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Protective Effects of α-Tocopherol and Ischemic Preconditioning on Hepatic Reperfusion Injury

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This study evaluated the effect of α-tocopherol (α-TC), ischemic preconditioning (IPC) or a combination on the extent of mitochondrial injury caused by hepatic ischemia/reperfusion (I/R). Rats were pretreated with α-TC (20 mg/kg per day, i.p.) for 3 days before sustained ischemia. A rat liver was preconditioned with 10 min of ischemia and 10 min of reperfusion, and was then subjected to 90 min of ischemia followed by 5 h or 24 h of reperfusion. I/R increased the aminotransferase activity and mitochondrial lipid peroxidation, whereas it decreased the mitochondrial glutamate dehydrogenase activity. α-TC and IPC individually attenuated these changes. α-TC combined with IPC (α-TC+IPC) did not further attenuate the changes. The mitochondrial glutathione content decreased after 5 h reperfusion. This decrease was attenuated by α-TC, IPC, and α-TC+IPC. The significant production of peroxides observed after 10 min reperfusion subsequent to sustained ischemia was attenuated by α-TC, IPC, and α-TC+IPC. The mitochondria isolated after I/R were rapidly swollen. However, this swelling rate was reduced by α-TC, IPC, and α-TC+IPC. These results suggest that either α-TC or IPC reduces the level of mitochondrial damage associated with oxidative stress caused by hepatic I/R, but α-TC combined with IPC offers no significant additional protection.

Key words: α-Tocopherol, Ischemic preconditioning, Hepatic ischemia/reperfusion, Oxidative stress, Mitochondrial permeability transition

INTRODUCTION

Most injuries associated with ischemia and reperfusion (I/R) occur during the period of reperfusion, when reactive oxygen species (ROS) are generated. These ROS cause nonspecific damage to lipids, proteins, and DNA, leading to an alteration or loss of the cellular function. The mitochondria are continuously exposed to ROS and experience oxidative damage more rapidly than the remainder of the cell. This is particularly so because ROS are highly reactive and short-lived (Kowaltowski and Vercesi, 1999). Many studies have reported that ischemic cell death is a consequence of irreversible mitochondrial injury (Clemens *et al.*, 1985). Therefore, there is a need to develop strategies for preventing mitochondrial damage caused I/R during the transplant process in order to improve the graft outcome.

Several therapeutic strategies have been successfully developed to prevent liver tissue damage after I/R. These include pharmacological intervention with antioxidants as well as alternative clamping techniques. Enhancing the liver antioxidant capacity was reported to be a promising therapeutic strategy for I/R injury (Bilzer and Gerbes, 2000). Approaches involving the administration of various antioxidants have been suggested but the efficacy of these interventions has varied (Lehmann *et al.*, 2000). α-Tocopherol (α-TC) is an endogenous lipid-soluble chain-breaking antioxidant, which is known to protect cells from the diverse actions of free oxygen radicals by donating its hydrogen atom (Burton *et al.*, 1988). When administered over several days prior to I/R, α-TC protects the liver from lipid peroxidation and improves the survival in rats (Lee and Clemens, 1992). Recently, it was reported that α-TC is involved in signal transduction and growth regulation (Azzi and Stocker, 2000). However, there is limited information available on the effect of α-TC on the oxidative damage to the mitochondria during hepatic I/R.

Ischemic preconditioning (IPC), which is defined as brief periods of I/R before sustained ischemia, has been reported to confer a state of protection in many organs,

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resulting in increased tolerance towards hypoxia. IPC has been shown to attenuate the tissue injury observed after reperfusion of the heart, brain and skeletal muscles (Tsai *et al.*, 2004). This procedure was recently extended to experimental models of hepatic vessel interruption as well as liver resections (Clavien *et al.*, 2003). ROS, which are released during sublethal ischemia, have been suggested to induce the defense mechanisms against oxidative stress (Rüdiger *et al.*, 2003). It has also been reported that IPC protects the liver from hepatic I/R injury by preserving the mitochondrial redox-state (Glanemann, 2003). Moreover, Javadov *et al.* (2003) showed that the protection of the heart from I/R injury afforded by IPC is associated with the indirect inhibition of the mitochondrial permeability transition (MPT) pore.

Therefore, this study examined the effects of α-TC, IPC, or their combination on the level of a postischemic injury, with particular focus on the deterioration of the mitochondrial function.

MATERIALS AND METHODS

Hepatic ischemic procedure

Male Sprague-Dawley rats, 270-300 g, were fasted for 18 h prior to the experiment but were provided with tap water *ad libitum*. The rats were anesthetized with ketamine (60 mg/kg body weight) and xylazine (10 mg/kg body weight) intraperitoneally. Throughout the anesthesia, the body temperature was monitored by a rectal probe and was maintained at 37°C using a heating pad. All the animals were treated humanely under the Sungkyunkwan University Animal Care Committee guidelines.

A midline incision to the abdomen was made, and the left branches of the portal vein and hepatic artery were clamped to induce complete ischemia of the median and left hepatic lobes. The right lobes remained perfused in order to prevent intestinal congestion. After 90 min of ischemia, the clip around the left branches of the portal vein was removed to allow reperfusion. Prior to prolonged ischemia, IPC was performed by 10 min of ischemia followed by 10 min of reperfusion, and IPC without the prolonged ischemia did not affect the normal hepatic function (data not shown). The sham-operated animals were prepared in a similar manner with the exception that a clip was not placed on the median and left lobes. After 10 min, 5 h, and 24 h of reperfusion, the left and median lobe of the liver were removed and examined. Blood samples were taken from the abdominal aorta 5 h and 24 h after reperfusion.

Administration of α-tocopherol

α-TC was dissolved in soybean oil (20 mg/mL) and administered *via* an intraperitoneal injection of 20 mg/kg of body weight per day for 3 days before the sustained ischemia. This α-TC dose was selected because it had been evaluated previously (Lee *et al.*, 2000). In the vehicle-treated animals, the same volume of soybean oil was injected in the same manner as with the α-TC. The following 6 treatment groups were examined: (a) vehicle-treated sham (sham), (b) α-TC-treated sham (α-TC), (c) vehicle-treated ischemic (I/R), (d) α-TC-treated ischemic (α-TC+I/R), (e) IPC/vehicle-treated ischemic (IPC+I/R), and (f) α-TC/IPC-treated ischemic (α-TC+IPC+I/R).

Analytical procedures

The serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured spectrophotometrically using the Sigma kits INFINITY™ 52-UV and 51-UV, respectively (Sigma Chemical Co., St. Louis, MO, U.S.A.). The hepatic peroxide level was measured from the liver samples taken during IPC and after 0, 1, 3, 5, 10, 20, 30, and 300 min of reperfusion. The H_2O_2-mediated oxidation of Fe^{2+} to Fe^{3+} was measured spectrophotometrically at a wavelength of 560 nm under acidic conditions using a xylenol orange dye using the method reported by Wolff (1994). The liver mitochondrial fraction was prepared using the method reported by Johnson and Lardy (1967). The glutamate dehydrogenase (GDH) activity in the suspension was determined according to the method reported by Ellis and Goldberg (1972). The total glutathione level was measured spectrophotometrically at a wavelength of 412 nm using yeast glutathione reductase and 5,5'-dithio-bis(2-nitrobenzoic acid), as described by Tietze (1969). The oxidized glutathione (GSSG) level was measured using the same method but in the presence of 2-vinylpyridine (Griffith, 1980). The level of reduced glutathione (GSH) was determined by the difference between the total glutathione and GSSG levels. The steady-state level of malondialdehyde (MDA), which is the end product of lipid peroxidation, in the liver mitochondria was determined by measuring the level of the thiobarbituric acid-reactive substances spectrophotometrically at 535 nm using the method described by Buege and Aust (1978). The rate of mitochondrial swelling, which indicates the level of the mitochondrial permeability transition (MPT), was determined by measuring the change in the absorbance of a mitochondrial suspension at 520 nm using the procedure reported by Elimadi *et al.* (2001). The protein content was estimated using the Bradford method (1976).

Statistical analysis

The data was examined using two-way ANOVA. The differences between the groups were considered significant at $P<0.05$ with the appropriate Bonferroni correction made for multiple comparisons. All results are presented as a mean ± SEM.

Table I. The effects of α-tocopherol and ischemic preconditioning on the serum aminotransferases in the rat liver after ischemia and reperfusion

Group	ALT (IU/L)		AST (IU/L)	
	5 h	24 h	5 h	24 h
Sham	46.5 ± 3.9	39.8 ± 2.4	205.1 ± 5.2	161.4 ± 13.3
α-TC	40.7 ± 1.2	39.6 ± 1.3	208.0 ± 11.8	143.8 ± 5.7
I/R	5393.1 ± 438.1**	2985.7 ± 229.8**	12986.0 ± 1145.6**	6328.8 ± 846.8**
α-TC+I/R	3687.5 ± 274.7**,+	1762.1 ± 99.1**,+	9674.9 ± 581.2**,+	3562.5 ± 555.9**,+
IPC+I/R	2999.1 ± 346.1**,+	1393.4 ± 287.4**,+	7642.2 ± 1067.9**,+	3598.5 ± 538.5**,+
α-TC+IPC+I/R	3840.7 ± 308.5**,+	1417.3 ± 194.9**,+	8791.4 ± 1005.0**,+	3792.4 ± 604.4**,+

The rat liver was preconditioned with 10 min of ischemia followed by 10 min of reperfusion. The liver was then subjected to 90 min of sustained ischemia followed by 5 h of reperfusion. The values are shown as a mean ± SEM for 8-10 rats per group. ** Significantly different ($P<0.01$) from the sham. + Significantly different ($P<0.05$) from the I/R.

RESULTS

Serum aminotransferase activity

As shown in Table I, the serum level of ALT in the vehicle-treated sham animals 5 h and 24 h after reperfusion was 46.5 ± 3.9 IU/L and 39.8 ± 2.4 IU/L, respectively. In contrast, the serum ALT levels in the ischemic group increased approximately 116 and 75 times those observed in the sham, respectively. These increases were suppressed by α-TC, IPC, and α-TC+IPC. Similarly, the serum AST levels were also markedly higher both 5 h and 24 h after reperfusion than those of the sham animals but these increases were also attenuated by α-TC, IPC, and α-TC+IPC.

Tissue peroxide levels

Fig. 1 and 2 show very low peroxide levels in the vehicle- and α-TC-treated sham animals (0.83 ± 0.18 – 0.97 ± 0.18 μmol/g liver). The peroxide level increased immediately after 10 min of ischemia, and was normalized during 10 min of reperfusion. A pretreatment with α-TC suppressed the transient increase during IPC (Fig. 1). An increase in the peroxide level in the ischemic rats was also observed after 90 min of ischemia. This was followed by a further increase after reperfusion and reached a peak after 10 min of reperfusion (Fig. 2A). α-TC, IPC, and α-TC+IPC attenuated the increase in the peroxide level (Fig. 2B).

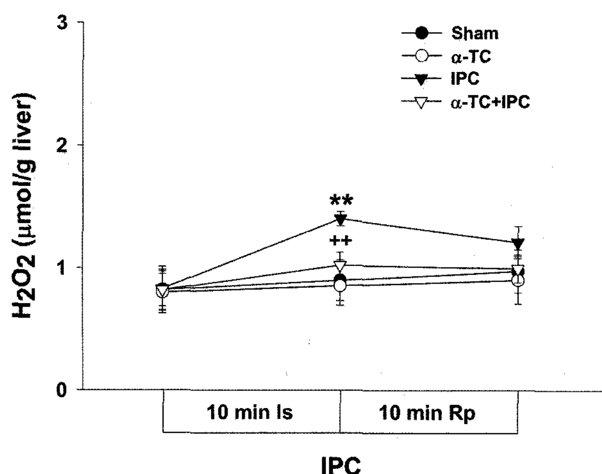

Fig. 1. The peroxide levels in the tissues indicating the level of oxidative stress during IPC. The values are represented as a mean ± SEM for 8-10 rats per group. ** Significantly different ($P<0.01$) from the sham. ++ Significantly different ($P<0.01$) from the IPC group. Is, ischemia; Rp, reperfusion.

Mitochondrial glutamate dehydrogenase activity

The mitochondrial GDH activity in the vehicle- or α-TC-treated sham animals ranged from 2.82 ± 0.15 to 2.91 ± 0.21 IU/mg protein, and was significantly lower in the ischemic group after 5 h and 24 h of reperfusion. This decrease in GDH activity was attenuated by α-TC, IPC, and α-TC+IPC (Fig. 3).

Mitochondrial malondialdehyde level and glutathione content

The MDA level in the liver mitochondria of the vehicle-treated sham animals was 0.95 ± 0.04 nmol/mg protein 5 h after reperfusion and 0.91 ± 0.05 nmol/mg protein 24 h after reperfusion. In the ischemic rats, the MDA level after 5 h reperfusion increased 1.4 and 1.7 times those of the sham values. This increase was attenuated by α-TC, IPC, and α-TC+IPC (Table II). The mitochondrial GSH and GSSG levels in the vehicle- and α-TC-treated sham animals were similar. The GSH concentration decreased significantly after 5 h of reperfusion. α-TC, IPC, and α-TC+IPC attenuated the decrease in the GSH level. The GSSG content was similar in all the experimental groups after 5 h and 24 h of reperfusion. The GSH to GSSG ratio, which indicates the mitochondrial redox state, decreased markedly during reperfusion. After 5 h of reperfusion, the

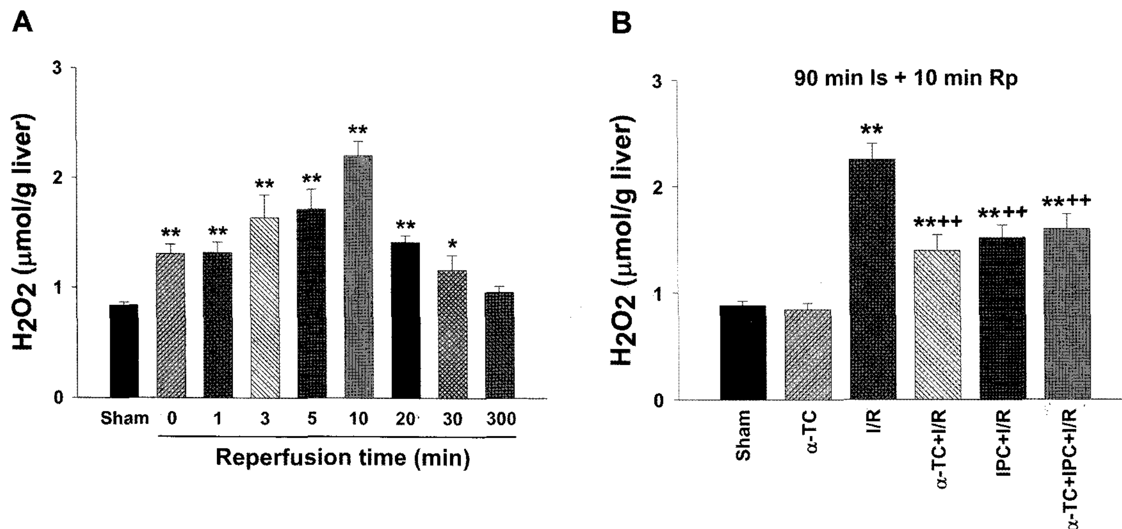

Fig. 2. Changes in the tissue peroxide levels in an ischemic rat liver. (A) The peroxide levels in the tissues measured after 90 min of ischemia and subsequent reperfusion. (B) The effects of α-tocopherol and ischemic preconditioning on the peroxide levels in the tissues measured after 90 min of ischemia followed by 10 min of reperfusion. The values are represented as a mean ± SEM for 7-10 rats per group. *,** Significantly different ($P<0.05$, $P<0.01$) from the sham. ++ Significantly different ($P<0.01$) from the I/R group. Is, ischemia; Rp, reperfusion.

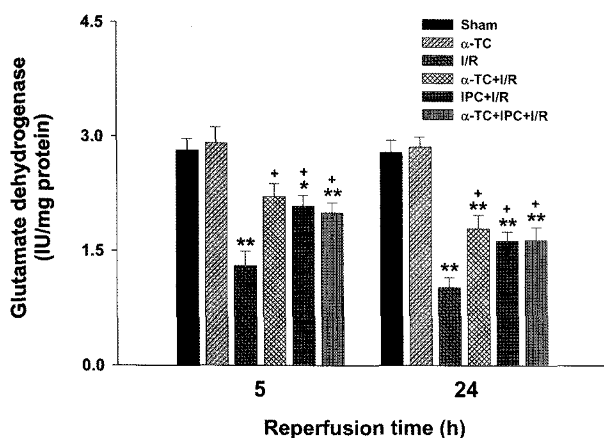

Fig. 3. The effects of α-tocopherol and ischemic preconditioning on the glutamate dehydrogenase activity in a rat liver after 90 min of ischemia followed by reperfusion. The values are represented as a mean ± SEM for 8-10 rats per group. *,** Significantly different ($P<0.05$, $P<0.01$) from the sham. + Significantly different ($P<0.05$) from the I/R group.

Table II. The effects of α-tocopherol and ischemic preconditioning on the malondialdehyde level in the rat liver after ischemia and reperfusion

Group	MDA (nmol/mg protein)	
	5 h	24 h
Sham	0.95 ± 0.04	0.91 ± 0.05
α-TC	0.97 ± 0.05	0.88 ± 0.02
I/R	1.32 ± 0.02**	1.55 ± 0.12**
α-TC+I/R	1.10 ± 0.04*,++	1.17 ± 0.10**,+
IPC+I/R	1.08 ± 0.02**,++	1.22 ± 0.06**,+
α-TC+IPC+I/R	1.14 ± 0.04**,++	1.21 ± 0.02**,+

The rat liver was preconditioned with 10 min of ischemia followed by 10 min of reperfusion. The liver was then subjected to 90 min of ischemia followed by 5 h of reperfusion. The values are shown as a mean ± SEM for 8-10 rats per group. *,** Significantly different ($P<0.05$, $P<0.01$) from the sham. +,++ Significantly different ($P<0.05$, $P<0.01$) from the I/R.

decrease in the GSH to GSSG ratio was attenuated by α-TC, IPC, and α-TC+IPC, but there was no effect observed after 24 h of reperfusion (Table III).

Mitochondrial swelling

Fig. 4 shows the initial swelling rates of the mitochondria isolated from each experimental group. The mitochondria in all the groups swelled after being energized with succinate. The rate of mitochondrial swelling in the I/R group was significantly higher than that observed in the sham-operated group. This rapid increase was attenuated by α-TC, IPC, and α-TC+IPC.

DISCUSSION

ROS production during I/R of the liver is a major pathophysiological component of acute liver failure in I/R situations. In particular, the postischemic oxidative stress and cell damage observed in the liver during reperfusion has been as attributed to the overproduction of ROS in activated Kupffer cells, as well as in the mitochondria of hepatocytes (Jaeschke, 1991). I/R injury in the liver occurs in a biphasic pattern, which consists of both acute- and subacute-phase responses. The acute-phase, which is characterized by hepatocellular injury after 3-6 h of

Table III. The effects of a-tocopherol and ischemic preconditioning on mitochondrial glutathione content in the rat liver after ischemia and reperfusion

Group	GSH (nmol/mg protein)		GSSG (nmol/mg protein)		GSH/GSSG ratio	
	5 h	24 h	5 h	24 h	5 h	24 h
Sham	9.42 ± 0.64	10.42 ± 1.16	0.65 ± 0.03	0.56 ± 0.06	14.56 ± 1.76	18.70 ± 1.56
α-TC	9.70 ± 1.04	9.98 ± 0.99	0.68 ± 0.05	0.60 ± 0.03	14.23 ± 3.03	16.53 ± 3.22
I/R	1.98 ± 0.38**	7.62 ± 1.84	0.87 ± 0.14	0.82 ± 0.14	2.52 ± 0.90**	9.07 ± 2.22**
α-TC+I/R	5.20 ± 0.48$^{**,+}$	7.08 ± 0.95	0.63 ± 0.06	0.60 ± 0.06	8.34 ± 0.93$^{**,++}$	12.01 ± 2.28**
IPC+I/R	6.35 ± 0.87^{++}	12.17 ± 2.00	0.64 ± 0.04	0.81 ± 0.13	9.74 ± 0.72$^{**,++}$	15.41 ± 3.92
α-TC+IPC+I/R	5.98 ± 1.05$^{*,++}$	11.01 ± 2.64	0.68 ± 0.08	0.70 ± 0.22	8.82 ± 1.29$^{**,++}$	15.55 ± 6.28

The values are shown as a mean ± SEM for 8-10 rats per group. *,** Significantly different (P<0.05, P<0.01) from the sham. $^{+,++}$ Significantly different (P<0.05, P<0.01) from the I/R.

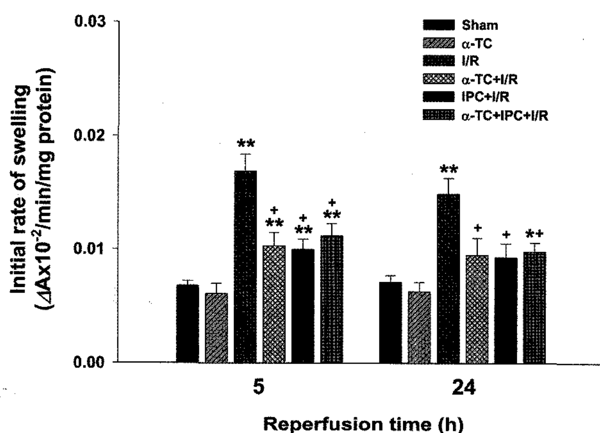

Fig. 4. The effects of α-tocopherol and ischemic preconditioning on the rate of mitochondrial swelling in a rat liver after 90 min of ischemia followed by reperfusion. The values are represented as a mean ± SEM for 8-10 rats per group. ** Significantly different (P<0.01) from the sham. + Significantly different (P<0.05) from the I/R group.

reperfusion, is associated with the generation of free radicals with T-lymphocytes and Kupffer cell activation. Many studies have suggested that the burst of ROS generated after reperfusion may initiate postischemic liver injury, and the subsequent inflammatory infiltration (Zwacka *et al.*, 1997). The subacute-phase response following liver I/R is characterized by massive neutrophil infiltration, which reaches a peak after 18-24 h of reperfusion. The pro-inflammatory cytokines secreted by neutrophils during the subacute-phase bring about the organ damage and generation of intracellular ROS in the damaged tissue through the various receptor-mediated pathways (Kurokawa *et al.*, 1996).

Among the currently tested therapeutic strategies, IPC or pharmacological interventions that mimic these effects have the greatest potential to eliminate the postischemic oxidant stress and reduce the level of hepatic I/R injury. α-Tocopherol (α-TC) is the major chain-breaking antioxidant in mammalian cellular membranes as a result of its efficient antioxidant capacity, which interrupts the chain of membrane lipid peroxidation (Packer, 1992). It was previously reported that a pretreatment with α-TC substantially attenuated the increases in hepatic lipid peroxidation during I/R, which correlated with the improvement in some of the indicators of liver injury and cytochrome P450 activity (Lee and Clemens, 1992). Recently, it was reported that α-TC ameliorates the hepatic secretory functions and microsomal drug metabolizing systems *in vivo*, which is associated with level of lipid peroxidation (Lee *et al.*, 2000). In addition, a low level of α-TC in both the liver and plasma is indicative of oxidative stress (Koneru *et al.*, 1995; Giakoustidis *et al.*, 2002).

There is evidence suggesting that ischemic preconditioning (IPC) leads to a lower inflammatory response with less postischemic oxidant stress (Peralta *et al.*, 2002). IPC is achieved by subjecting the liver to stress resulting in intracellular adaptation. This is unlike the conventional techniques, where therapeutic interventions are used extracellularly to preserve the liver. The initial stressful events enhance the endogenous defense system, making the liver more tolerant to a subsequent I/R injury (Rüdiger *et al.*, 2003). The energy metabolism in hepatocytes occurs in the mitochondria. In response to the I/R process, a mitochondrion produces excess ROS, which causes lipid peroxidation of the mitochondrial membrane, resulting in mitochondrial damage. However, the cellular effects of α-TC and IPC on the I/R-induced mitochondrial injury are largely unknown. This study shows that α-TC and IPC alleviates the postischemic oxidative injury to the liver in both the acute- (5 h) and the subacute-phase (24 h) of I/R.

These results show that the ALT and AST activities increased significantly after 5 h reperfusion. After 24 h of reperfusion, the increase in the ALT activity declined but the level remained high compared with that of the sham-operated rats. This hepatoprotective effect against a warm I/R injury was clearly demonstrated in the rats pretreated

with both α-TC and IPC. A combination of α-TC and IPC did not have any synergistic effects against I/R-induced hepatocyte damage. The enzyme GDH is located in the mitochondrial matrix and indicates damage to the mitochondrial integrity (Lohse *et al.*, 1984). Frederiks *et al.* (1984) reported that an increase in the serum GDH level indicates the presence of cell necrosis in an ischemic liver. There was a decrease in GDH activity in the mitochondria after 5 h and 24 h of reperfusion. In contrast to the ischemic livers, the mitochondria from the liver treated with α-TC, IPC, and α-TC+IPC showed significantly higher GDH activity. These results demonstrate that α-TC and IPC improve the mitochondrial function after hepatic I/R but a combination of α-TC and IPC offers no further improvement in the functional recovery of the mitochondria.

The protective effects of IPC might be mediated by a short sublethal burst of ROS (Rüdiger *et al.*, 2003). During IPC, the hepatic peroxide level increases after 10 min of ischemia, which is significantly suppressed by α-TC. After 90 min of ischemia, the hepatic peroxide level increased with increasing reperfusion time, reaching a peak 10 min after reperfusion. Both α-TC and IPC attenuated this increase. No additional inhibition of the generation of H_2O_2 after reperfusing the livers, which had been subjected to a prolonged period of ischemia, was observed when α-TC was preceded by IPC. α-TC suppressed the sublethal ROS production by IPC, which has been suggested to trigger the protective effects of IPC. The results in this study are similar to those reported by Sindram *et al.* (2002) in that a pretreatment with *N*-acetylcysteine, which is an effective radical scavenger, reversed the beneficial effects of IPC on the sinusoidal endothelial cell detachment and apoptosis.

Although ROS play a role in a number of liver diseases, the detailed mechanisms of ROS involvement are unclear. The most convincing hypothesis of ROS-induced cell injury is the destruction of the cellular membranes *via* lipid peroxidation (Omar *et al.*, 1989). All the mitochondrial constituents including proteins, lipids, and mitochondrial DNA are potential targets of ROS-mediated damage. The redox status inside the cells is tightly controlled, and remains relatively constant unless the cells are exposed to a massive oxidant stress. The GSH/GSSG redox system is a major regulator of the intracellular redox status. In the ischemic rats, the mitochondrial GSH concentration decreased markedly during reperfusion with a concomitant increase in the level of mitochondrial lipid peroxidation. It is likely that the mitochondrial GSH decreases during reperfusion as a consequence of the reduced uptake from the cytosol. Indeed, a marked decrease in the cytosolic GSH (data not shown) occurs for two reasons. It is exported outside of the cell and there is a deficiency in GSH *ex novo* synthesis. The latter is due to the poor availability of ATP caused by the decreased mitochondrial synthesis in the postischemic livers (Metzger and Lauterburg, 1988). α-TC, IPC, and α-TC+IPC attenuated this decrease in the mitochondrial GSH level and mitochondrial lipid peroxidation. This suggests that they increase the mitochondrial pool of GSH and decrease the level of oxidative stress.

Many studies have associated the mitochondrial dysfunctions caused by ROS with both necrotic and apoptotic cell death (Zamzami *et al.*, 1997). One possible explanation is that oxidant stress induces a MPT, which is a key event preceding cell death (Lemasters *et al.*, 1998). Although few studies have evaluated the effect of an opening of the MPT pore on the mitochondrial functions *in situ* (Leducq *et al.*, 1998), the MPT is believed to play a major role in the cell death associated with I/R. Indeed, I/R is closely associated with a Ca^{2+} overload, ROS overproduction, an increase in Pi and a decrease in the cellular ATP concentration, which are conditions favoring the opening of the MPT (Zoratti and Szabo, 1995). In this study, I/R disrupted the mitochondrial structure, which is characterized by an increase in the swelling rate. Both α-TC and IPC protected the mitochondria by inhibiting the MPT, which suggests that it might involve the inhibition of ROS production and have pronounced antioxidant properties. The combination of α-TC and IPC did not further inhibit the opening of the MPT pore.

In conclusion, both α-TC and IPC offered significant hepatoprotection against postischemic injury, which was illustrated by the attenuated mitochondrial oxidant stress, and the preserved mitochondrial integrity. However, there was no additive effect observed when α-TC and IPC were used in combination. It is possible that IPC becomes insignificant when ROS are reduced by the use of α-TC.

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REFERENCES

Azzi, A. and Stocker, A., Vitamin E: non-antioxidant roles. *Prog. Lipid Res.*, 39, 231-255 (2000).

Bilzer, M. and Gerbes, A., Preservation injury of the liver: mechanisms and novel therapeutic strategies. *J. Hepatol.*, 32, 508-515 (2000).

Bradford, M. M., A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, 72, 248-254 (1976).

Buege, J. A. and Aust, S. D., Microsomal lipid peroxidation. *Methods Enzymol.*, 52, 302-310 (1978).

Burton, G. W., Ingold, K. U., and Foster, D. O., Comparison of

free α-tocopherol and α-tocopherol acetate as sources of vitamin E in rats and humans. *Lipids*, 23, 834-840 (1988).

Clavien, P. A., Selzner, M., Rüdiger, H. A., Graf, R., Kadry, Z., Rousson, V., and Jochum, W., A prospective randomized study in 100 consecutive patients undergoing major liver resection with versus without ischemic preconditioning. *Ann. Surg.*, 238, 843-850 (2003).

Clemens, M. G., McDonagh, P. F., Chaudry, I. H., and Baue, A. E., Hepatic microcirculatory failure after ischemia and reperfusion: improvement with ATP-$MgCl_2$ treatment. *Am. J. Physiol.*, 248, H804-H811 (1985).

Elimadi, A., Sapena, R., Settaf, A., Le Louet, H., Tillement, J., and Morin, D., Attenuation of liver normothermic ischemia-reperfusion injury by preservation of mitochondrial functions with S-15176, a potent trimetazidine derivative. *Biochem. Pharmacol.*, 62, 509-516 (2001).

Ellis, G. and Goldberg, D. M., Optimal conditions for the kinetic assay of serum glutamate dehydrogenase activity at 37. *Clin. Chem.*, 18, 523-527 (1972).

Frederiks, W. M., Vogels, I. M., and Fronik, G. M., Plasma ornithine carbamyl transferase level as an indicator of ischaemic injury of rat liver. *Cell Biochem. Funct.*, 2, 217-220 (1984).

Giakoustidis, D., Kontos, N., Tsantilas, D., and Botsoglou, N., Intramuscular administration of very high dose of a-tocopherol protects liver from severe ischemia/reperfusion injury. *World J. Surg.*, 26, 872-877 (2002).

Glanemann, M., Vollmar, B., Nussler, A. K., Schaefer, T., Neuhaus, P., and Menger, M. D., Ischemic preconditioning protects from hepatic ischemia/reperfusion-injury by preservation of microcirculation and mitochondrial redox-state. *J. Hepatol.*, 38, 59-66 (2003).

Griffith, O. W., Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. *Anal. Biochem.*, 106, 207-212 (1980).

Jaeschke, H., Reactive oxygen and ischemia/reperfusion injury of the liver. *Chem. Biol. Interact.*, 79, 115-136 (1991).

Javadov, S. A., Clarke, S., Das, M., Griffiths, E. J., Lim, K. H., and Halestrap, A. P., Ischaemic preconditioning inhibits opening of mitochondrial permeability transition pores in the reperfused rat heart. *J. Physiol.*, 549, 513-524 (2003).

Johnson, D. and Lardy, H., Isolation of liver and kidney mitochondria. *Methods Enzymol.*, 10, 456-470 (1967).

Koneru, B., Reddy, M. C., dela Torre, A. N., Patel, D., Ippolito, T., and Ferrante, R. J., Studies of hepatic warm ischemia in the obese Zucker rat. *Transplantation*, 59, 942-946 (1995).

Kowaltowski, A. J. and Vercesi, A. E., Mitochondrial damage induced by conditions of oxidative stress. *Free Radic. Biol. Med.*, 26, 463-471 (1999).

Kurokawa, T., Nonami, T., Harada, A., Nakao, A., and Takagi, H., Mechanism and prevention of ischemia-reperfusion injury of the liver. *Semin. Surg. Oncol.*, 12, 179-182 (1996).

Leducq, N., Delmas-Beauvieux, M. C., Bourdel-Marchasson, I., Dufour, S., Gallis, J. L., Canioni, P., and Diolez, P., Mitochondrial permeability transition during hypothermic to normothermic reperfusion in rat liver demonstrated by the protective effect of cyclosporin A. *Biochem. J.*, 336, 501-506 (1998).

Lee, S.-M. and Clemens, M. G., Effect of α-tocopherol on hepatic mixed function oxidases in hepatic ischemia/reperfusion. *Hepatology*, 15, 276-281 (1992).

Lee, S.-M., Park, M.-J., Cho, T.-S., and Clemens M. G., Hepatic injury and lipid peroxidation during ischemia and reperfusion. *Shock*, 13, 279-284 (2000).

Lehmann, T. G., Wheeler, M. D., Schwabe, R. F., Connor, H. D., Schoonhoven, R., Bunzendahl, H., Brenner, D. A., Jude Samulski, R., Zhong, Z., Thurman, R. G., Gene delivery of Cu/Zn-superoxide dismutase improves graft function after transplantation of fatty livers in the rat. *Hepatology*, 32, 1255-1264 (2000).

Lemasters, J. J., Nieminen, A. L., Qian, T., Trost, L. C., Elmore, S. P., Nishimura, Y., Crowe, R. A., Cascio, W. E., Bradham, C. A., Brenner, D. A., and Herman, B., The mitochondrial permeability transition in cell death: a common mechanism in necrosis, apoptosis and autophagy. *Biochim. Biophys. Acta*, 1366, 177-196 (1998).

Lohse, W., Otto, G., Pahlig, H., Winkler, H., and Wolff, H., Prognostic value of glutamyl dehydrogenase activity in clinical liver transplantation. *Transplantation*, 38, 559 (1984).

Metzger, J. and Lauterburg, B. H., Postischemic ATP levels predict hepatic function 24 hours following ischemia in the rat. *Experientia*, 44, 455-457 (1988).

Omar, R., Nomikos, I., Piccorelli, G., Savino, J., and Agarwal, N., Prevention of postischaemic lipid peroxidation and liver cell injury by iron chelation. *Gut*, 30, 510-514 (1989).

Packer, L., Interactions among antioxidants in health and disease: Vitamin E and its redox cycle. *Proc. Soc. Exp. Biol. Med.*, 200, 271-276 (1992).

Peralta, C., Bulbena, O., Xaus, C., Prats, N., Cutrin, J. C., Poli, G., Gelpi, E., and Rosello-Catafau, J., Ischemic preconditioning: a defense mechanism against the reactive oxygen species generated after hepatic ischemia reperfusion. *Transplantation*, 73, 1203-1211 (2002).

Rüdiger, H. A., Graf, R., and Clavien, P. A., Sub-lethal oxidative stress triggers the protective effects of ischemic preconditioning in the mouse liver. *J. Hepatol.*, 39, 972-977 (2003).

Sindram, D., Rüdiger, H. A., Upadhya, A. G., Strasberg, S. M., and Clavien, P. A., Ischemic preconditioning protects against cold ischemic injury through an oxidative stress dependent mechanism. *J. Hepatol.*, 36, 78-84 (2002).

Tietze, F., Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. *Anal. Biochem.*, 27, 502-522 (1969).

Tsai, B. M., Wang, M., March, K. L., Turrentine, M. W., Brown, J. W., and Meldrum, D. R., Preconditioning: evolution of basic

mechanisms to potential therapeutic strategies. *Shock*, 21, 195-209 (2004).

Wolff, S. P., Ferrous ion oxidation in presence of ferric ion indicator xylenol orange for measurement of hydroperoxides. *Methods Enzymol.*, 233, 182-189 (1994).

Zamzami, N., Hirsch, T., Dallaporta, B., Petit, P. X., and Kroemer, G., Mitochondrial implication in accidental and programmed cell death: apoptosis and necrosis. *J. Bioenerg. Biomembr.*, 29, 185-193 (1997).

Zoratti, M. and Szabo, I., The mitochondrial permeability transition. *Biochem. Biochim. Biophys. Acta*, 1241, 139-176 (1995).

Zwacka, R. M., Zhang, Y., Halldorson, J., Schlossberg, H., Dudus, L., and Engelhardt, J. F., $CD4^+$ T-lymphocytes mediate ischemia/reperfusion-induced inflammatory responses in mouse liver. *J. Clin. Invest.*, 100, 279-289 (1997).