

Preservation of Steatotic Livers in IGL-1 Solution

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A new Institut Georges Lopez (IGL-1) solution was used to preserve steatotic livers. Steatotic (obese [Ob]) and nonsteatotic (lean [Ln]) livers from Zucker rats ($n = 16$, 8 Ln and 8 Ob) were preserved for 24 hours at 4°C in University of Wisconsin (UW) or IGL-1 solution, respectively, and then perfused *ex vivo* for 2 hours at 37°C. Additionally, Ob and Ln livers ($n = 16$, 8 Ln and 8 Ob) were preserved in IGL-1 plus *N* ω -nitro-L-arginine methyl ester hydrochloride (L-NAME). Hepatic injury and function (aminotransferases, bile production, bromosulphophthalein clearance), and factors potentially involved in the susceptibility of steatotic livers to ischemia-reperfusion injury, such as oxidative stress, mitochondrial damage, and vascular resistance, were studied. Nitric oxide (NO) production and constitutive and inducible NO synthase were also measured. Steatotic and nonsteatotic livers preserved in IGL-1 solution showed lower transaminases, malondialdehyde, glutamate dehydrogenase levels, and higher bile production than UW-solution-preserved livers. IGL-1 solution protected against oxidative stress, mitochondrial damage and the alterations in vascular resistance associated with cold ischemia-reperfusion. Thus, at the end of reperfusion period, aspartate aminotransferase levels in steatotic livers were 281 ± 6 U/L in UW vs. 202 ± 10 U/L in IGL-1 solution. Glutamate dehydrogenase was 463 ± 75 U/L in UW vs. 111 ± 4 U/L in IGL-1 solution, and oxidative stress was 3.0 ± 0.1 nmol/mg prot in UW vs. 2.0 ± 0.1 nmol/mg prot in IGL-1 solution. These beneficial effects of IGL-1 solution were abolished by the addition of L-NAME, which implicates NO in the benefits of IGL-1. In conclusion, IGL-1 solution provided steatotic livers with better protection against the deleterious effects of cold ischemia-reperfusion injury than did UW solution. *Liver Transpl* 12:1215-1223, 2006. © 2006 AASLD.

Received October 11, 2005; accepted February 25, 2006.

The lack of quality donor organs has led to the used of steatotic livers for transplantation. This practice is accepted despite the higher risk of graft dysfunction or nonfunction associated with ischemia-reperfusion (I/R).^{1,2-6}

Steatosis is due to the fatty accumulation in the cytoplasm of hepatocytes resulting in partial or complete obstruction of hepatic sinusoidal space.^{1,7} This leads to a severe reduction of blood flow with the subsequent microcirculation impairment that may be an important

factor in the decreased tolerance of steatotic liver to cold ischemia (preservation) and rewarming-reperfusion injury.⁷⁻¹⁰ Moreover, the induction of severe mitochondrial damage^{11,12} and increased reactive oxygen species production^{8,13} have also been proposed as factors that might leave steatotic livers vulnerable to I/R injury. Recent studies in experimental models of hepatic I/R indicate that nitric oxide (NO) could modulate the factors implicated in the vulnerability of steatotic livers to hepatic I/R.^{13,14}

Abbreviations: I/R, ischemia-reperfusion; NO, nitric oxide; UW, University of Wisconsin; IGL-1, Institut Georges Lopez; PEG, polyethylene glycol; Ob, obese; Ln, lean; ALT, alanine aminotransferase; AST, aspartate aminotransferase; L-NAME, *N* ω -nitro-L-arginine methyl ester hydrochloride; BSP, bromosulphophthalein; GLDH, glutamate dehydrogenase; MDA, malondialdehyde. Supported by the Ministerio de Ciencia y Tecnología (project grants SAF 2005-00385 and 2005SGR/00781 and BFI 2003-00912, and Ramón y Cajal research contract to Carmen Peralta), the Ministerio de Asuntos Exteriores y Cooperación /Agencia Española de Cooperación Internacional (A4251/05 and HP 2003-0051 research project) and the Ministerio de Sanidad y Consumo (project V2003-REDC03G-O).

Ismail Ben Mosbah holds a fellowship from the AECl.

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DOI 10.1002/lt.20788

Published online in Wiley InterScience (www.interscience.wiley.com).

The composition of preservation solutions is critical for the preservation of the quality of livers kept for prolonged ischemic periods. The University of Wisconsin (UW) solution is considered the gold standard for abdominal organ preservation,^{15,16} as it prevents organ damage during cold ischemia and extends the threshold for prolonged storage.^{17,18} UW solution can improve graft and patient survival in liver transplantation.¹⁹ However, some of the properties of UW solution do not favor the organ preservation, such as high potassium levels (required to flush the organ before graft reperfusion in the recipient)²⁰ and the presence of hydroxyethyl starch as oncotic support, which could be responsible for red blood cell aggregation.^{21,22}

Recently, a modified UW preservation solution called Institut Georges Lopez (IGL-1), characterized by the inversion of K⁺ and Na⁺ concentration and the replacement of hydroxyethyl starch by polyethylene glycol (PEG) in the original UW solution, has been successfully used in kidney transplantation.²³⁻²⁵ In our study, we examined the ability of IGL-1 preservation solution to protect steatotic liver grafts against the deleterious effects of I/R injury, as well as the underlying mechanisms responsible for this protection.

METHODS AND MATERIALS

Experimental Animals

Homozygous (obese [Ob]) and heterozygous (lean [Ln]) Zucker rats aged 16-18 weeks old were purchased from Iffa-Credo (L'Abresle, France).^{8,13} We used a perfused rat liver model to evaluate hepatic function, isolated from the influence of other organ systems, undefined plasma constituents, and neural-hormonal effects. Hepatic architecture, microcirculation, and bile production are preserved in this experimental model.^{26,27} All procedures were performed under isoflurane inhalation anesthesia. The study respected the European Union regulations (Directive 86/609 EEC) for animal experiments.

Preservation Solutions

The composition of the preservation solutions is shown in Table 1. UW solution (ViaSpan, Madrid, Spain) is the original Belzer solution without dexamethasone, insulin, or antibiotics. IGL-1 solution is a modified UW solution manufactured by the Institut Georges Lopez in Lyon, France that was obtained by replacing hydroxyethyl starch with PEG and changing the concentrations of Na⁺ and K⁺.

Liver Procurement

The surgical technique was performed as previously described.²⁸ Briefly, the abdomen was opened by midline incision after cannulation of the common bile duct; the portal vein was isolated, and the splenic and gastroduodenal veins were ligated.

TABLE 1. Composition of the Preservation Solutions Tested

Component	UW	IGL-1
Hydroxyethyl starch (mmol/L)	0.25	—
PEG (35 Kd) (mmol/L)	—	0.03
Na ⁺ (mmol/L)	30	125
K ⁺ (mmol/L)	125	30
Lactobionic acid (mmol/L)	100	100
Raffinose (mmol/L)	30	30
MgSO ₄ (mmol/L)	5	5
KH ₂ PO ₄ (mmol/L)	25	25
Glutathione (mmol/L)	3	3
Adenosine (mmol/L)	5	5
Allopurinol (mmol/L)	1	1
pH	7.2-7.4	7.2-7.4

Protocol I: Effect of IGL-1 Solution on Steatotic Liver Injury After 24 Hours of Cold Ischemia

After 24 hours of cold storage, livers from 16 Zucker rats (8 Ln and 8 Ob) preserved in UW solution (UW group) and livers from 16 Zucker rats (8 Ln and 8 Ob) preserved in IGL-1 solution (IGL-1 group) were flushed with Ringer's lactate solution. The aliquots of the effluent flush were sampled for measurements of cumulative alanine aminotransferase (ALT) and aspartate aminotransferase (AST) after prolonged ischemia. Control livers (CONT 1 group) (16 Zucker rats; 8 Ln and 8 Ob) were flushed the portal vein without ischemic preservation.

Protocol II: Effect of IGL-1 Solution on Steatotic Liver Injury After 24 Hours of Cold Ischemia Followed by 2 Hours of Normothermic Reperfusion

After 24 hours of cold preservation, livers from 16 Zucker rats (8 Ln and 8 Ob) preserved in UW solution, livers from 16 Zucker rats (8 Ln and 8 Ob) preserved in IGL-1 solution, and livers from 16 Zucker rats (8 Ln and 8 Ob) preserved in IGL-1 solution with N ω -nitro-L-arginine methyl ester hydrochloride (L-NAME), an inhibitor of NO synthesis at a dose of 60 μ g/g liver²⁹ (Sigma Co., St Louis, MO) were left for 30 minutes at 22°C prior to reperfusion to account for the period of rewarming during surgical implantation in vivo.^{30,31} Livers were connected via the portal vein to a recirculating perfusion system for 120 minutes at 37°C.^{28,32} Control livers (CONT 2 group) (16 Zucker rats; 8 Ln and 8 Ob) were flushed and immediately perfused ex vivo without ischemic preservation. Time 0 was the point at which the portal catheter was satisfactorily connected to the circuit. As previously reported,^{28,33} during the first 15 minutes of perfusion (initial equilibration period), the flow was progressively increased to stabilize the portal pressure at 12 mmHg (Pression Monitor BP-1, Instruments, Inc., Sarasota, FL). The flow was controlled using a peristaltic pump (Minipuls 3, Gilson, France)^{28,32}

The reperfusion liquid consisted of a cell culture medium (William's medium E, Bio Whitaker, Madrid, Spain), with Krebs-Heinseleit-like electrolyte composition enriched with 5% albumin, as osmotic support. The buffer was continuously ventilated with a 95% O₂ and 5% CO₂ gas mixture. The buffer was subsequently passed through a heat exchanger (37°C) and a bubble trap prior to entering the liver.^{28,31} After the initial equilibration period, flow rate and vascular resistance were assessed continuously throughout the reperfusion period. Transaminases were evaluated throughout the reperfusion period. Bile output, hepatic clearance (expressed as percentage of bromosulphophthalein [BSP] in bile samples), lipid peroxidation, glutamate dehydrogenase (GLDH) activity, nitrite and nitrate, and constitutive and inducible NO synthase were determined at 120 minutes of reperfusion.

Biochemical Determinations

Transaminase Assay

Hepatic injury was evaluated according to transaminase levels using a commercial kit from Boehringer Mannheim (Munich, Germany).

Glutamate Dehydrogenase Activity

Mitochondrial enzymes release at the end of reperfusion was measured in perfusate using enzymatic method. GLDH is a mitochondrial enzyme that indicates mitochondrial damage.³¹

Bile Output

Liver function was assessed by measurement of bile production.^{6,34} Bile was collected through the cannulated bile duct and output reported as $\mu\text{L/g}$ liver.

Hepatic Clearance

Hepatic clearance was considered another parameter of hepatic function.^{35,36} Thirty minutes after the onset of the perfusion (t_{30}), 1 mg BSP (Sigma Co.) was added to the perfusate. The concentration of BSP in bile samples (t_{120}) was measured at 580 nm with a ultraviolet-visible spectrometer. Bile BSP excretion was expressed as a percentage of perfusate content (t_{120} bile/ t_{30} perfusate \cdot 100).^{35,36}

Flow Rate and Vascular Resistance

Liver circulation was assessed by measuring perfusion flow rate and vascular resistance.^{6,9} Perfusion flow rate was assessed continuously throughout the reperfusion period. Vascular resistance was defined as the ratio of portal venous pressure to flow rate and expressed as $\text{mmHg} \cdot \text{minute} \cdot \text{g/mL}$.^{6,9}

Lipid Peroxidation Assay

Lipid peroxidation, used as an indirect measurement of the oxidative injury induced by reactive oxygen species,^{13,37} was determined by measuring the formation

of malondialdehyde (MDA) with the thiobarbiturate reaction.^{13,37}

Determination of Nitrite and Nitrate

NO production was determined by tissue accumulation of nitrite and nitrate.^{38,39}

Western Blot Analysis of NO Synthase

Liver tissue was homogenized as previously described,⁴⁰⁻⁴² and proteins were separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes. Membranes were immunoblotted with antibodies anti-eNOS (1:500) and anti-inducible NO synthase (1:1000) (Transduction Laboratories, Lexington, KY). Signals were detected by enhanced chemiluminescence and quantified by scanning densitometry. All signals were normalized to the corresponding ponceau S.⁴⁰⁻⁴²

Statistics

Data are expressed as means \pm standard error and were compared statistically by variance analysis, followed by the Student-Newman-Keuls test. $P < 0.05$ was considered significant.

RESULTS

Protocol I: Effect of IGL-1 Solution on Steatotic Liver Injury After 24 Hours of Cold Ischemia

Hepatic damage due to the cold ischemia process was established by the measurement of AST and ALT levels in liver effluents.^{32,43} As shown in Figure 1, AST and ALT levels measured in steatotic and nonsteatotic livers stored in UW and IGL-1 preservation solutions for 24 hours were significantly more impaired than levels in unstored livers (CONT 1). The higher AST and ALT levels in steatotic livers preserved in UW and IGL-1 solutions compared with nonsteatotic ones are consistent with the increased vulnerability of steatotic livers to cold ischemia injury. Significantly lower AST and ALT levels were found in steatotic and nonsteatotic livers preserved in IGL-1 solution than in UW solution (Fig. 1).

Protocol II: Effect of IGL-1 Solution on Steatotic Liver Injury After 24 Hours of Cold Ischemia Followed by 2 Hours of Normothermic Reperfusion

As shown in Figure 2, higher perfusate transaminase levels were observed in steatotic livers as the reperfusion period progressed, compared with those found in nonsteatotic livers. During normothermic reperfusion, the AST and ALT levels in both types of liver preserved in IGL-1 were lower than in UW-preserved livers, especially steatotic. L-NAME addition to IGL-1 preservation solution led to AST and ALT levels similar to those observed for UW solution for steatotic and nonsteatotic livers, respectively.

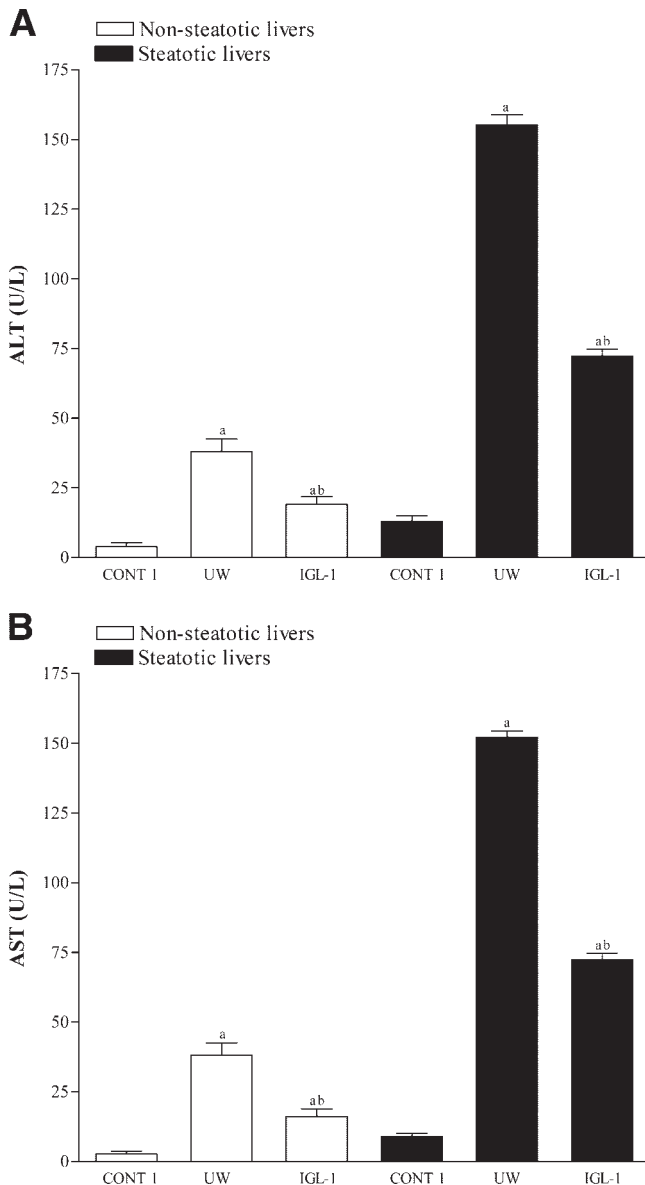


Figure 1. (A) ALT and (B) AST levels in flushing effluent after 24 hours of cold storage. White bars represent nonsteatotic livers. Black bars represent steatotic livers. CONT 1, liver flushed without cold preservation; UW, liver preserved in UW solution; IGL-1, liver preserved in IGL-1 solution.. * $P < 0.05$ vs. CONT 1, ** $P < 0.05$ vs. UW.

Steatotic and nonsteatotic liver function was assessed by measuring bile production and BSP clearance. Bile output and the percentage of BSP in bile were lower in livers preserved in UW and IGL-1 solution than in control livers (CONT 2) (Fig. 3). Both liver function parameters improved significantly when steatotic and nonsteatotic livers were preserved in IGL-1 solution. L-NAME addition to IGL-1 solution led to similar bile output (1.72 ± 0.13 uL/g/liver/120 min) and percentage of BSP (4.0 ± 0.9) values in steatotic livers preserved in UW solution. A similar pattern was obtained for nonsteatotic livers.

We also evaluated the liver oxidative stress (mea-

sured as lipoperoxides) at the end of the reperfusion period, as shown in Figure 4. Steatotic and nonsteatotic livers preserved in IGL-1 and UW solution showed greater MDA than their respective control groups. MDA levels were higher in steatotic livers. IGL-1 solution prevented the increase of MDA levels significantly more than UW in both steatotic and nonsteatotic livers. L-NAME addition to IGL-1 solution gave values of MDA for steatotic and nonsteatotic livers similar to those observed in livers preserved in UW solution.

We also evaluated liver vascular resistance during liver reperfusion. As shown in Figure 5, marked increases in the vascular resistance and lower perfusion flow rate (data not shown) were observed in steatotic livers preserved in UW solution during the reperfusion period ($t_{30,60,90,120}$) compared with the rates in nonsteatotic livers. Both liver types preserved in IGL-1 solution showed lower vascular resistance and higher perfusion flow rate than those preserved in UW solution. L-NAME addition to IGL-1 solution gave values of vascular resistance and perfusion flow rate for steatotic and nonsteatotic livers similar to those found in livers preserved in UW solution.

Mitochondrial damage was evaluated by measuring GLDH activity levels in perfusate at the end of reperfusion period. Steatotic and nonsteatotic livers preserved in IGL-1 and UW solution showed greater GLDH activity than their respective control groups (Fig. 6). IGL-1 solution prevented the increase of GLDH activity levels significantly more than UW in steatotic and nonsteatotic livers. The beneficial effects of IGL-1 were reverted when NO synthesis was inhibited with L-NAME.

The use of IGL-1 solution significantly increased the NO production (reflected in the values of tissues nitrites and nitrates) (Fig. 7). Steatotic and nonsteatotic livers preserved in IGL-1 solution showed greater constitutive NO synthase expression than the UW-preserved group (Fig. 8). The new NO synthesis originated mainly from constitutive NO synthase. Western blot did not reveal inducible NO synthase changes in any of study groups (Fig. 8).

DISCUSSION

The data reported here confirmed that steatotic livers were more susceptible to cold ischemia injury than nonsteatotic livers. Both liver types were better preserved in IGL-1 solution than in standard UW solution. This beneficial effect of IGL-1 solution was maintained during reperfusion, as the parameters of hepatic injury and functionality show. Preservation in IGL-1 solution was associated with lower transaminase levels, a higher final volume of bile, and better hepatic BSP clearance compared with UW. This confirms the suitability of IGL-1 for liver preservation.

As previously reported,^{9,13,44} steatotic livers were more susceptible to oxidative stress injury than were nonsteatotic livers. Our results indicate a higher resistance to flow in steatotic, as opposed to nonsteatotic, livers, suggesting that steatotic livers offer greater impediments to perfusion. Fat accumulation in the cyto-

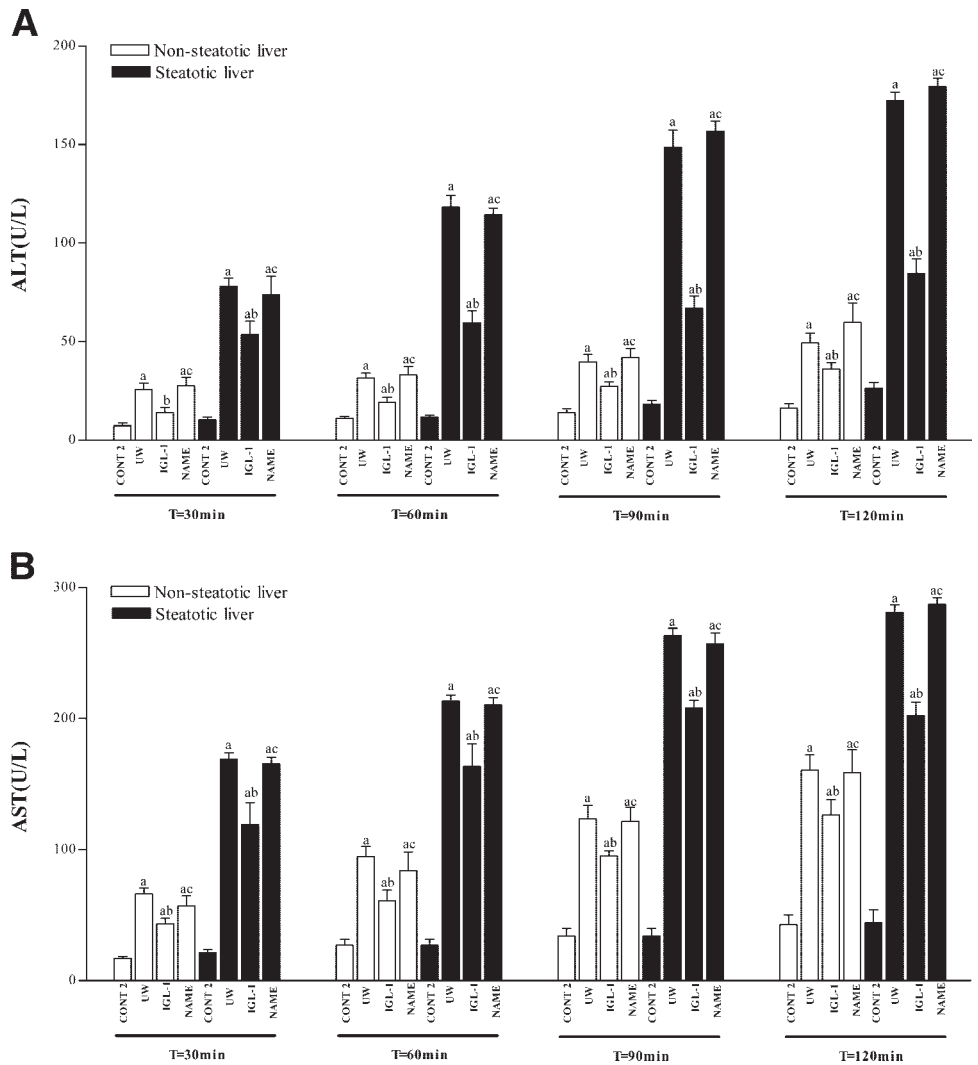


Figure 2. (A) ALT and (B) AST levels in perfusate during the normothermic reperfusion. White bars represent nonsteatotic livers. Black bars represent steatotic livers. CONT 2, liver flushed and perfused ex vivo without cold preservation; UW, liver preserved in UW solution; IGL-1, liver preserved in IGL-1 solution; NAME, liver preserved in IGL-1 solution with L-NAME; T, time.. *P < 0.05 vs. CONT 2, **P < 0.05 vs. UW, *P < 0.05 vs. IGL-1.**

plasm of hepatocytes is associated with an increase in cell volume, which may result in the partial or complete obstruction of the hepatic sinusoidal space.^{1,7} In addition, fatty degeneration, which induces a series of ultrastructural and biochemical alterations both in human and animal mitochondria,^{12,45} may render these organelles intrinsically more susceptible to I/R injury. Our results indicate that the IGL-1 preservation solution was more efficient than UW solution in preventing lipid peroxidation associated with hepatic I/R. Another important consequence of the use of IGL-1 for liver preservation is its beneficial effect on vascular resistance. The benefits of IGL-1 were also observed in the mitochondrial damage caused by I/R. Thus, lower GLDH activity for the livers preserved in IGL-1 solution was found compared with the results obtained when UW solution was used. Our results show that the use of IGL-1 solution reduced hepatic injury and ameliorated the parameters of liver functionality. IGL-1 also controlled the mechanisms potentially responsible for the vulnerability of steatotic livers to I/R injury, including oxidative stress, vascular resistance, and mitochondrial alterations.

It has been noticed that NO, a small molecule with multiple physiological functions, plays an important role in modulating hepatic I/R injury.^{46,47} The benefits of NO on oxidative stress in steatotic livers undergoing warm ischemia and livers grafts undergoing 6 hours of cold ischemia have been previously reported. Using an isolated perfused liver model, NO donors added in UW solution improved flow rate of nonsteatotic livers preserved during 24 hours.^{48,49} The benefits of NO on mitochondrial damage in isolated hepatocytes have been also reported in different experimental models of I/R.^{50,51} For these reasons, we hypothesized that the benefits of IGL-1 solution in steatotic livers preserved in IGL-1 solution could be mediated by NO. In our model, increased NO production (reflected by liver nitrite and nitrate levels), was observed when steatotic and nonsteatotic livers were preserved in IGL-1 solution compared with the values obtained for UW solution. NO derived mainly from constitutive NO synthase. The inhibition of NO synthesis with L-NAME in livers preserved in IGL-1 solution abolished the benefits of IGL-1, resulting in parameters of hepatic injury, liver functionality, oxidative stress, vascular resistance, and mito-

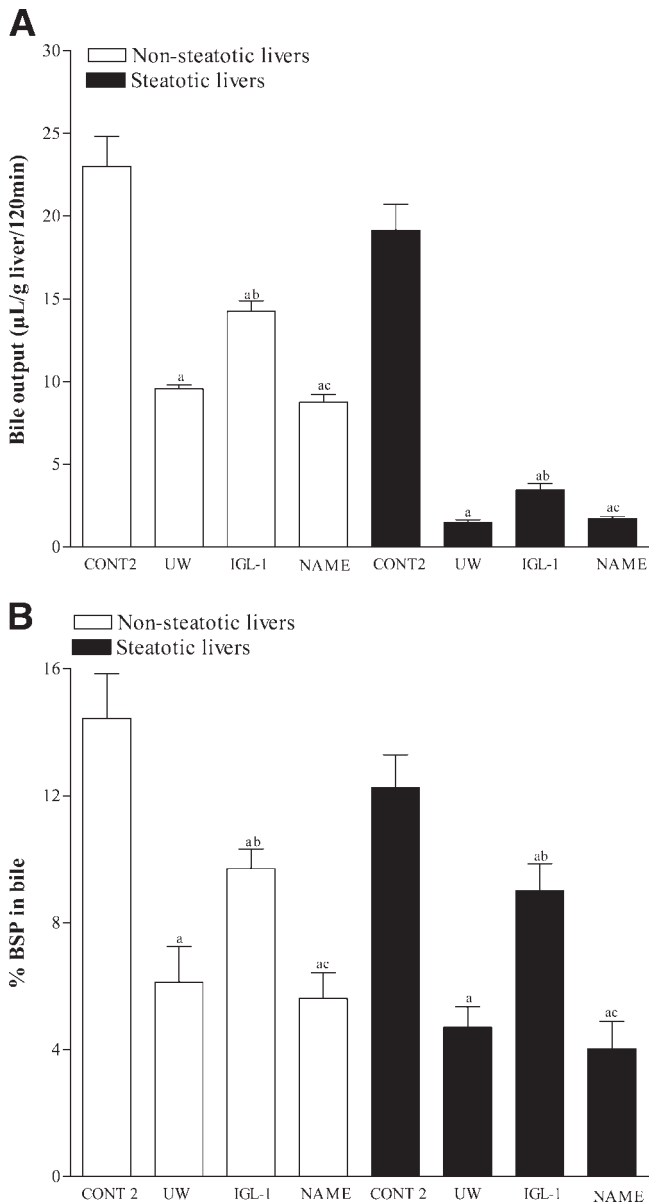


Figure 3. (A) Bile output (B) and percentage of BSP in bile of steatotic livers (black bars) and nonsteatotic livers (white bars) after 120 minutes of normothermic reperfusion. CONT 2, liver flushed and perfused ex vivo without cold preservation; UW, liver preserved in UW solution; IGL-1, liver preserved in IGL-1 solution; NAME, liver preserved in IGL-1 solution with L-NAME. *P < 0.05 vs. CONT 2, **P < 0.05 vs. UW, *P < 0.05 vs. IGL-1.**

chondrial alterations similar to those observed when livers were preserved in UW solution. These results indicate the involvement of NO in the beneficial effects of IGL-1 solution on hepatic injury and liver functionality.

In addition to NO, the possibility that the benefits of IGL-1 solution is also due to the effects of PEG and the lower potassium concentration should not be ruled out. Previous reports indicate that PEG reduced lipid peroxidation in isolated hepatocytes and experimental models of ischemia-reperfusion in kidney and

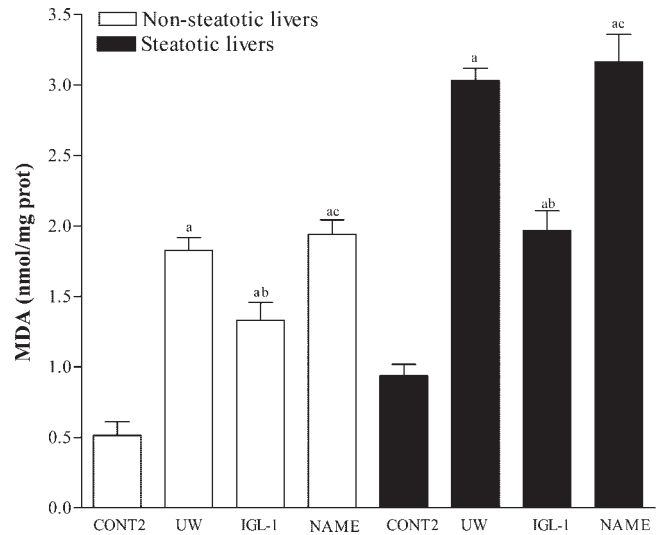


Figure 4. Hepatic MDA levels after 120 minutes of normothermic reperfusion. White bars represent nonsteatotic livers. Black bars represent steatotic livers. CONT 2, liver flushed and perfused ex vivo without cold preservation; UW, liver preserved in UW solution; IGL-1, liver preserved in IGL-1 solution; NAME, liver preserved in IGL-1 solution with L-NAME. *P < 0.05 vs. CONT 2, **P < 0.05 vs. UW, *P < 0.05 vs. IGL-1.**

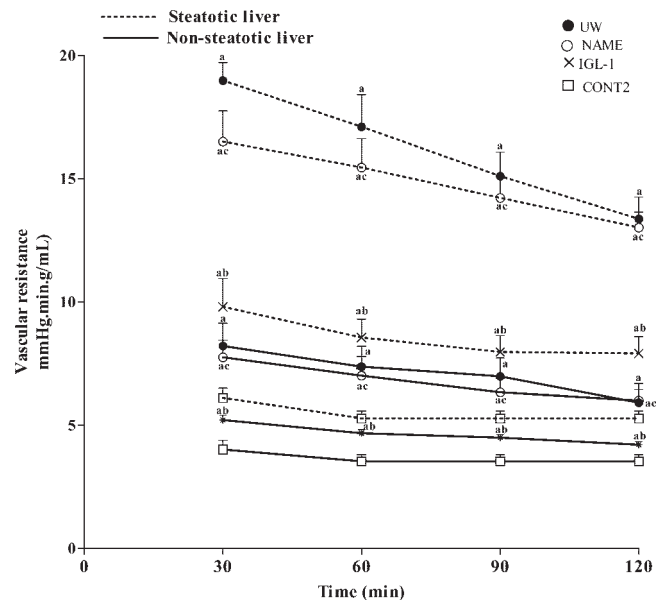


Figure 5. Vascular resistance of steatotic livers (dotted line) and nonsteatotic livers (solid line) during 120 minutes of normothermic reperfusion. UW (closed circle), liver preserved in UW solution; NAME (open circle), liver preserved in IGL-1 solution; IGL-1 (x) liver preserved in IGL-1 solution; CONT 2 (open square), liver flushed and perfused ex vivo without cold preservation. *P < 0.05 vs. CONT 2, **P < 0.05 vs. UW, *P < 0.05 vs. IGL-1.**

lung.⁵²⁻⁵⁴ In experimental models of isolated perfused liver and kidney, PEG and the lower potassium concentration contributed to prevention of vascular constriction, as well as improving the better wash-out of the organ and subsequent graft perfusion impairment dur-

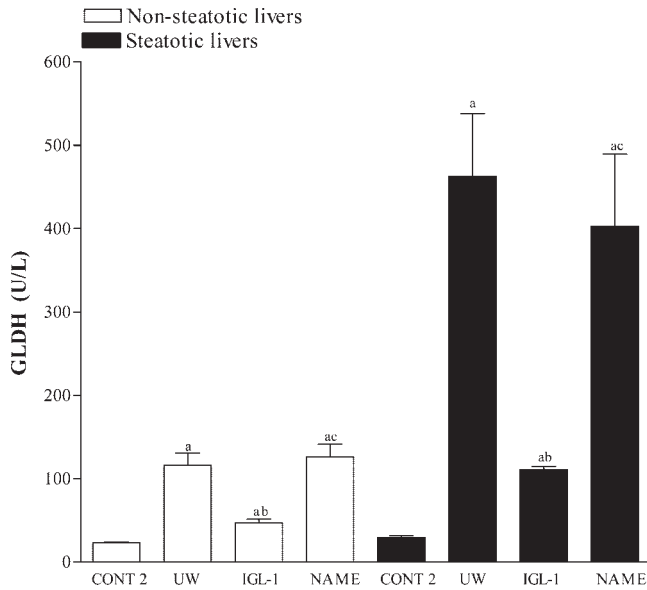


Figure 6. Glutamate dehydrogenase (GLDH) activity in perfusate after 120 minutes of normothermic reperfusion. White bars represent nonsteatotic livers. Black bars represent steatotic livers. CONT 2, liver flushed and perfused ex vivo without cold preservation; UW, liver preserved in UW solution; IGL-1, liver preserved in IGL-1 solution; NAME, liver preserved in IGL-1 solution with L-NAME. *P < 0.05 vs. CONT 2, **P < 0.05 vs. UW, *P < 0.05 vs. IGL-1.**

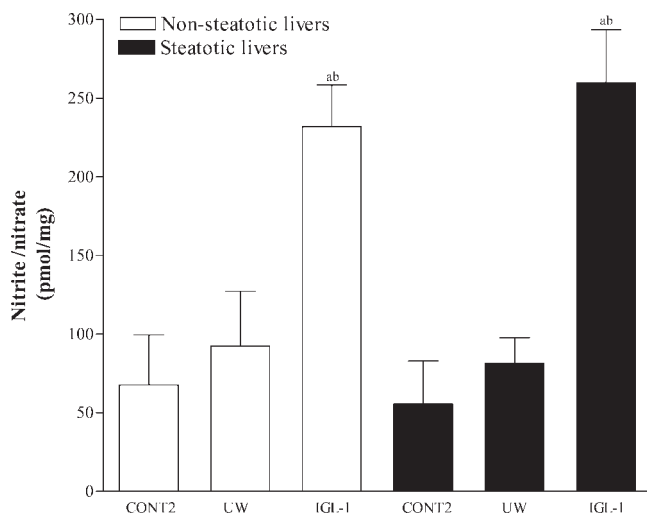


Figure 7. NO production, measured by nitrite and nitrate after 120 min of normothermic reperfusion. White bars represent nonsteatotic livers. Black bars represent steatotic livers. CONT 2, liver flushed and perfused ex vivo without cold preservation; UW, liver preserved in UW solution; IGL-1, liver preserved in IGL-1 solution. *P < 0.05 vs. CONT 2, **P < 0.05 vs. UW.

ing organ procurement.²⁰⁻²² In addition, PEG has been shown to have properties that may make it suitable for cold storage of organs, including its ability to suppress cell swelling.^{55,56} There are also other preservation solutions such as HTK or Celsior, which are either void of hydroxyethyl starch and/or present different ion patterns than UW.⁵⁷ In this line, in isolated perfused liver

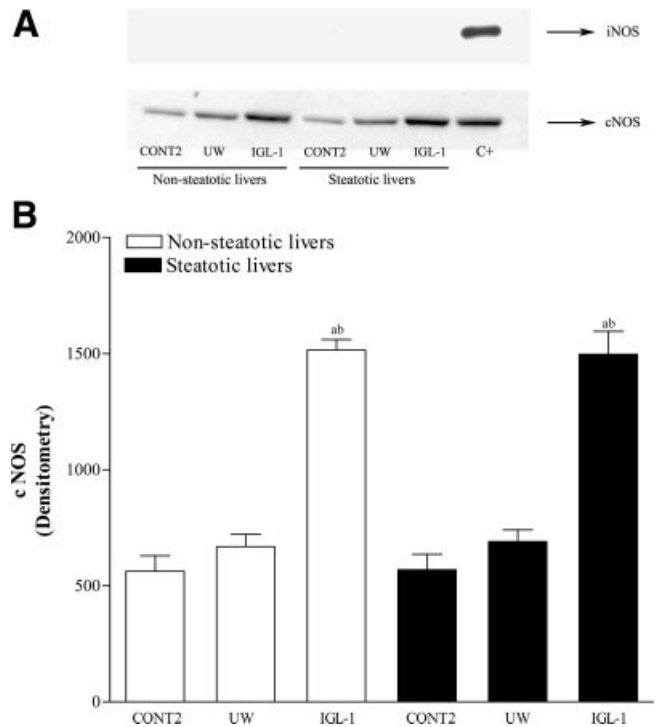


Figure 8. (A) Representative Western blot of inducible NO synthase and constitutive NO synthase and (B) densitometric analysis of constitutive NO synthase. Blots shown are representative of 3 similar experiments. Densitometric results are reported as integrated values (area × density of the band). White bars represent nonsteatotic livers. Black bars represent steatotic livers. iNOS, inducible NO synthase; cNOS, constitutive NO synthase; CONT 2, liver flushed and perfused ex vivo without cold preservation; UW, liver preserved in UW solution; IGL-1, liver preserved in IGL-1 solution; C+, positive control. *P < 0.05 vs. CONT 2, **P < 0.05 vs. UW.

model, Celsior and UW were equally effective in preventing cell death after 0-16 hours of cold preservation as compared with the less effective HTK solution. After 24 hours of cold storage, livers were best preserved in UW solution.²⁰ In the same line, another study indicated that UW solution was superior to Celsior and HTK in the protection of human liver endothelial cells against preservation injury.⁵⁷ In our conditions, IGL-1 solution was superior to UW solution. Further studies will be required to evaluate the relevance of PEG and the lower potassium concentration in the benefits of IGL-1 preservation solution.

In conclusion, the data reported here demonstrate that IGL-1 solution is useful for steatotic liver preservation. IGL-1 solution protected against hepatic injury, ameliorated liver injury, and modulated the mechanisms potentially responsible for the vulnerability of steatotic livers to cold I/R injury. The benefits of IGL-1 solution appear to be mediated by NO.

ACKNOWLEDGEMENTS

The authors thank the Language Advisory Service of the University of Barcelona for revising the English text.

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