Increased Susceptibility to Oxidant Injury in Hepatocytes from Rats with Intra-abdominal Hypertension

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Background: **Intra-abdominal hypertension leads to visceral organ hypoperfusion, and subsequent decompression may cause ischemia-reperfusion, releasing toxic metabolites. This study focuses on the effect of intra-abdominal hypertension on hepatic antioxidant store and the susceptibility of hepatocytes to oxidant injury.**

Methods: **Sprague-Dawley rats (150–180 g) were acclimatized to an environment for 3 days and then divided into two groups according to challenge based on intra-abdominal pressure (0 and 30 cm H_2O for control and experimental groups, respectively). After a 90-minute challenge, the rats underwent immediate laparotomy for decompression; after a further 30 minutes, one fragment of liver from the lingual lobe (>0.1 g) was excised to measure glutathione (GSH) in vivo before portal vein perfusion. After hepatocyte isolation (viability rate > 85%), the cell density was set at 1×10^5/mL for each well. The samples were cultured in an incubator for 12 hours, after which varying concentrations of t-butyl hydroperoxide (TBHP) (0.0, 0.5, 1.0, and 2.0 mmol/L) were added into the wells. After another 5-hour incubation, the total store of intracellular GSH in vitro (GSHVT) and the hepatocyte survival rates were measured for different groups of TBHP challenge using GSH assay and MTT kits.**

Results: **The control and experimental groups consisted of 10 and 8 rats, respectively, that successfully completed the entire experimental procedure. Compared with the control group, the in vivo GSH store was significantly reduced after the intra-abdominal pressure challenge (mean ± SE, 968.1 ± 63.5 vs. 1,581.0 ± 115.3 nmol/g of protein; $p = 0.001$). After the hepatocyte isolation, the GSHVT stores at various TBHP concentrations in the experimental rats were also similarly and significantly decreased relative to the control animals (894.4 ± 56.4, 804.2 ± 118.4, 586.9 ± 86.6, and 410.2 ± 87.4 nmol/g of protein vs. 1,282.2 ± 112.0, 1,156.6 ± 91.0, 995.2 ± 92.7, and 866.8 ± 62.4 nmol/g of protein for TBHPs of 0.0, 0.5, 1.0, and 2.0 mmol/L, respectively; all $p < 0.05$). Moreover, from photocytometry, the hepatocyte survival rates were significantly reduced for the experimental rats compared with the control animals after challenge with various TBHP concentrations (survival was 100%, 91.1%, 81.3%, and 72.8% vs. 100%, 99.2%, 95.0%, and 88.2%, respectively, for TBHPs of 0.0, 0.5, 1.0, and 2.0 mmol/L; $p < 0.05$ for the last two).**

Conclusion: **This animal study demonstrated that intra-abdominal hypertension and subsequent decompression deplete the total in vivo GSH store in rat livers, probably via the mechanism of ischemia-reperfusion injury, and the GSHVT after hepatocyte isolation, which makes the isolated hepatocytes of rats more susceptible to oxidant challenge.**

Key Words: **Intra-abdominal hypertension, Abdominal compartment syndrome, Hepatocyte isolation, Glutathione, Oxidant injury.**

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Acute expansion of intra-abdominal contents, in excess of the capacity of the abdominal cavity, will increase intra-abdominal pressure (IAP) and cause intra-abdominal hypertension (IAH). Despite having no unanimous definition, abdominal compartment syndrome (ACS) is considered to be IAH in which an adverse physiologic response is produced. In a clinical setting, ongoing intra-abdominal hemorrhage, retroperitoneal hematoma, intractable ascites, postresuscitation bowel edema, and retained packs after postsurgical damage control are encountered frequently and lead to this problem.[1–3] Many studies have demonstrated that IAP increased above a critical level detrimentally affects the physiologic function of multiple organ systems.[1,3–9] In their study of swine, Diebel and colleagues demonstrated that, when IAP was elevated above 20 mm Hg, both hepatic and superior mesenteric artery blood flow were decreased to less than one fourth of the original level.[4,5]

The development of ACS in abdomen injury patients after damage-control surgery has recently been associated with high rates of postinjury multiple organ failure (MOF).[1,10] The elevation of some proinflammatory cytokines, such as interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor-α in animal studies of IAH, are believed to be integral to MOF pathogenesis.[11,12] For patients with ACS, however, immediate decompression reportedly causes cardiovascular collapse, and even asystole. Some researchers have postulated that this problem may result, in part, from isch-

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emia-reperfusion (I/R) injury, which releases acid and metabolites from the reperfused ischemic viscera.[8,9] In the rat experiment designed by Eleftheriadis et al., the number of free oxygen radicals in the lungs increased after elevated IAP challenge.[13] Furthermore, glutathione (GSH), a tripeptide composed of glutamine, cysteine, and glycine, is a major intracellular antioxidant (especially in the liver) capable of neutralizing toxic oxygen metabolites and other free oxygen radicals.[14,15]

The purpose of this study was to evaluate the relationship between IAH and the antioxidant consumption of rat hepatocytes in an IAH animal model and to examine the effects of IAH on the susceptibility of hepatocytes to oxidant injury. This work hypothesizes the following: (1) IAH and subsequent decompression will cause I/R and deplete the in vivo GSH store in the liver; (2) after hepatocyte isolation and challenge with an oxidant agent, the in vitro intracellular GSH store will be depleted in accordance with the increased oxidant concentration; and (3) the hepatocytes isolated from rats after IAH insult will be more susceptible to oxidant challenge.

MATERIALS AND METHODS

Animal Preparation

Male, virus-free, Sprague-Dawley rats (body weight, 150–180 g) (the animal center of the National Science Council, Taiwan) were housed in an animal facility at a constant room temperature and in a 12-hour light-dark cycle. They were cared for in accordance with principles contained in the *Guide to the Care and Use of Experimental Animals*. The rats were acclimatized to laboratory conditions for at least 3 days before use. All animals received rat chow and water ad libitum throughout the acclimatization period and until the time of the experiment.

Study Design

During the first 3 days, the animals were acclimatized in animal cages with access to water and normal chow ad libitum. At 8:00 AM on the fourth day, the elevated IAP challenge was performed.

Rat IAH Challenge

Animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg), the abdominal hair was clipped, and the skin was prepared with povidone-iodine. Under aseptic conditions, one intravenous set catheter was inserted into the peritoneal cavity via the lower left quadrant of the abdomen and fixed using two-layer pursestring sutures in the abdominal skin to achieve a water-tight seal and secure the connection to a central venous pressure (CVP) set, directly reflecting catheter pressure. An 18-gauge needle was inserted into the peritoneal cavity of the lower right quadrant of the abdomen to infuse 0.9% normal saline and thus raise IAP.

Rat Groups

Thirty rats were divided into two groups. Infusion of 0.9% normal saline via an 18-gauge needle and measurement via the catheter connected to the CVP set were used to maintain the IAP at the required group level. For group 1 (control, 15 rats), the IAP was maintained at 0 cm H_2O without any saline infusion, whereas for group 2 (experimental, 15 rats), the IAP was maintained at 30 cm H_2O. The IAP was maintained for 90 minutes using CVP monitoring and additional saline infusion, if necessary.

Rat Liver Excision Biopsy and Hepatocyte Isolation

Each animal was weighed and anesthetized with intraperitoneal pentobarbital as described previously. After 90-minutes of IAP challenge at different levels, the rats underwent immediate laparotomy for decompression using a midline ventral incision made from the pubis to the xiphoid process. After an additional 30 minutes, the rat livers were harvested. Before portal venous perfusion, one fragment (weight, $>$0.1 g) of the lingual lobe of the liver was excised and hemostasis was performed using ligation with 3-0 silk at its proximal area. The rat hepatocyte isolation was performed with collagenase perfusion as described by Schuetz et al.[16] Specifically, the portal vein was cannulated using a 22-gauge intravenous catheter, and the liver was perfused with calcium-free Krebs bicarbonate buffer followed by collagenase (30 mg of 494 IU/mg collagenase IV [Sigma C-5138]) in 280 mL of Krebs bicarbonate containing 1.2 mmol/L $CaCl_2$ and 1.8% bovine serum albumin (Sigma A-4503). All solutions were maintained at 37°C and aerated using 95% oxygen and 5% carbon dioxide. The partially digested liver was excised, passed over 60-μm nylon mesh, and resuspended in Wilson medium (Sigma W-4125) with insulin (Sigma I-0516; 1 U/dL) and certified fetal bovine serum (GIBCO BRL 16000-044; 10%). Hepatocytes were purified by centrifugation through the Wilson medium at 50*g* for 5 minutes and then resuspended in Wilson medium after a second centrifugation at the same speed through a gradient of 50% Percoll-Wilson medium. The viability of the hepatocytes, which were maintained above 85% during the experiment, was determined using trypan blue exclusion. The cell concentration was adjusted to 1×10^5 cells/mL, with the isolated hepatocytes then placed in a microtiter plate in an incubator in 5% carbon dioxide at a temperature of 37°C.

Measurement of Total In Vivo Hepatic GSH Content

To assay total hepatic GSH, the excised liver sample was rapidly deproteinized by vigorous mixing with an adequate volume of 3.33% 5-sulfosalicylic acid, followed by centrifugation at 2.000*g* for 2 minutes. GSH was measured by 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB)–glutathione disulfide (GSSG) reductase recycling assay, which combines the selectivity of GSSG with the sensitivity of DTNB using a microtiter plate reader (Uvmax, Molecular Devices, Palo Alto, CA) to assay total GSH. The DTNB reacts with GSH to

form the highly colored 5-thio-2-nitrobenzoic acid anion and GSSG, where the GSSG is reduced by GSSG reductase coupled with nicotinamide adenine dinucleotide phosphate to produce GSH. A typical assay mixture consisted of 70 μL daily buffer (0.3 mmol/L nicotinamide adenine dinucleotide phosphate, 6.3 mmol/L disodium EDTA, 125 mmol/L sodium phosphate), 10 μL 6 mmol/L DTNB solution, 10 μL sample, and 10 μL GSSG reductase (10 U/mL). Meanwhile, the instrument settings for the microtiter plate reader were single-wavelength kinetics using a 405-nm filter, read for 10 minutes at 30°C incubation.

Measurement of Total GSH in Rat Hepatocytes in Wells: In Vitro Study (GSHVT)

To assay total GSH in the rat hepatocytes, the samples were also deproteinized using 3.33% 5-sulfosalicylic acid. In addition, total GSH was also determined using DTNB-GSSG reductase recycling as described above.

Measurement of Hepatocyte Survival after Oxidant Challenge Via the MTT (Thiazole Blue) Technique

Isolated hepatocytes were cultured in Wilson medium for 12 hours in an incubator, after which the original medium was removed and a new one (1.0 mL) containing various concentrations of t-butyl hydroperoxide (TBHP) (0.0, 0.5, 1.0, and 2.0 mmol/L) was added to the well. The hepatocytes were then co-cultured with TBHP for a further 5 hours in an incubator, after which an MTT stock solution equivalent to one tenth of the original culture volume was added to each culture being assayed, with subsequent incubation for 2 to 4 hours at 37°C. At the end of the incubation period, the culture media were removed from the plates, and the resulting MTT formazan crystals were dissolved using dimethyl sulfoxide. Samples were read within 1 hour of the addition of the MTT solvent to ensure accuracy. Furthermore, tests performed in 24-well plates were transferred to 96-well variants and measured using an enzyme-linked immunosorbent assay-type plate reader equipped with the appropriate filters. The absorbance of the converted dye was measured at a wavelength of 570 nm.

Statistical Analysis

Data are expressed as mean ± SE. Meanwhile, the two-way analysis of variance test was used to investigate the effects of IAP and TBHP on GSH in vitro and hepatocyte survival rate, and the interaction between IAP and TBHP. Statistical analyses were performed using the Mann-Whitney *U* test for between-group comparison of the viability rates of the isolated hepatocytes, GSH in vivo and in vitro, and survival rates after the oxidant challenge. Finally, the level of statistical significance was set at $p < 0.05$.

RESULTS

The rats were able to tolerate intra-abdominal pressures of up to 30 cm H_2O for 90 minutes very well, except for one animal that died after 54 minutes of insult at pressure. Each group originally contained 15 rats that underwent challenge, with all survivors undergoing hepatocyte isolation. However, a viability rate of over 85% after hepatocyte isolation was only achieved for 10 rats in the control group and for 8 rats in the experimental group. The viability rate for the control rats was 91.9 ± 0.9%, compared with 89.9 ± 0.9% for the experimental analogs ($p > 0.05$). The data obtained from the 10 control rats and the 8 experimental analogs were used for the procedures detailed below.

The in vivo GSH of the control group was 1,581.0 ± 115.3 nm/g of protein, significantly higher than the experimental analog at 968.1 ± 63.5 nmol/g of protein ($p = 0.001$) (Fig. 1). The effects of IAP and TBHP on GSHVT were statistically significant (both $p < 0.001$); however, the interaction between IAP and TBHP was not significant ($p = 0.953$). After the hepatocyte isolation, incubation for 5 hours, and TBHP challenge at various concentrations (0.0, 0.5, 1.0, and 2.0 mmol/L), the in vitro GSH for each group was reduced with respect to the increased TBHP concentration. The GSHVT decreased significantly for each group in accordance with the increased concentration of TBHP ($p < 0.001$). After completion of the above-mentioned procedure, together with the addition of oxidant at various concentrations for 5 hours, the levels of GSHVT in the control group for 0.0, 0.5, 1.0, and 2.0 mmol/L of TBHP were 1,282.2 ± 112.0, 1,156.6 ± 91.0, 995.2 ± 92.7, and 866.8 ± 62.4 nmol/g of protein, respectively, significantly higher than the respective levels for the experimental group, which were 894.4 ± 56.4, 804.2 ± 118.4, 586.9 ± 86.6, and 410.2 ± 87.4 nmol/g of protein; $p = 0.011$, $p = 0.033$, $p = 0.006$, and $p < 0.001$) (Table 1

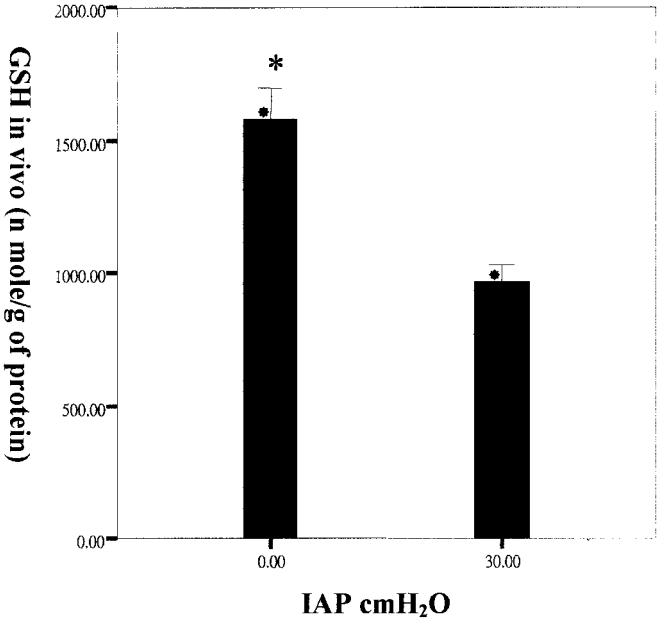

Fig. 1. *The level of GSH in vivo of livers in both control and experimental rats.* *p < 0.05, *compared with experimental rats using the Mann-Whitney* U *test.*

Table 1 GSH In Vitro in Various Concentrations of TBHP (Mean ± SE)

TBHP (mmol/L)	IAP (cm H_2O)		*p* Value
	0	30	
0	1,282.2 ± 112.0*	894.4 ± 56.4	0.008
0.5	1,156.6 ± 91.0*	804.2 ± 118.4	0.041
1.0	995.2 ± 92.7*	586.9 ± 86.6	0.011
2.0	866.8 ± 62.4*	410.2 ± 87.4	0.002

The unit of GSH is nmol/g of protein. Mann-Whitney *U* test is used for statistical analysis.

* $p < 0.05$.

and Fig. 2). The GSHVT levels declined sharply, with high oxidant concentrations (0.5–2.0 mmol/L) in rats subjected to IAP challenge relative to controls.

The sensitivity of the isolated hepatocytes with respect to the oxidant challenge was represented by the survival rate of hepatocytes detected using the MTT technique. The effects of IAP and TBHP on cell viability rate were statistically significant (both $p < 0.001$), but the interaction between IAP and TBHP was not significant ($p = 0.472$). Furthermore, the gradual decrease in hepatocyte survival rate was consistent with the elevated concentration of added TBHP ($p < 0.001$). The hepatocyte survival rates for the 0.0, 0.5, 1.0, and 2.0 mmol/L in the control group were 100.0 ± 0.0%, 99.2 ± 2.6%, 95.0 ± 2.9%, and 88.2 ± 3.8%, respectively, significantly higher than that for the experimental rats at the higher concentrations (1.0 and 2.0 mmol/L) of the added oxidants (100.0 ± 0.0%, 91.1 ± 3.0%, 81.3 ± 3.0%, and 72.8 ± 2.6%, respectively, with $p < 0.05$ for the last two comparisons). The two groups did not differ significantly for the 0.5-mmol/L oxidant concentration; however, the *p* value approached 0.05 (Table 2 and Fig. 3).

DISCUSSION

It has long been recognized that IAH causes a significant decrease in visceral organ function. Furthermore, it has been known since the latter part of the 19th century that increased IAP can result in respiratory impairment, with IAH-associated deterioration of the other organ systems determined in the early 1900s.[17] In 1984, Kron and colleagues introduced ACS, which characterizes the most devastating consequences of IAH, recommending immediate decompression laparotomy for patients in whom postoperative IAP was greater than 25 mm Hg with associated oliguria.[18] In recent years, inten-

Table 2 Hepatocyte Viability Rate Detected by MTT in Various Concentration of TBHP (Mean ± SE)

TBHP (mmol/L)	IAP (cm H_2O)		*p* Value
	0 (%)	30 (%)	
0	100.0 ± 0.0	100.0 ± 0.0	1.0
0.5	99.2 ± 2.6	91.09 ± 3.0	0.068
1.0	95.0 ± 2.9*	81.3 ± 3.0	0.013
2.0	88.2 ± 3.8*	72.8 ± 2.6	0.006

Mann-Whitney *U* test is used for statistical analysis.

* $p < 0.05$.

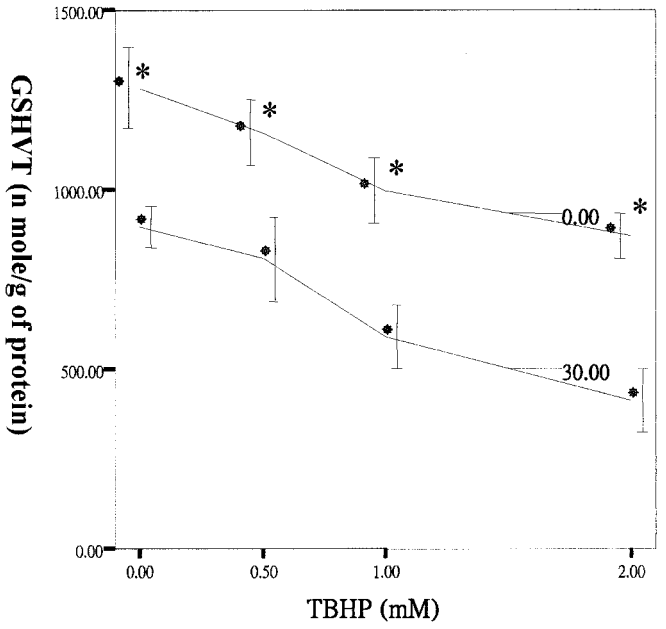

Fig. 2. *The level of GSH in vitro (GSHVT) of hepatocytes in various concentrations of TBHP in both control and experimental rats.* *p < *0.05, compared with experimental rats at corresponding TBHP concentrations using the Mann-Whitney* U *test.*

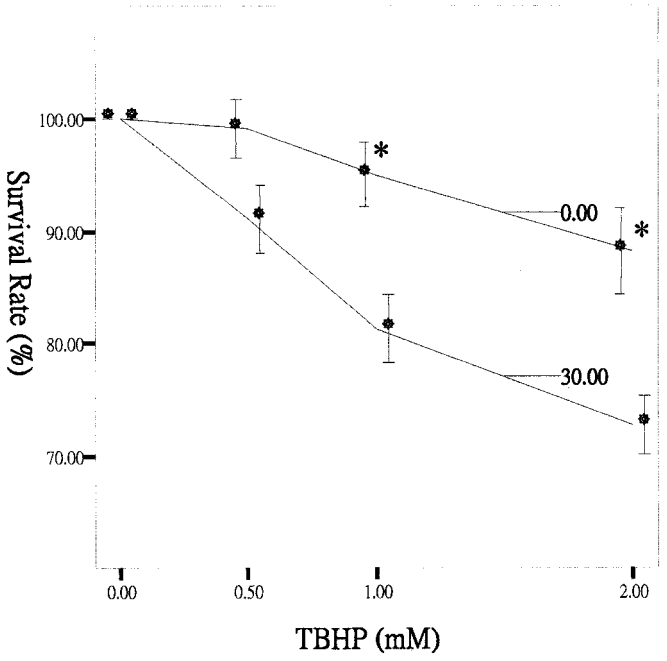

Fig. 3. *The survival rate of hepatocytes detected by the MTT method in various concentrations of TBHP in both control and experimental rats.* *p < *0.05, compared with experimental rats at corresponding TBHP concentrations using the Mann-Whitney* U *test.*

sive care physicians have emphasized that IAP measurement should be part of routine monitoring in the intensive care unit, and it has become a prognostic parameter for understanding abdominal problems and the physiologic status of patients.[19–21]

Despite the lack of a universally accepted definition, ACS is considered IAH when it produces an adverse physiologic response. The point at which IAH becomes ACS is still not well defined, however.[8,22] Clinically, when IAP reaches this undetermined cutoff point, clinical presentations occur that include oliguria, raised pulmonary pressure, hypoxia, decreased cardiac output, hypotension, and acidosis. The cutoff point for IAP in ACS remains controversial; however, Burch et al., Ivatory et al., and Chen et al. have all suggested that immediate decompression becomes mandatory when IAP exceeds 25 cm H_2O.[2,23,24] Meldrum et al. have recommended surgical intervention when IAP rises above 20 mm Hg (27 cm H_2O) and clinical symptoms reveal renal (urine output $<$ 0.5 mL/kg/h), pulmonary (peak airway pressure $>$ 40 cm H_2O), and cardiac dysfunction (oxygen delivery index $<$ 600 mL/min/m^2).[22] Mayberry proposes that surgical decompression becomes urgent when IAP has increased to 25 mm Hg (34 cm H_2O) and organ dysfunction, hypotension, and acidosis have occurred.[25] For the current rat study, an IAP of 30 cm H_2O was used as the cutoff point for ACS. It was experimentally determined that rats were unable to tolerate IAPs above 30 cm H_2O, which frequently and rapidly led to apnea during manipulation. When the IAP was set at 30 cm H_2O for 90 minutes, only one rat suffered IAP insult, with this animal dying as a result of apnea during the procedure.

As is well known, GSH is the most prevalent cellular thiol and the most abundant low-molecular-weight peptide in cells, especially the hepatic variants.[14,15] Several studies have shown that the rapid depletion of GSH in the liver in vivo and in freshly isolated hepatocytes is associated with lipid peroxidation and cell death.[26–28] Furthermore, GSH is a major intracellular antioxidant by virtue of the donation of a hydrogen ion and an unimpaired electron from its reactive sulfhydryl group to neutralize toxic oxygen metabolites and other free radicals.[15] This peptide plays a role in protection against tissue damage resulting from exposure to oxidizing environments. Because GSH is the major antioxidant in the liver, this chemical was used as an index of the antioxidant level of rat hepatocytes in our experiment.

It has been noted that increased IAP adversely affects many organ systems, decreasing visceral blood flow in animal models. Diebel and colleagues used a swine experiment to demonstrate a dramatic decrease in hepatic artery blood flow of more than 50%, with a reduction of superior mesenteric artery blood flow of more than one fourth where the IAP was above 20 mm Hg.[4,5] Abdominal decompression, which is the preferred treatment for ACS, may induce tissue reperfusion.[20–24] Immediate IAP decompression leads to hypotension because of decreased vascular resistance, reduced cardiac output, and peripheral vascular dilation.[29] In addition, Offner and colleagues have demonstrated that, of 17 trauma patients with ACS, 12 (71%) developed adult respiratory distress syndrome and MOF, with 6 (35%) eventually dying.[10] Furthermore, an amplified response in proinflammatory cytokines, such as IL-1β, IL-6, IL-8, and tumor necrosis factor-α, and lung injury with elevated myeloperoxidase activity or demonstration of histopathologic findings of intense pulmonary inflammatory infiltration, were determined in IAH, or sequential hemorrhage and IAH using animal models.[11,12] IAH provokes the release of proinflammatory cytokines, which may serve as a second insult for the induction of MOF.[12] Furthermore, immediate postdecompression asystole has also been reported.[9] Eddy reported that 12% (4 of 34) of patients with ACS developed asystole after decompression laparotomy.[30] Cullen et al. and Morris et al. proposed that this postdecompression cardiovascular collapse may result from I/R because of the release of toxic acids and metabolites from the reperfused ischemic viscera.[8,9] In an animal study of the effects of IAH on remote organ injury designed by Eleftheriadis et al., increased levels of free oxygen radicals were demonstrated in the lungs of rats after elevated IAP challenge.[13]

The current study has demonstrated that a 90-minute challenge with IAP of 30 cm H_2O significantly reduced total GSH stores in the liver in vivo, compared with unchallenged rats. Furthermore, after hepatocyte isolation, the intracellular GSHVT stores in the IAP challenge animals were also decreased significantly compared with the control rats. These results may be explained by the fact that, when the IAP reaches a critical level, blood flow to the visceral organs decreases in the rats, with relative ischemic change resulting. At the same time, immediate decompression leads to reperfusion of the visceral organs and the release of numerous toxic free radicals, which consume antioxidant stores (such as GSH) in the liver. The above results indirectly verify the hypothesis of an I/R syndrome in immediate decompression of the abdominal compartment.

With respect to the strong oxidant challenge after hepatocyte isolation, GSHVT and the cell survival rate gradually decreased as oxidant concentration was increased. Furthermore, the GSHVT was significantly higher in the control rats than in the experimental analogs after the oxidant challenge. However, the cell survival rates for the control animals were significantly higher than those for the experimental analogs under higher oxidant concentrations (1.0 vs. 2.0 mmol/L). Moreover, the decrease in GSHVT slope and cell survival rate were greater for the rats with IAP insult in comparison with controls when the oxidant concentrations were higher (1.0 vs. 2.0 mmol/L). Hepatocyte survival rates after the oxidant challenge were measured via absorbance of the converted dye using MTT kits. The survival rate of the initial isolated hepatocytes with no added oxidant was set at 100%, with the other hepatocyte survival rates at various TBHP concentrations calculated from the ratio of the optical absorbance to this standard value. Given the low concentrations of oxidant challenge (0.5 mmol/L), the in vitro hepatocytes only consumed a small amount of antioxidant (GSHVT) and, thus,

the two groups did not differ significantly in terms of the tolerance of the isolated hepatocytes to oxidant challenge. At higher concentrations of oxidant challenge, however, excessive antioxidant consumption caused a relatively large and sharp decrease in total antioxidant reserves for the IAP challenge rats and a significant between-group difference in survival rates. The above phenomenon reflects that the reserves of total cell GSH store (antioxidant) in vivo and in vitro decreased after IAP challenge, making the isolated cells more susceptible to oxidant injury when the oxidant concentrations were higher.

The current study has a number of problems. First, the number of experimental animals was small, and thus the likelihood of a type II statistical error must be considered. Second, apart from GSH, other antioxidants, such as ascorbic acid and α-tocopherol, are also extant in the hepatocytes, and the current study did not investigate these analogs. The survival rate of cells after oxidant challenge cannot be explained by GSH alone, and so measuring all of the antioxidants may be a more convincing strategy. Third, the free oxygen radicals were not measured in serum or liver, so the decrease in hepatic antioxidant store (GSH) could not be completely attributed to reactive oxygen intermediates, which were produced after the tissue I/R.

Studies of I/R injury during ACS have been few and far between. Although much work has been conducted on IAH, proinflammatory cytokines, postinjury MOF, and even free oxygen radicals in the lungs of rats in animal experiments, the relationship between ACS and visceral I/R is still not clearly delineated. The link between antioxidant store, ACS, and hepatocyte susceptibility to oxidant injury has not been previously reported, however. It seems reasonable to suggest, therefore, that this initial work may provide potentially valuable information.

On the basis of the results of this study, we conclude that IAH and subsequent decompression will deplete total hepatocyte GSH store both in vivo and in vitro. Antioxidant consumption can probably be attributed to the release of toxic metabolites via I/R, increasing the susceptibility of the hepatocytes to oxidant challenge.

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