Pretreatment with intrathecal or intravenous morphine attenuates hepatic ischaemia–reperfusion injury in normal and cirrhotic rat liver

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Editor’s key points

- Opioid preconditioning protects against ischaemia–reperfusion injury (IRI) in many organs.
- Morphine given i.v. or intrathecally protected against IRI in both normal and cirrhotic rat liver.
- This effect required peripheral μ-opioid receptor activation and multiple prosurvival intracellular signalling pathways.
- Further studies are required to establish the clinical significance of these effects.

Background. Opioids have been shown to attenuate ischaemia–reperfusion injury (IRI) in a number of organs. We evaluated the effect of morphine pretreatment on IRI in both normal and cirrhotic rat liver.

Methods. Morphine was administered either i.v. or intrathecally (i.t.) 10 min before initiating 1 h of ischaemia followed by 6 h reperfusion in normal rat liver. Hepatic injury was assessed histologically using Suzuki’s criteria. These manoeuvres were repeated using the optimal dose of morphine after administration of naloxone methiodide and wortmannin. Serum levels of transaminases were measured, and expression of phosphorylated Akt, Jak2, and STAT3 were assessed by immunoblotting. Similar procedures were repeated on rats with carbon tetrachloride-induced liver cirrhosis, and the levels of phosphorylated protein kinase C (PKC), haem oxygenase-1 (HO-1), and inducible nitric oxide synthase (iNOS) were also evaluated, as these proteins have beneficial effects during IRI.

Results. Morphine pretreatment at 100 μg kg⁻¹ (i.v.) or 10 μg (i.t.) reduced necrosis, apoptosis, and serum transaminase levels, and increased phosphorylated Akt and STAT3 but not JAK2 expression in normal liver. These changes were reversed by prior administration of naloxone methiodide and wortmannin. Although morphine preconditioning was also protective in cirrhotic liver, STAT3 and JAK2 phosphorylation status was unchanged. There was, however, increased expression of phosphorylated PKC and HO-1, and a reduction in iNOS.

Conclusions. Morphine preconditioning protects against IRI in both normal and cirrhotic rat liver. This involves opioid receptors, phosphatidylinositol-3-kinase, and Akt. The downstream pathways involved are different for cirrhotic liver, with preliminary evidence suggesting involvement of HO-1.

Keywords: hepatic ischaemia–reperfusion injury; intrathecal morphine; liver, cirrhosis; liver, damage; opioids

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Exogenous opioid administration can precondition many different tissues against ischaemia–reperfusion injury (IRI) through opioid receptor activation and recruitment of prosurvival pathways such as the PI3K/Akt/eNOS or JAK/STAT pathways. Neuraxial administration of morphine has also been shown to initiate remote cardioprotection without involving peripheral opioid receptors. Although the liver also contains opioid receptors and activation of prosurvival signalling pathways has been shown to be hepatoprotective, only recently has a clinical opioid been shown to reduce hepatic IRI in rats with normal livers. Previously, only the experimental δ-opioid agonist DADLE was shown to confer protection against IRI.

Patients prone to hepatic ischaemia often have concomitant cirrhotic disease. Cirrhotic livers can develop more extensive injury after ischaemia–reperfusion, probably because of diminished anti-oxidative capacity. Haem oxygenase 1 (HO-1), an inducible enzyme involved with haem catabolism, has anti-inflammatory, anti-apoptotic and anti-oxidative properties, and the induction of HO-1 has been shown to reduce hepatic IRI in both normal and cirrhotic liver. Protein kinase C (PKC) translocation HO-1 and inducible nitric oxide synthase (iNOS) induction have shown to be involved in the late phase of hepatic ischemic preconditioning. Whether these pathways are involved with other modes of hepatic protection has not been examined.

In this study, we aimed to determine whether morphine, administered via either the i.v. or intrathecal (i.t.) routes, can ameliorate hepatic IRI in rats with either normal or cirrhotic livers. We also tested whether morphine-mediated
hepato-protection involves μ-opioid receptor activation and the PI3K/Akt and Jak2/STAT3 pathways as these pathways have previously been shown to be involved in morphine-mediated cardioprotection. The haem oxygenase system has been implicated as a modulator of hepatobiliary function and HO-1 might be important in modulating iNOS activity, thus playing a protective role in liver cirrhosis; so, we also evaluated the role of HO-1 induction in IRI protection of the cirrhotic liver.

Methods

All procedures were performed with approval from our institutional animal ethics committee. Male Sprague–Dawley rats weighing 250–300 g were used for experiments on normal liver. The same strain of rats weighing between 160 and 180 g was used first for the induction of liver cirrhosis and later for IRI studies. Animals were housed in separate cages and given free access to food and water, except for 1 h before the study, and exposed to 12 h light and dark cycles.

I.T. catheter insertion

Anaesthesia was induced by injecting pentobarbital 50 mg kg⁻¹ body weight intraperitoneally. After cleaning the skin with 10% povidone iodine, a 4 cm polyethylene-10 (external radius 0.4 mm and internal radius 0.2 mm) catheter was placed carefully through a puncture in the middle of the atlanto-occipital membrane to the thoracic spinal cord level. Correct catheter placement was confirmed by backflow of cerebrospinal fluid. The wound was closed by interrupted 3-0 silk sutures. The rat returned to its cage and recovered from anaesthesia (2 mg kg⁻¹ body weight intraperitoneally). The forelimbs and hind limbs were monitored daily for gross motor and sensory deficits. Those rats displaying neurological deficits were excluded from further studies and euthanized.

Induction of liver cirrhosis

Sprague–Dawley rats received biweekly subcutaneous injection of 99% carbon tetrachloride (CCl₄) diluted 1:1 with olive oil at a dose of 0.2 ml 100 g⁻¹ of body weight for 5 weeks. To confirm the effect of CCl₄ injection, liver biopsies (~20 mm³) were taken from the left lobe just before the induction of IRI. Animals that did not display histological characteristics of cirrhosis were excluded from analysis.

Induction of hepatic IRI

After induction of anaesthesia with pentobarbital 50 mg kg⁻¹ intraperitoneally, the liver was exposed through a midline laparotomy. The right branches of the hepatic artery and portal vein were occluded with a microvascular clamp that resulted in complete interruption of blood supply to the right and caudate lobes. The clamp was removed after 60 min for 6 h of reperfusion. At baseline and at the end of the reperfusion period, 1–2 ml of blood was collected from the vena cava using non-heparinized syringes. The ischaemic liver lobes were removed and washed with cold phosphate-buffered saline and dried. The caudate lobe was fixed in 10% formalin for 48 h then embedded in paraffin for haematoxylin–eosin staining. The remainder of the ischaemic liver was frozen in liquid nitrogen immediately and then stored at −80 °C for batch processing for immunoblotting and terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling (TUNEL) staining analysis. After removal of the liver, the rat was euthanized by i.v. injection of pentobarbital (150 mg kg⁻¹).

Preconditioning protocols and study groups

I.V. morphine at doses of 1, 10, or 100 μg kg⁻¹ or i.t. morphine at doses of 0.1, 1, or 10 μg kg⁻¹ were administered to rats with normal liver 10 min before the onset of hepatic ischaemia to determine whether morphine preconditioning confers hepatic protection, and if so whether it had any dose–response characteristics. Subsequent mechanistic and cirrhotic liver studies used only the optimal protective dose (lowest Suzuki score, see below). I.V. or i.t. morphine was administered after prior i.v. administration of the peripherally restricted opioid receptor antagonist naloxone methiodide (50 mg kg⁻¹) (Sigma-Aldrich, St. Louis, MO, USA), or the PI3K inhibitor wortmannin (15 μg kg⁻¹, 900 μg dissolved in 1 ml DMSO; Tocris Bioscience, Bristol, UK) in rats with normal livers. The control group received saline and underwent hepatic IRI only.

Histopathological examination

The effect of morphine preconditioning on hepatic architecture was assessed by haematoxylin–eosin staining. Small samples (20 mm³) were embedded in cassettes and fixed in 10% formalin for 48 h and then embedded in paraffin and cut into 4 μm slices for examination by a blinded investigator. Histological changes were graded from 0 to 4 based on Suzuki’s criteria.¹¹ No changes in sinusoidal congestion, hepatocyte vacuolation, and necrosis were marked as 0 while severe congestion/vacuolation and more than 60% necrotic areas were scored as 4.

Assessment of apoptosis

The presence of apoptotic cells in liver samples was quantified by TUNEL staining. The results were scored by counting the number of apoptotic cells as a percentage of the total number of hepatocytes in 30 random high power fields (200×) per sample. The expression of cleaved Caspase-3, a critical cell signal for apoptosis, was also assessed using immunoblotting (see below).

Measurement of serum aminotransferase

Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured using the commercially available VITROS 250/350/950/5, 1 FS and 4600 Chemistry Systems and the VITROS 5600 Integrated System that uses reflectance spectrophotometry technology.
Immunoblot analysis of pAkt, pJAK2, pSTAT3, pPKC, HO-1, and iNOS

Fresh ischaemic liver tissue (50 mg) was homogenized and placed in microcentrifuge tubes with 500 μl 1× cell lysis buffer (20mM Tris-HCl, 150mM NaCl, 1mM EDTA, 1mM EGTA, 1% Triton, 2.5mM sodium pyrophosphate, 1mM beta-glycerophosphate, 1mM Na3VO4, 1μg/ml leupeptin; Cell Signaling Technology, Inc. Danvers, MA, USA). Proteins were separated on a 10% sodium dodecyl sulphate–polyacrylamide gel to target molecular weight above 60 kDa and a 12% gel buffer for those below 60 kDa. After completion of electrophoresis, the gel was transferred to blotted polyvinylidene fluoride (PVDF) membrane and was blocked with 5% non-fat milk for 1 h. The PVDF membrane was then incubated with primary antibodies to the above proteins [1:1000 diluted in 5% Bovine Serum Albumin (BSA) in 1× Tris-Buffered Saline Tween 20 (TBST)] at 4°C overnight [primary antibodies: phospho-Akt (pAkt, Ser473), total-Akt, phospho-JAK2 (pJAK2), total JAK2, phospho-STAT3 (pSTAT3, S727), phospho-STAT3 (pSTAT3, T705), phospho-PKC (pPKC), HO-1 and iNOS were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA)]. After washing with 1× TBST trice, the PVDF membrane was incubated with the horseradish peroxide-conjugated secondary antibody for 1 h [secondary antibodies: goat anti-rabbit IgG, Merck Millipore (Billerica, MA, USA)]. The signal was detected by the ECL kit (ECL, GE Healthcare Biosciences, Pittsburgh, PA, USA). The membrane was soaked in the ECL solution and wrapped for 5 min in a dark room. Protein bands were analysed using a computer software program (Quantity One, Bio-Rad Company, Hercules, CA, USA).

Statistics

All results are presented as mean (standard deviation). For immunoblot analysis, the protein expression of the control group is marked as 100%, and the experimental groups are expressed relative to this group. One-way analysis of variance followed by the Tukey test was used for comparing differences in values between the groups and P<0.05 was considered to be significant. Calculations were performed using GraphPad software version 5.0.

Results

A total of 215 animals were used for these studies. Five rats demonstrated neurological injury after i.t. catheter insertion, and were excluded from further experimentation. Of the 40 animals assigned for the induction of cirrhosis, 13 were excluded as they had negative biopsy results for cirrhosis. A total of 197 completed the studies with at least six rats per group.

Effect of morphine pretreatment on preservation of liver architecture and apoptosis

Morphine at 100 μg kg⁻¹ i.v. or 10 μg kg⁻¹ i.t. significantly reduced the degree of congestion and necrosis in normal livers after IRI as reflected by the lower Suzuki scores compared with their respective control groups (Fig. 1a and a). This enhanced preservation of hepatic structure was not apparent at the lower doses for both routes of administration. At these protective doses, morphine also reduced the degree of apoptosis evident in a reduction in the percentage of positive cells on TUNEL staining and reduced expression of cleaved caspase-3 (Fig. 1c).

Effect of peripheral opioid receptor antagonism and inhibition of PI3K inhibition in normal livers

Blood samples obtained from the 100 μg kg⁻¹ i.v. and 10 μg kg⁻¹ i.t. morphine groups for the measurement of AST/ALT did not differ at baseline compared with the control (results not shown), but their levels were markedly reduced compared with the control group at 6 h reperfusion (Table 1). Pretreatment with naloxone methiodide and wortmannin before morphine preconditioning of normal livers attenuated the beneficial effects of morphine preconditioning, with histological appearance, apoptotic cell count, caspase-3 expression, and serum levels of liver enzymes being no different from that of their respective controls. The sole administration of these two compounds did not have any intrinsic detrimental effect on any of these parameters (Fig. 2a–d).

Effect of morphine pretreatment on expression of pAkt, pJAK2, and STAT3 in normal livers

Morphine preconditioning did not affect expression of total Akt, JAK2, and STAT3 in normal liver. However, morphine increased phosphorylated Akt and STAT3 at the S727 site. Interestingly, it had no effect on expression of phosphorylated Jak2 (Fig. 3). Prior PI3K inhibition with wortmannin reversed these changes in phosphorylation due to morphine administration.

Effect of morphine pretreatment on cirrhotic liver

The induction of cirrhosis was confirmed in 28 out of the 40 animals treated with CCl₄ by characteristic histological findings in liver biopsies taken from the left lobes immediately before experimentation. IRI caused more severe vacuolization in cirrhotic liver control group compared with normal liver. Although both i.v. and i.t. morphine reduced the degree of congestion, only i.t. morphine reduced the degree of necrosis (Fig. 4a).

The apoptotic index of hepatocytes was also decreased by morphine pretreatment in cirrhotic liver for both i.v. and i.t. morphine groups when compared with controls (Fig. 4b). Interestingly, there were no differences in the expression of cleaved caspase 3 (Fig. 4c).

Morphine pretreatment also reduced liver enzyme release after IRI in cirrhotic rats. There was no difference in serum enzymes between the groups at baseline (data not shown), but both i.v. and i.t. morphine reduced the serum levels of AST and ALT at 6 h after reperfusion (Fig. 4d).
Fig 1 Dose–response of morphine preconditioning on hepatic IRI in normal liver. (a) Representative haematoxylin and eosin-stained sections of ischaemic livers at different morphine doses and their corresponding Suzuki score. IVCON, i.v. control; IVMPC, i.v. morphine preconditioning; ITCON, i.t. control; ITMPC, i.t. morphine preconditioning; n=6 per group. Data expressed as mean (SD). *P<0.05 vs IVCON; **P<0.05 vs IVMPC; #P<0.05 vs ITCON; ##P<0.05 vs IVCON.
TABLE 1 Serum levels of transaminases at 6 h after reperfusion. ALT, alanine aminotransferase; AST, aspartate aminotransferase; IU, international units; IVCON, i.v. control; IVMPC, i.v. morphine preconditioning at 100 μg kg⁻¹; ITCON, i.t. control; ITMPC, i.t. morphine preconditioning at 10 μg kg⁻¹; NM, naloxone methiodide (50 mg kg⁻¹); W, wortmannin. *P<0.05 compared with control. All data corrected to 3 significant figures.

<table>
<thead>
<tr>
<th>Group</th>
<th>ALT (IU litre⁻¹)</th>
<th>AST (IU litre⁻¹)</th>
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<tr>
<td>IVCON</td>
<td>663 (161)</td>
<td>8410 (7280)</td>
</tr>
<tr>
<td>IVMPC</td>
<td>193 (50)*</td>
<td>728 (90)*</td>
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<tr>
<td>IVMPC + NM</td>
<td>754 (662)</td>
<td>9440 (7960)</td>
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<tr>
<td>NM</td>
<td>784 (474)</td>
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<td>656 (229)</td>
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<tr>
<td>W</td>
<td>770 (292)</td>
<td>12 300 (4810)</td>
</tr>
<tr>
<td>ITCON</td>
<td>659 (294)</td>
<td>8841 (6750)</td>
</tr>
<tr>
<td>ITMPC</td>
<td>254 (95)*</td>
<td>1760 (783)*</td>
</tr>
<tr>
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<tr>
<td>NM</td>
<td>607 (398)</td>
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<tr>
<td>ITMPC + W</td>
<td>575 (145)</td>
<td>9977 (9200)</td>
</tr>
<tr>
<td>W</td>
<td>517 (282)</td>
<td>13 400 (5010)</td>
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</table>

**Effect of morphine pretreatment on hepatic expression of pAkt, pJAK2, pSTAT3, pPKC, HO-1, and iNOS in cirrhotic liver**

As in normal liver, morphine pretreatment did not affect total levels of Akt, JAK2, and STAT3. Although morphine increased expression of pAkt, it did not have any significant effect on pJAK2 and pSTAT3 at either the S727 or the Y705 site, unlike in the normal liver. However, morphine increased expression of phosphorylated pPKC α/βII and HO-1 and decreased iNOS expression (Fig. 5).

**Discussion**

We have shown that morphine pretreatment, by either i.v. or i.t. routes, offers protection against hepatic IRI in normal and cirrhotic liver. Morphine administration better preserved hepatic structures and reduced necrosis, apoptosis, and release of liver enzymes compared with control groups. These beneficial effects were reversible in normal livers by the prior administration of naloxone methiodide and wortmannin, suggesting that this protection is peripheral µ-opioid receptor-mediated and involves phosphatidylinositol-3-kinase (PI3K) and Akt activity.

We considered it prudent to separately examine the two routes of morphine administration as both can be used perioperatively to provide analgesia, but their doses differ by a large order of magnitude. We have previously shown that activation of central opioid receptors by i.t. morphine alone without involving peripheral receptors is sufficient to trigger cardiac preconditioning, whereas i.v. morphine acts on opioid receptors in both the periphery and the central nervous systems to produce this. In contrast, peripheral block of µ-opioid receptors prevented the hepato-protective effects of morphine by both routes. This implies that both i.t. and i.v. morphine-mediated hepato-protection requires peripheral µ-opioid receptor activation.

One possible explanation for the above observation is that i.t. morphine can diffuse across the blood–brain barrier into the blood and bind directly to hepatic opioid receptors. However, this is rather unlikely, given that i.v. morphine required a dose of at least 100 μg kg⁻¹ to be protective, whereas only 10 μg kg⁻¹ was protective by the i.t. route. Further, given the slow kinetics and low systemic concentration of morphine achieved after neuraxial administration, it is improbable that i.t. morphine can achieve a sufficiently high systemic concentration in a timely manner to trigger hepato-protection peripherally. It is more likely that i.t. morphine modulates the activity of opioidergic fibres innervating the liver. This explanation is consistent with previous reports suggesting that the hepatobiliary effects of morphine are, at least in part, mediated in the brain and central opioid receptor stimulation can modulate metabolic activity in the liver. For example, intracerebroventricular injection of morphine has been shown to alter hepatic glutathione levels, an effect which is not affected by prior administration of hexamethonium, atropine, propranolol, or destruction of peripheral adrenergic fibres. Extracellular adenosine generation has been shown to play an important part in the protection of the liver against IRI. The inhibition of ecto-5 nucleotidase, an enzyme involved in adenosine generation, and the induction of ectonucleoside triphosphate diphosphohydrolase (CD39), respectively, reduce or enhance the protection afforded by ischaemic preconditioning of the liver.

Even though the in vivo effects of morphine-mediated hepato-protection are µ-opioid receptor-dependent, these receptors might not be limited to those on hepatocytes. Despite µ-opioid receptors being expressed in the rat liver, the distribution of these receptors among the different cell types within the liver is not well defined. Expression might even be species-specific as other investigators have failed to detect any opioid receptor subtypes in mouse liver using reverse transcriptase–polymerase chain reaction. A previous study using a hepatocyte anoxia/reoxygenation injury in vitro demonstrated that morphine’s cytoprotective effects are independent of opioid receptor activation. Non-parenchymal cells such as Kupffer cells or stellate cells are known to express opioid receptors any could also be the target cells of morphine preconditioning. Their role in in vivo hepato-protection might not be reflected in cell line studies involving only hepatocytes.

The mechanisms of morphine-induced hepato-protection are most likely multifactorial. Akt activation in the liver can enhance cellular survival and reduce apoptosis by phosphorylating BAD (Bcl-2-associated death promoter) and inhibiting the release of cytochrome c. As in morphine-induced cardioprotection, we demonstrated that morphine-induced hepato-protection involved Akt phosphorylation in normal

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**Table 1** Serum levels of transaminases at 6 h after reperfusion. ALT, alanine aminotransferase; AST, aspartate aminotransferase; IU, international units; IVCON, i.v. control; IVMPC, i.v. morphine preconditioning at 100 μg kg⁻¹; ITCON, i.t. control; ITMPC, i.t. morphine preconditioning at 10 μg kg⁻¹; NM, naloxone methiodide (50 mg kg⁻¹); W, wortmannin. *P<0.05 compared with control. All data corrected to 3 significant figures.
The JAK/STAT pathway has been identified as a major signalling pathway activated in response to cytokines and interferons and has been suggested as playing a role in hepatic IRI and hepatocytic apoptosis. Similar to morphine cardioprotection, there was a PI3K-dependent increase in STAT3 phosphorylation. However, unlike that for myocytes, phosphorylation of STAT3 after morphine exposure occurred at the serine site. Further, there was no change in liver JAK2 phosphorylation with morphine pretreatment. These findings support the involvement of the PI3/Akt pathway in

Fig 2 Effect of opioid receptor and Akt inhibition on the effects of morphine pretreatment in normal livers evaluated by Suzuki scores (A), apoptotic index (B), and expression of cleaved caspase-3 (C). The mean band density was normalized against glyceraldehyde-3-phosphate dehydrogenase (GAPDH). IVCON, i.v. control; IVMPC, i.v. morphine preconditioning at 100 μg kg⁻¹; ITCON, i.t. control; ITMPC, i.t. morphine preconditioning at 10 μg kg⁻¹; NM, naloxone methiodide (50 mg kg⁻¹); W, wortmannin (15 μg kg⁻¹); RD, relative density. Data expressed as mean (±SD). n=6 per group, *P<0.05 vs IVCON; #P<0.05 vs ITCON.
Fig 3  Effect of morphine preconditioning on expression of total and phosphorylated Akt, JAK2, and STAT3, in the presence or absence of wortmannin. (a) I.V. morphine preconditioning and (b) i.t. morphine preconditioning groups. The mean band density was normalized against glyceraldehyde-3-phosphate dehydrogenase (GAPDH). IVCON, i.v. control; IVMPC, i.v. morphine preconditioning at 100 μg kg⁻¹; ITCON, i.t. control; ITMPC, i.t. morphine preconditioning at 10 μg kg⁻¹; W, wortmannin (15 μg kg⁻¹). Data expressed as mean (SD). *P < 0.05 vs IVCON; **P < 0.01 vs IVCON; †P < 0.05 vs ITCON; ##P < 0.01 vs ITCON.
Fig 4 Effect of morphine preconditioning on hepatic IRI in cirrhotic liver. (a) Representative haematoxylin and eosin and TUNEL-stained sections of ischaemic cirrhotic liver and their corresponding Suzuki score and apoptotic index. (b) ALT and AST levels at 6 h reperfusion. (c) Cleaved caspase-3 levels, with the mean band density normalized against glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Data expressed as mean (SD). IVMPC, i.v. morphine preconditioning at 100 μg kg⁻¹; ITCON, i.t. control; ITMPC, i.t. morphine preconditioning at 10 μg kg⁻¹; *P<0.05 vs IVCON; **P<0.05 vs ITCON.
the phosphorylation of STAT3, but this activation of STAT3 is independent of JAK2.

Despite morphine having similar beneficial effects on the liver after IRI, there appear to be differences in the downstream effects between normal and cirrhotic liver. Although there was still an increase in phosphorylated Akt, there was no associated increase in JAK2 or STAT3 phosphorylation in cirrhotic liver. We therefore investigated the

**Fig 5** Effect of morphine preconditioning on the expression of total and phosphorylated Akt, JAK2, STAT3, and PKCα/βII, and also HO-1 and iNOS. (A) Immunoblot analysis of the expression of total and phosphorylated Akt, JAK2, and STAT3. (B) Expression of phosphorylated PKC (α/βII subunit), HO-1, and iNOS. The mean band density was normalized against glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Data expressed as mean ± SD. *P<0.05 vs IVCON; **P<0.01 vs IVCON; #P<0.05 vs ITCON.
pathway involving HO-1, as the induction of this enzyme has been associated with a reduction in hepatocyte IRI, including one of its upstream proteins PKC. Analysis showed elevation in phosphorylated PKC (α/βII subunit) along with HO-1 expression, suggesting activation of this pathway. The associated reduction in iNOS expression is consistent with the previous work indicating negative modulation of the products of HO-1 on iNOS expression. The reduction in iNOS expression has been suggested as a mechanism for the hepato-protective effects of HO-1 induction. Therefore, induction of the HO-1 pathway might also be the underlying mechanism of morphine hepato-protection in cirrhotic liver.

This study is limited in that the precise mechanism of how i.t. morphine confers hepato-protection remains unexplained. Possibilities involve neural or humoral pathways or even alteration in systemic inflammatory response, but require further investigation. One of the more interesting implications of this study is that a single dose of i.t. morphine can protect more than one organ against IRI. From a clinical perspective, i.t. morphine is well known to produce prolonged postoperative analgesia and we have now identified another possible benefit. However, this was an animal study and clinical evaluation would be required to evaluate its consistency in humans.

In summary, we provide evidence that i.v. or i.t. morphine pretreatment attenuates hepatic IRI in normal and cirrhotic liver. The cellular protective effects of morphine might be multifactorial and, apart from the involvement of phosphorylated Akt, its effects on the liver are different depending on whether the tissue is normal or cirrhotic.

Declaration of interest

M.G.I. is an Editorial board member of CPD Anaesthesia (UK), CPD Anaesthesia Taiwanica, Case Reports in Medicine Pain Research and Treatment, Expert Opinion on Pharmacotherapy, and Perioperative Medicine, on the International Advisory Board of Anaesthesia, a Board member of the International Society of Anaesthetic Pharmacology, and a Faculty of 1000 Medicine reviewer in the field of Cardiovascular medicine in anaesthesia. G.T.C.W. is an Editorial board member of The Open Anesthesiology Journal and World Journal of Anesthesiology.

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Morphine-mediated hepatic protection


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