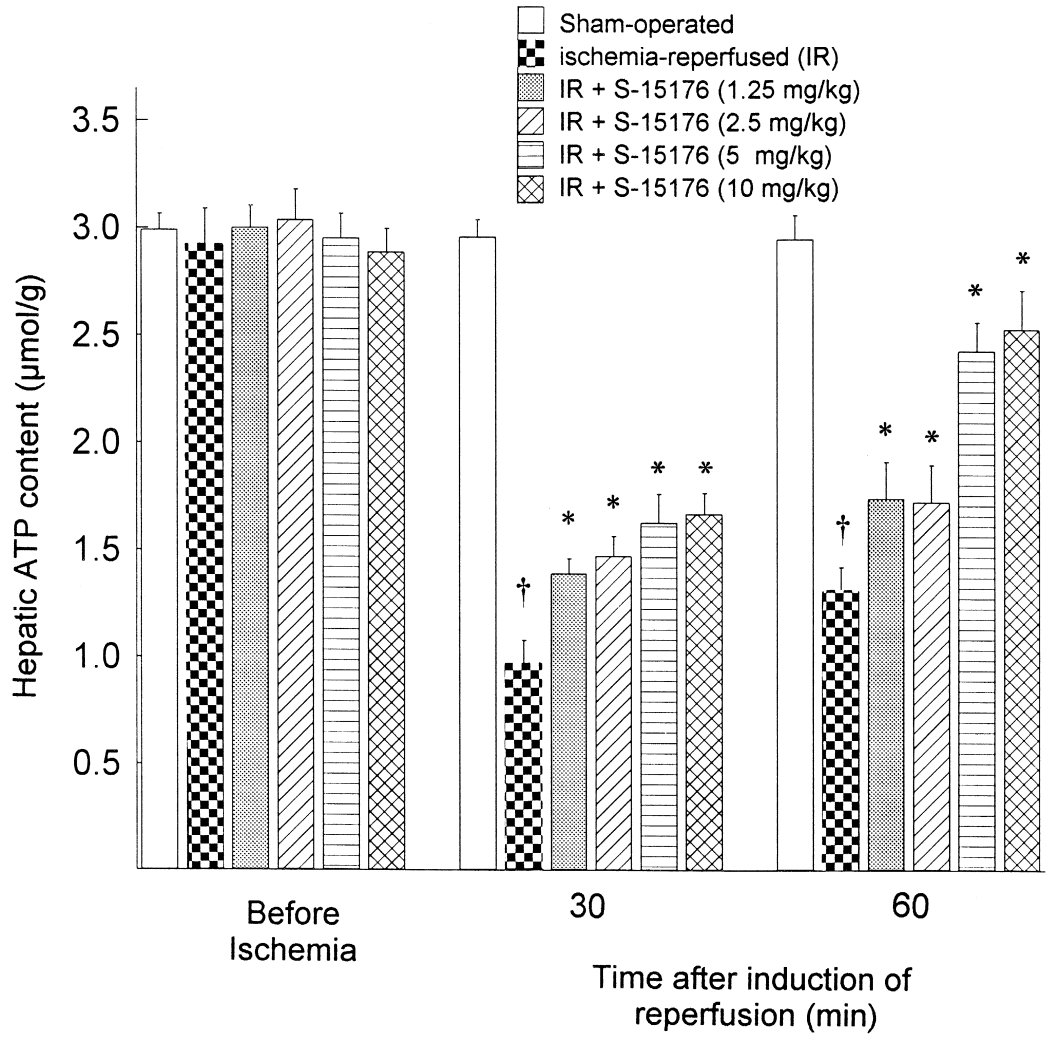

Fig. 7. Protective effect by S-15176 administration against the decrease in ATP level caused by ischemia–reperfusion. Rats were pretreated with increasing doses of S-15176 for 5 days before the induction of 120 min ischemia followed by different reperfusion times. †$P < 0.001$: statistically different from sham-operated rats. *$P < 0.001$: statistically different from non-treated (no S-15176) ischemia-reperfused rats.

chemia–reperfusion (Karwinski et al., 1996) or in a model simulating pure oxidative injury by intravenous injection of tertbutyl hydroperoxide (Poggetti et al., 1995). At the biliary level, ischemic liver injury is known to inhibit total glutathione efflux (Jaeschke et al., 1988). In the present study, it was considerably reduced and corresponded to an increase in the biliary GSSG/GSH ratio, consistent with previous studies (Lauterburg et al., 1984; Fujikawa et al., 1994; Karwinski and Soreide, 1997).

S-15176 treatment causes a clear decrease in plasma GSSG levels, and even normalized the plasma GSSG/GSH ratio in the group treated with the higher dosage. This strongly suggests that the antioxidant properties demonstrated for the compound in vitro could play a major role in its protective effect.

S-15176 also significantly attenuated the drop of glutathione efflux into the bile but concomitantly increased the biliary GSSG/GSH ratio because of the higher increase in biliary GSSG. We were surprised to find that S-15176 increased a parameter indicator of oxidative stress and, therefore, appeared to amplify the effect of the ischemia–reperfusion process. However, this phenomenon is not inconsistent with a protective mechanism; it may be explained by an increase of the efficiency of the enzymatic defenses of the cell, the amount of GSSG excreted into the bile being proportional to the amount of hydroperoxides reduced (Akerboom et al., 1982). This observation may indicate that S-15176 would be capable of accelerating the detoxification process and to enhance GSSG formation and secretion into the bile. This may also explain why the increase in biliary GSH concentration remained constant whatever the dose of S-15176 (Fig. 6).

The hepatic ATP concentration has also been used extensively in several studies as an indicator of liver function (Kamiike et al., 1982; Metzger and Lauterburg, 1988). A marked decrease of hepatic ATP levels was

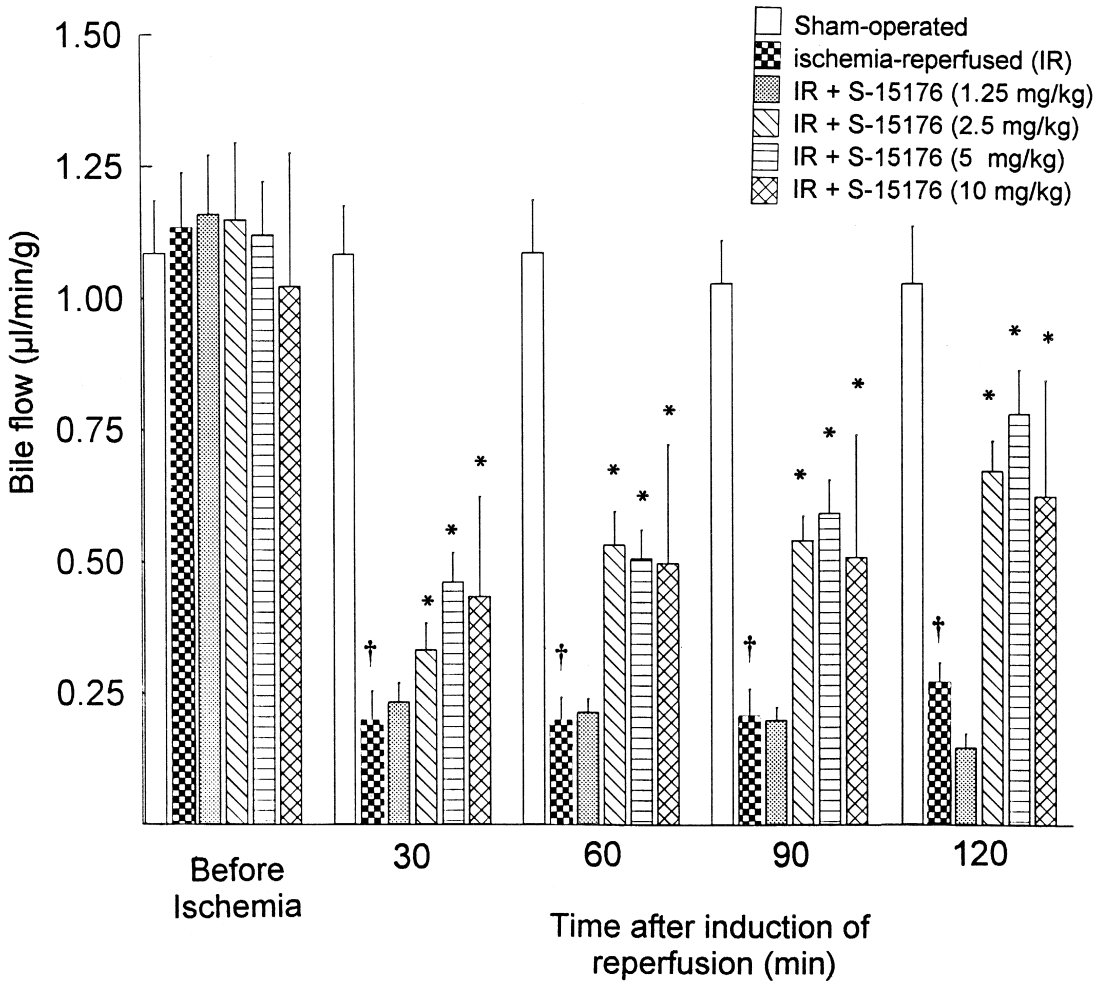

Fig. 8. Protective effect by S-15176 administration against the decrease in bile flow caused by ischemia–reperfusion. Rats were pretreated with increasing doses of S-15176 for 5 days before the induction of 120 min ischemia followed by different reperfusion times. †$P < 0.001$: statistically different from sham-operated rats. *$P < 0.001$: statistically different from non-treated (no S-15176) ischemia-reperfused rats.

observed in non-treated ischemic livers confirming that oxidative phosphorylation is rapidly and seriously affected by ischemia–reperfusion (Kurokawa et al., 1996; Xia et al., 1996). S-15176 partially restored the ATP content in the liver regardless of the time after reperfusion, 30 or 60 min. In this test, S-15176 appeared more effective than its parent drug since it maintained higher ATP levels in ischemic cells at a lower dosage.

It is known that bile production is correlated with the generation of ATP and is therefore also a reliable index of liver function (Karwinski et al., 1989). S-15176 improved both parameters to the same extent, suggesting that the drug effectively attenuates ischemia–reperfusion injury by improving energy synthesis recovery. Thus, one can hypothesize that the protective effect of S-15176 could be related to its mitochondrial effects. This idea was reinforced by the observation that its parent drug, trimetazidine, protects liver against warm ischemia–reperfusion injuries by preserving mitochondrial functions, preventing the occurrence of the increase of membrane mitochondrial permeability which seems to be responsible for the events leading to cell death (Crompton, 1999).

Our present data do not provide detailed information on the mechanism of action of the compound since it is impossible to establish if the reduction in ATP decrease is due to a decrease in the loss of ATP, or resynthesis of ATP during reperfusion. S-15176 may act by two different pathways, one with antioxidant and one with non-antioxidant effects.

Our future studies will focus on the effects of this drug on mitochondrial functions.

In summary, when administered prior to ischemia–reperfusion, S-15176 protects hepatocytes from ischemic injury. It improves ATP regeneration, bile flow, total glutathione efflux in the bile and limits the release of liver enzymes. Additional work is required to characterize the

mechanism of this protective effect, but S-15176 appears to be a promising drug for limiting the tissue damage resulting from hepatic ischemia followed by reperfusion.

Acknowledgements

The authors would like to thank Dr A. Le Ridant, Institut de Recherches Internationales Servier, for his help and his gift of S-15176. We also gratefully acknowledge Dr WS. Neckameyer (Department of Pharmacology, Saint Louis University School of Medicine, USA) and Dr E. Schenker (Institut de Recherches Internationales Servier) for rereading the manuscript. This work was supported by grants from the Réseau de Pharmacologie Clinique, the Ministère de l'Education Nationale (EA 427) and by the Institut de Recherches Internationales Servier.

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