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European Journal of Pharmacology 403 (2000) 243–250

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The beneficial effect of ATP–$MgCl_2$ on hepatic ischemia/reperfusion-induced mitochondrial dysfunction

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Received 13 April 2000; received in revised form 5 July 2000; accepted 7 July 2000

Abstract

The present study was undertaken to determine whether ATP–$MgCl_2$ administration in rats could protect hepatic mitochondrial function and improve energy metabolism during hepatic ischemia and subsequent reperfusion. Global hepatic ischemia was produced for 60 min followed by reperfusion. The rats then received 0.5 ml of saline or ATP–$MgCl_2$ intravenously. In saline-treated ischemic rats, serum alanine-aminotransferase levels peaked at 5 h. The aminotransferase level was significantly reduced in the ATP–$MgCl_2$ treatment group. The wet weight-to-dry weight ratio of the liver was significantly increased by ischemia/reperfusion. ATP–$MgCl_2$ treatment minimized the increase in this ratio. The ketone body ratio in blood, which reflects the mitochondrial free NAD^+/NADH ratio, decreased after ischemia and at 1 h following reperfusion. This decrease was somewhat improved by ATP–$MgCl_2$ infusion. At 1 and 5 h after reperfusion, mitochondrial monoamine oxidase and glutamate dehydrogenase activities decreased. ATP–$MgCl_2$ infusion following ischemia restored the lost activities. Hepatic ATP levels in saline-treated rats were found to be 50% lower 5 h following reperfusion; however, treatment with ATP–$MgCl_2$ resulted in significantly higher ATP levels and energy charge. The accumulation of purine catabolites in ischemic tissues was reduced during reperfusion. ATP–$MgCl_2$ infusion resulted in accumulation of adenosine in reperfused liver. Mitochondrial lipid peroxidation was elevated in the saline-treated ischemic group, but this elevation was inhibited by ATP–$MgCl_2$ infusion. The present results lead us to conclude that the amelioration of liver function which occurs with ATP–$MgCl_2$ infusion following ischemia may be mediated through improvement in ischemia-induced mitochondrial energy metabolism.

Keywords: ATP–$MgCl_2$; Mitochondrial energy metabolism; Mitochondrial enzyme; Lipid peroxidation; Hepatic ischemia/reperfusion

1. Introduction

Temporary interruption of hepatic blood flow is often required in the management of acute hepatic trauma and is obligatory during liver transplantation. However, such manipulations may result in hepatic hypoxia and hepatic dysfunction. Due to the high metabolic rate, hepatic cells are vulnerable to the deleterious influence of anoxia. The pathophysiological mechanism of the cellular injury that follows ischemia is still incompletely understood, although hepatic ischemia has been implicated in the cause of these abnormalities.

It has also been shown that hepatic mitochondrial function and cell membrane transport of sodium and potassium are depressed during early hemorrhagic shock (Baue et al., 1974), indicating the susceptibility of the liver to even small insults. Moreover, a number of studies have suggested that ischemic cell death is a consequence of irreversible mitochondrial injury (Trump et al., 1976). The impairment of the oxidative phosphorylation system in mitochondria is the most significant change (Mittnacht et al., 1979). A decrease in the cellular level of ATP is common to all ischemic tissues and has been confirmed by many researchers (Marubayashi et al., 1980). The production of adenosine and other purine metabolites in ischemic kidney, heart and muscle has been reported (Warnick and Lazarus, 1981). However, the catabolic pathway of the nucleotide in ischemic liver has not been extensively investigated.

It has been shown that, after 3 h of ischemia to the left lateral and median lobes of the liver, various ultrastructural changes take place in the mitochondria which are not restored upon reestablishment of hepatic blood flow (Mittnacht et al., 1979). However, in animals pretreated with

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0014-2999/00/$ - see front matter
PII: S0014-2999(00)00485-4

chlorpromazine, all of the mitochondrial alterations were reversible after 3 h of ischemia. This suggest that some aspects of ischemic cellular damage are reversible. Furthermore, studies by Hirasawa et al. (1978) have shown that infusion of ATP–$MgCl_2$ following 60 or 90 min of global hepatic ischemia significantly decreased serum enzyme levels and increased survival rate in the test population. However, the precise mechanism of action remains unknown.

The present study was undertaken to elucidate the possible mechanism of the salutary effects of ATP–$MgCl_2$. The influence of ATP–$MgCl_2$ infusion on adenine nucleotide levels and mitochondrial function was also studied in rats.

2. Materials and methods

2.1. Hepatic ischemia procedure

Male Sprague–Dawley rats, 250–300 g, were fasted 24 h before the experiment and allowed to drink tap water ad libitum. The rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.), and the abdomen was opened by midline incision. The left branches of the portal vein and hepatic artery were clamped to induce complete ischemia of the median and left hepatic lobes, while the right lobes remained perfused to prevent intestinal congestion. At the end of 60 min of ischemia, the clip around the left branch of the portal vein was removed, and the branch to the right lobes was ligated. This resulted in restoration of all portal and hepatic arterial flow, except for the very small amount to the caudate lobe, through the previously ischemic lobes. After 1 and 5 h of reperfusion, blood was taken from the abdominal aorta. The median lobe of the liver was removed and then frozen in liquid nitrogen for the assay of purine nucleotides. The left lobe of the liver was removed and used for other experiments.

2.2. Experimental groups

Four groups of animals were studied. Rats in group 1 (sham) received the same manipulations as group 3, except that the clamp around the hepatic vessels was not placed. Group 2 was subjected to 60 min of hepatic ischemia. Group 3 was subjected to 60 min of hepatic ischemia followed by 1 and 5 h of reperfusion. At the beginning of the reperfusion period, groups 1 and 3 rats were administered 0.5 ml saline intravenously. Group 4 was subjected to the same ischemia and reperfusion procedures as group 3, except that during the first 45 min of reperfusion the rats were treated with ATP plus $MgCl_2$ (12.5 μmol each dissolved in 0.5 ml and administered intravenously). Due to potent hypotensive effects associated with administration of ATP–$MgCl_2$ (Chaudry et al., 1984), series of 0.05 ml boluses were given over a 45 min period.

2.3. ATP and its catabolites analysis

The median lobe of liver samples was frozen in liquid nitrogen and freeze-dried. The tissue was then minced to a powder and extracted with 1.5 M perchloric acid. Following centrifugation, neutralization and final centrifugation, extracts were kept in an ice bath until injected into a high-performance liquid chromatography (HPLC) system for analysis. The hepatic concentrations of ATP, ADP, AMP, adenosine, inosine, hypoxanthine and xanthine were measured by HPLC (Gilson model). The HPLC system consisted of two high-performance pumps, model 306, a buffer mixer, model 811C and a motor valve, Rheodyne 77251, for loading and injecting the samples. The nucleotides, nucleosides and purine bases were separated on a reverse-phase C_{18} silica column, with 0.1 M ammonium dihydrogen phosphate buffer (pH 4.0) and methanol/acetonitrile (1:1) solution as eluant. Flow rate was 1 ml/min and detection wavelength was 254 nm. Separation was accomplished within 30 min. The metabolite concentrations were calculated from the computer-integrated areas of the peaks in the sample chromatogram in relation to the areas obtained for standard solutions, as previously detailed by Maessen et al. (1988). Based on ATP, ADP and AMP concentrations, the energy charge (EC) was calculated as $EC = (ATP + 0.5\ ADP)/(ATP + ADP + AMP)$ (Atkinson, 1966).

2.4. Analytical procedures

Concentrations of arterial ketone bodies were measured in blood samples mixed with 4 ml ice-cold 10% (w/v) perchloric acid. After centrifugation at $8000 \times g$ for 5 min at 4°C, the supernatant was adjusted to pH 6.0 with ice-cold 69% (w/v) K_2CO_3 and recentrifuged at $8000 \times g$ for 5 min at 4°C. The final supernatant was used in the analyses of the concentrations of acetoacetate and 3-hydroxybutyrate (Mellanby and Williamson, 1974). The ketone body ratio (acetoacetate/3-hydroxybutyrate) was calculated. A piece of the left lateral lobe was excised for determination of the tissue water content. The tissue was weighed, dried for 48 h at 80°C, and then reweighed to obtain the wet weight-to-dry weight ratio. For the measurements of mitochondrial enzyme activity and lipid peroxidation, the mitochondria were prepared by differential centrifugation from a 10% liver homogenate in 0.25 M sucrose. After sedimentation of the nuclear fraction at $600 \times g$ for 15 min, the mitochondria were sedimented from the supernatant by centrifugation at $7000 \times g$ for 15 min. The fluffy layer was carefully discarded, and the pellet was washed twice with 1/2 and 1/4 the initial volume of 0.25 M sucrose followed by centrifugation at $7000 \times g$ for 15 min. The final mitochondrial pellets were suspended in 0.25 M sucrose. All operations of preparation were carried out at 4°C. Monoamine oxidase was estimated by measuring the aldehyde formed during the enzymatic

oxidation of tyramine (Green and Haughton, 1961). For the measurement of mitochondrial glutamate dehydrogenase, the mitochondrial suspension was diluted in a solution containing Triton X-100 (0.05% v/v), 50 mM KH_2PO_4, and 1 mM EDTA, pH 7.5. Glutamate dehydrogenase activity was measured spectrophotometrically in the direction of glutamate formation and the oxidation of nicotinamide adenine dinucleotide (NADH) was monitored at 340 nm at 30°C (Ellis and Goldberg, 1972). Succinate dehydrogenase was assayed spectrophotometrically by using the procedure of Green et al. (1955), in which the rate of reduction of dichlorophenolindophenol was measured at 600 nm. The extent of lipid peroxidation in the mitochondria was determined by measuring malondialdehyde, an end product of lipid peroxidation. The malondialdehyde content in homogenates prepared as described by Masugi and Nagamura (1976) was measured using thiobarbituric acid. The protein content was calculated according to the method of Bradford (1976). Serum alanine-aminotransferase activity was determined using standard spectrophotometric procedures using kit # 59-UV (Sigma, St. Louis, MO, USA).

2.5. Statistical analysis

Significant changes in various parameters measured were determined using the one-way analysis of variance (ANOVA) method. Differences between experimental groups were considered significant at $P < 0.05$ with the appropriate Student–Newman–Keuls test made for multiple comparisons. All results are presented as means ± S.E.M.

3. Results

3.1. Serum aminotransferase activity

The serum level of alanine aminotransferase in the sham-operated rats was 55 ± 5 IU/l, which was similar to that of normal rats, and increased to 600 ± 84 IU/l after 5 h of reperfusion. After 60 min of ischemia without reperfusion, no changes were observed in serum aminotransferase in ischemic rats compared with preischemic values. However, when the blood flow to the ischemic lobe of the liver was restored, increased aminotransferase activity was noted after 1 h, in the saline-treated group, with a maximum at 5 h (4174 ± 468 IU/l, $P < 0.01$). Treatment with ATP–$MgCl_2$ slightly prevented the increase in aminotransferase activity after 1 h of reperfusion following ischemia; however, it significantly suppressed the aminotransferase activity at 5 h (Fig. 1).

3.2. Wet weight-to-dry weight ratio of liver

The wet weight-to-dry weight ratio of the livers in the sham-operated rats was 3.27 ± 0.09. However, the ratio

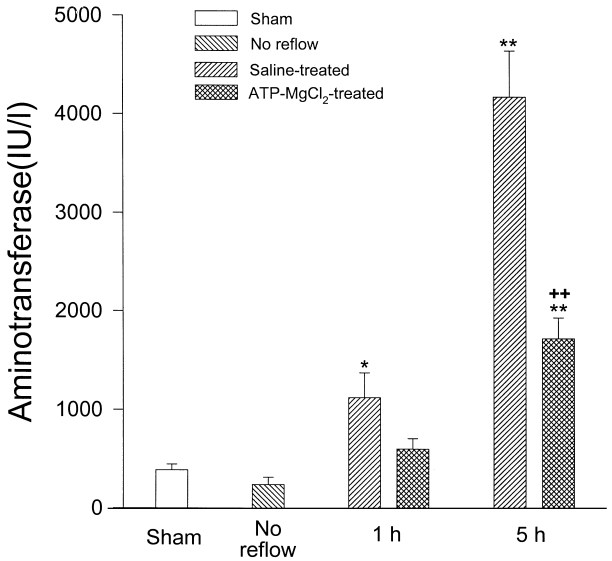

Fig. 1. Effect of ATP–$MgCl_2$ on serum alanine aminotransferase activity after ischemia and subsequent reperfusion. Values are means ± S.E.M. for 7–10 rats/group. $^{*}P < 0.05$, $^{**}P < 0.01$, difference from sham-operated rats. $^{++}P < 0.01$, difference from saline-treated rats.

significantly increased after ischemia and in the saline-treated rats after 1 h of reperfusion, i.e. 3.82 ± 0.07 at ischemia and 3.94 ± 0.05 after 1 h of reperfusion. The wet weight-to-dry weight ratio of the livers in the saline-treated rats recovered to that of the sham-operated rats after 5 h of reperfusion. The tissue edema induced by ischemia/reperfusion was attenuated by ATP–$MgCl_2$ treatment (Fig. 2).

3.3. Mitochondrial enzymes

The total activity of monoamine oxidase in the sham-operated rats was 1.47 ± 0.02 μmol/g liver. Following ischemia, the monoamine oxidase activity significantly decreased and remained depressed for 1 h after reperfusion, but recovered to that of the sham-operated rats activity after 5 h of reperfusion. The decrease in monoamine oxidase activity after 1 h of reperfusion was restored by ATP–$MgCl_2$ treatment. The mitochondrial glutamate dehydrogenase activity in the sham-operated rats was 486 ± 34 μmol/g liver. After 60 min of ischemia without reperfusion, no changes were observed in mitochondrial glutamate dehydrogenase activity when compared with the preischemic values. However, when blood flow to the ischemic lobe of the liver was restored, the glutamate dehydrogenase activity in the saline-treated group was decreased after 1 h and decreased further after 5 h of reperfusion. In contrast, the glutamate dehydrogenase activity was significantly increased at both time points in ATP–$MgCl_2$-treated animals. The activity of succinate dehydrogenase in sham-operated rats remained constant at approximately 117 μmol/g liver throughout the experiment. The activity of succinate dehydrogenase in saline-

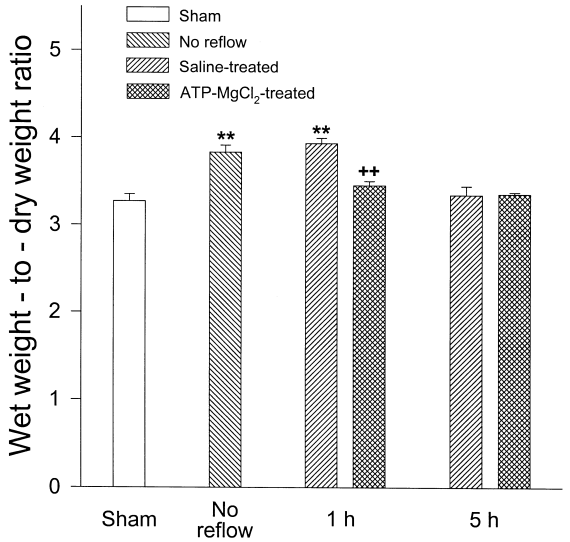

Fig. 2. Effect of ATP–$MgCl_2$ on the ratio of wet weight-to-dry weight in rat liver after ischemia and reperfusion. Values are means ± S.E.M. for 7–10 rats/group. $^{**}P < 0.01$, difference from sham-operated rats. $^{++}P < 0.01$, difference from saline-treated rats.

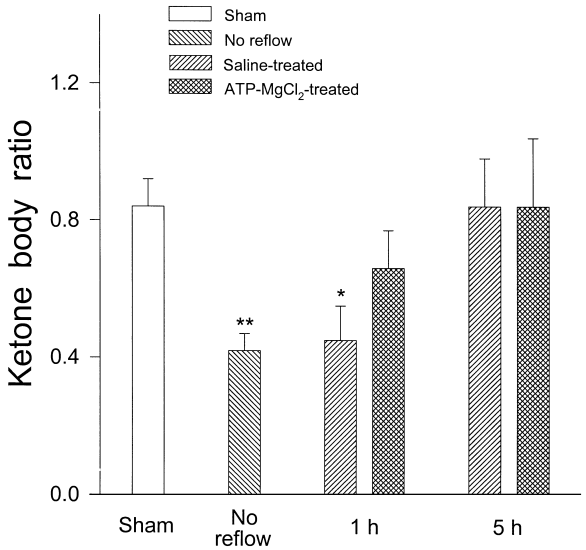

Fig. 3. Effect of ATP–$MgCl_2$ on the arterial ketone body ratio after ischemia and subsequent reperfusion. Values are means ± S.E.M. for 7–10 rats/group. $^{*}P < 0.05$, $^{**}P < 0.01$, difference from sham-operated rats.

treated rats and ATP–$MgCl_2$-treated rats did not differ from that of the sham animals (Table 1).

3.4. Ketone body ratio

The arterial ketone body ratio reflects the NAD^+/NADH ratio of the hepatic mitochondria. The ketone body ratio in the sham-operated rats remained unchanged during the period studied. Following ischemia for 60 min, the ketone body ratio in blood fell markedly from 0.84 ± 0.08 to 0.42 ± 0.05. In the saline-treated group, the ketone body

Table 1
Effect of ATP–$MgCl_2$ on mitochondrial enzyme activity in rat liver after ischemia and reperfusion

	End ischemia	1-h Reperfusion	5-h Reperfusion
Monoamine oxidase[1]			
Sham	1.47 ± 0.02	1.56 ± 0.12	1.84 ± 0.12
No reflow	0.25 ± 0.10[b]		
Saline-treated		1.00 ± 0.15[a]	1.82 ± 0.15
ATP–$MgCl_2$-treated		1.42 ± 0.11[c]	1.95 ± 0.20
Glutamate dehydrogenase[1]			
Sham	486.4 ± 34.2	535.1 ± 47.2	506.9 ± 41.6
No reflow	532.7 ± 70.8		
Saline-treated		376.5 ± 60.9	314.7 ± 49.5[a]
ATP–$MgCl_2$-treated		598.4 ± 47.7[c]	681.2 ± 57.4[a,d]
Succinate dehydrogenase[1]			
Sham	124.1 ± 18.9	109.1 ± 8.0	117.8 ± 9.9
No reflow	120.9 ± 19.4		
Saline-treated		116.6 ± 14.5	102.8 ± 17.6
ATP–$MgCl_2$-treated		103.4 ± 16.0	124.6 ± 8.9

Each value is the means ± S.E.M. for 7–10 rats/group.
[1] μmol/g liver.
[a] $P < 0.05$; difference from sham-operated rats.
[b] $P < 0.01$; difference from sham-operated rats.
[c] $P < 0.05$; difference from saline-treated rats.
[d] $P < 0.01$; difference from saline-treated rats.

Table 2
Effect of ATP–$MgCl_2$ on the cellular content of adenine nucleotides in ischemic rat liver

	End ischemia	1-h Reperfusion	5-h Reperfusion
ATP[1]			
Sham	6.82 ± 0.29	6.87 ± 0.24	6.92 ± 0.18
No reflow	1.10 ± 0.09[a]		
Saline-treated		2.05 ± 0.21[a]	3.39 ± 0.34[a]
ATP–$MgCl_2$-treated		3.92 ± 0.32[a,c]	5.07 ± 0.25[a,c]
ADP[1]			
Sham	3.06 ± 0.30	3.14 ± 0.26	3.04 ± 0.19
No reflow	0.76 ± 0.07[a]		
Saline-treated		1.46 ± 0.18[a]	2.37 ± 0.28
ATP–$MgCl_2$-treated		1.50 ± 0.12[a]	2.88 ± 0.12
AMP[1]			
Sham	3.56 ± 0.38	3.70 ± 0.28	3.47 ± 0.21
No reflow	5.70 ± 0.09[a]		
Saline-treated		2.65 ± 0.12[a]	2.22 ± 0.14[a]
ATP–$MgCl_2$-treated		2.27 ± 0.24[a]	2.54 ± 0.18[a]
Total adenine nucleotides[1]			
Sham	13.42 ± 0.92	13.67 ± 0.67	13.47 ± 0.59
No reflow	7.53 ± 0.26[a]		
Saline-treated		6.20 ± 0.49[a]	7.79 ± 0.79[a]
ATP–$MgCl_2$-treated		7.70 ± 0.58[a]	10.57 ± 0.48[a,b]

Each value is the means ± S.E.M. for 7–10 rats/group.
[1] μmol/g liver.
[a] $P < 0.01$; difference from sham-operated rats.
[b] $P < 0.05$; difference from saline-treated rats.
[c] $P < 0.01$; difference from saline-treated rats.

ratio remained depressed for 1 h after reperfusion but recovered to that of sham-operated rats after 5 h of reperfusion. The decrease in ketone body ratio after 1 h of reperfusion was restored by ATP–$MgCl_2$ treatment (Fig. 3).

3.5. Energy metabolism

Changes in adenine nucleotides and purine catabolites, reflecting energy metabolism in the hepatic tissue, during ischemia and after reperfusion are shown in Table 2, 3 and Fig. 4. Table 2 shows changes in the adenine nucleotide content of warm ischemic liver. The value of cellular ATP in sham-operated group was 6.82 ± 0.29 μmol/g liver weight. The level of ATP in liver decreased rapidly after 60 min of ischemia, while the level of ADP decreased gradually. A concomitant transient increase in the level of AMP was observed. At 5 h following reperfusion in the saline-treated rats, hepatic ATP levels were higher and were found to be approximately 50% of those of sham-operated rats. ADP levels recovered to 78% of sham levels

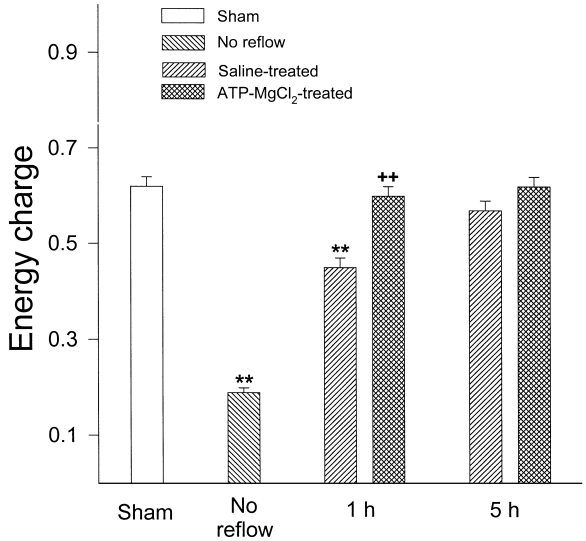

Fig. 4. Effect of ATP–$MgCl_2$ on the energy charge in rat liver after ischemia and subsequent reperfusion. Values are means ± S.E.M. for 7–10 rats/group. $^{**}P < 0.01$, difference from sham-operated rats. $^{++}P < 0.01$, difference from saline-treated rats.

Table 3
Effect of ATP–$MgCl_2$ on the cellular content of purine catabolites in ischemic rat liver

	End ischemia	1-h Reperfusion	5-h Reperfusion
Adenosine[1]			
Sham	0.31 ± 0.05	0.30 ± 0.08	0.28 ± 0.02
No reflow	0.66 ± 0.06[b]		
Saline-treated		0.24 ± 0.05	0.20 ± 0.04
ATP–$MgCl_2$-treated		0.42 ± 0.04[c]	0.58 ± 0.02[b,d]
Inosine[1]			
Sham	0.17 ± 0.03	0.18 ± 0.01	0.20 ± 0.03
No reflow	0.80 ± 0.09[b]		
Saline-treated		0.31 ± 0.02[b]	0.31 ± 0.07
ATP–$MgCl_2$-treated		0.24 ± 0.06	0.25 ± 0.05
Hypoxanthine[1]			
Sham	0.28 ± 0.04	0.26 ± 0.03	0.26 ± 0.04
No reflow	0.73 ± 0.10[b]		
Saline-treated		0.34 ± 0.02	0.24 ± 0.05
ATP–$MgCl_2$-treated		0.27 ± 0.02[c]	0.50 ± 0.17
Xanthine[1]			
Sham	0.24 ± 0.03	0.20 ± 0.02	0.23 ± 0.03
No reflow	2.07 ± 0.14[b]		
Saline-treated		0.26 ± 0.03	0.22 ± 0.02
ATP–$MgCl_2$-treated		0.37 ± 0.06[a]	0.38 ± 0.02[b,d]
Total purine bases[1]			
Sham	14.28 ± 1.06	14.32 ± 0.95	14.52 ± 0.68
No reflow	10.97 ± 0.59[a]		
Saline-treated		7.28 ± 0.50[b]	8.14 ± 0.96[b]
ATP–$MgCl_2$-treated		9.01 ± 0.82[b]	12.40 ± 0.88[c]

Each value is the means ± S.E.M. for 7–10 rats/group.
[1] μmol/g liver.
[a] $P < 0.05$; difference from sham-operated rats.
[b] $P < 0.01$; difference from sham-operated rats.
[c] $P < 0.05$; difference from saline-treated rats.
[d] $P < 0.01$; difference from saline-treated rats.

but AMP levels remained lower than the values observed in sham-operated rats. Corresponding hepatic ATP levels of rats treated with ATP–$MgCl_2$ after 5 h of reperfusion were found to be 73% of those of sham-operated rats. Hepatic AMP levels were still lower than those of sham-operated group. Hepatic energy charge was significantly reduced immediately after 60 min of ischemia. In the saline-treated rats, after 1 h of reperfusion, the energy charge was still lower but it was restored to the level of sham-operated rats after 5 h of reperfusion. The energy charge in the ATP–$MgCl_2$ group was similar to that of the sham-operated group (Fig. 4). Table 3 shows that during the ischemic period, there was a large accumulation of cellular concentrations of purine catabolites, which was paralleled by a large depletion of ATP. In this period, the level of xanthine markedly increased to 10 times the sham values. However, at 1 and 5 h following reperfusion of the previously ischemic liver, concentrations of purine catabolites significantly decreased in the saline-treated rats. The decrease in total concentrations of purine catabolites after 5 h of reperfusion was restored by ATP–$MgCl_2$ treatment. After 5 h of reperfusion the levels of adenosine and xanthine in ATP–$MgCl_2$-treated rats were higher than the values observed in the sham-operated rats.

3.6. Lipid peroxidation

The results of malondialdehyde determinations are presented in Fig. 5. In the sham animals, the level of malondialdehyde in the liver mitochondria remained constant at 0.36 ± 0.03 nmol malondialdehyde formed/mg protein throughout the experiment. In the saline-treated ischemic rats, the level of malondialdehyde in the mitochondria

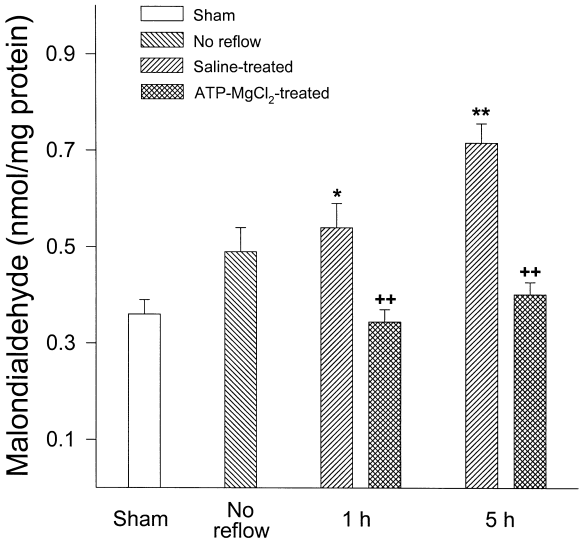

Fig. 5. Effect of ATP–$MgCl_2$ on mitochondrial lipid peroxidation in rat liver after ischemia and subsequent reperfusion. Values are means ± S.E.M. for 7–10 rats/group. $^{*}P < 0.05$, $^{**}P < 0.01$, difference from sham-operated rats. $^{++}P < 0.01$, difference from saline-treated rats.

started to increase after 1 h of reperfusion and was markedly increased to two times sham values after 5 h of reperfusion. ATP–$MgCl_2$ treatment prevented this elevation in mitochondria malondialdehyde after both 1 and 5 h of reperfusion.

4. Discussion

The results of this study indicate that hepatic ischemia causes severe damage to hepatic mitochondria in control animals in vivo. In contrast, treatment with ATP–$MgCl_2$ (but not with ATP or $MgCl_2$ alone) attenuated ischemic liver damage. This salutary effect of ATP–$MgCl_2$ was supported by a decrease in serum aminotransferase activity, restoration of mitochondrial enzyme activity, improvement of mitochondrial energy metabolism, and a decrease in mitochondrial lipid peroxidation level.

In this study, total hepatic ischemia was produced for 60 min by ligating the hepatic artery and portal vein as well as the bile duct. Previous work has shown no detectable blood flow to the liver after the occlusion of the above vessels (Hirasawa et al., 1978). However, despite the reestablishment of blood flow, abnormalities of hepatic function were still observed in ischemic animals (Hirasawa et al., 1978). Although pretreatment of animals with chlorpromazine has been shown to reverse hepatic dysfunction following ischemia (Mittnacht et al., 1979), postischemia treatment with therapeutic agents has not been effective in reversing ischemic damage. This suggests that the cellular damage resulting from ischemia is irreversible in nature. The results presented here, however, demonstrate that treatment of animals with ATP–$MgCl_2$ after hepatic ischemia reverses or prevents the depression of hepatic function. In saline-treated ischemic rats, the level of alanine aminotransferase increased after 1 h of reperfusion and reached a peak after 5 h of reperfusion. Treatment with ATP–$MgCl_2$ attenuated aminotransferase release after both 1 and 5 h of reperfusion. Thus, at least some critical components of ischemic damage can be improved by postischemia treatment with ATP–$MgCl_2$.

It has been shown that cell membrane transport of sodium and potassium is depressed during early hemorrhagic shock (Baue et al., 1974), indicating the susceptibility of the liver to even small insults. The properties of the plasma membrane as a permeability barrier and the presence of ATP–dependent ion pumps help to maintain ion gradients across the plasma membrane. In the present study, the ratio of wet weight-to-dry-weight in the saline-treated livers significantly increased at both the end of ischemia and after 1 h of reperfusion. The postischemia treatment with ATP–$MgCl_2$, however, resulted in a near normalization of the cellular water content after 1 h of reperfusion, suggesting that such treatment reduces the swelling of cells through an improvement in plasma membrane permeability or in Na^+K^+ATPase activity.

It is well established that the viability of mitochondrial function deteriorates with prolonged ischemia and that it is also one of the hallmarks for determining the reversibility of cell injury (Wang et al., 1988). According to Frederiks and Marx (1987), after as little as 1 h of reperfusion, cytosolic lactate dehydrogenase activity decreased in a certain proportion of the liver parenchymal cells, whereas glutamate dehydrogenase activity in the mitochondrial matrix started to decrease after 5 h of reperfusion. There are some indications that the appearance of mitochondrial enzymes in the blood can be viewed as a sign of irreversible liver cell damage (Lohse et al., 1984). The mitochondrial enzymes investigated here were monoamine oxidase, a marker for the outer membrane of the mitochondria, glutamate dehydrogenase localized in the mitochondrial matrix, and succinate dehydrogenase, a marker for the inner membrane of the mitochondria. Analysis of the mitochondrial enzymes of rat liver tissue during reperfusion after ischemia showed at first a decrease in monoamine oxidase activity during ischemia associated with mitochondrial swelling, followed by a decrease in glutamate dehydrogenase during reperfusion. The activity of succinate dehydrogenase remained unchanged. Frederiks and Marx (1987) have shown that the decrease in succinate dehydrogenase activity observed 24 h after ischemia occurs under conditions in which overt necrosis occurs, with the disappearance of nuclei. Although we did not measure the activity of these mitochondrial enzymes in the blood, it seems likely that the decrease in enzyme activity was caused by the leakage of the enzyme into the blood and/or a denaturation of the enzyme. In contrast to those of the saline-treated rats, the mitochondria from the rats treated with ATP–$MgCl_2$ exhibited significantly higher mono-

amine oxidase and glutamate dehydrogenase activities after 1 and 5 h of reperfusion. Thus, although reperfusion following hepatic ischemia results in severe deterioration of mitochondrial function, ATP–$MgCl_2$ improves hepatic mitochondrial function.

In liver mitochondria, acetoacetate is produced in the matrix compartment and undergoes reduction to β–hydroxybutyrate by β–hydroxybutyrate dehydrogenase in the mitochondrial inner membrane (Ernster and Kaylenstierna, 1970). Several studies have shown that the mitochondrial NAD oxidoreduction state is of major significance in energy production and in the control of cell metabolism. The arterial blood ketone body ratio reflects the free NAD^+/NADH ratio of the liver mitochondria via the 3-hydroxybutyrate dehydrogenase system (Taki et al., 1990). Although many factors are thought to contribute to changes in the ratio of ketone bodies in the blood, the liver is the only organ that makes a net contribution of ketone bodies to the blood stream. During ischemia without reperfusion, the arterial ketone body ratio rapidly decreased after 60 min of ischemia. It remained depressed for 1 h after reperfusion, followed by restoration to normal at 5 h. In contrast to that of the saline-treated rats, blood from the ATP–$MgCl_2$-treated group exhibited a higher ketone body ratio after 1 h of reperfusion.

Under aerobic conditions, cellular function is regulated by the level of high-energy phosphate produced mainly oxidative phosphorylation in the mitochondria. When tissue becomes ischemic, ATP is progressively degraded to other adenine nucleotides, nucleosides, and finally, purine catabolites. Levels of ATP are restored after reflow via the salvage pathway and through the slow, but more efficient pathway of de novo synthesis (Ishizaki et al., 1997). At the end of the ischemic period, ATP levels in the liver were very low; however, a marked increase in AMP levels was observed. Following reperfusion of the previously ischemic liver, the AMP levels decreased significantly and the ATP and ADP levels increased. Thus, ATP resynthesis does occur following reperfusion in previously ischemic liver. The hepatic ATP level in the saline-treated group was 50% of normal after 5 h of reperfusion, indicating that a rate-limiting factor dependent on hepatic flow or resynthesis of ATP existed.

As proposed by Atkinson (1966), the adenylate energy charge is an expression of the equilibrium between the energy-generating and energy-consuming reactions in a cell. In this study, the energy charge significantly decreased after ischemia and after 1 h of reperfusion but recovered after 5 h of reperfusion, suggesting that the equilibrium between high-energy production and/or utilization was distorted following ischemia. Treatment with ATP–$MgCl_2$ resulted in an increase of the hepatic ATP levels to 73% of normal and in an improvement of the energy charge within 5 h.

Changes in the cellular levels of adenine nucleotides and their metabolites have been analyzed in ischemic tissues by many researchers. However, these studies have usually been confined to only certain metabolites. In this study, we found that adenine nucleotides were finally degraded to xanthine with an accumulation of intermediate purine metabolites, such as adenosine, inosine and hypoxanthine, due to a shortage of molecular oxygen in the ischemic rat liver. Kamiike et al. (1982) reported that the levels of intermediate purine metabolites accumulated in the rat liver during ischemia decrease during reperfusion. When ATP was available, these metabolites may also be reused for nucleotide synthesis. However, using ATP–$MgCl_2$, we found that the elevation of adenosine levels was maintained during reperfusion. This is of special interest because adenosine is known to exert various biological actions (Mckie et al., 1994; Mullane and Bullough, 1995), such as increase in blood flow, vasodilation, inhibition of free radical production, suppression of neutrophil activation, and prevention of platelet aggregation. Over the last decade, the importance of the protective role of adenosine in ischemia and reperfusion injury has caught the attention of many researchers. A recent study has shown that preischemic administration of a nucleoside transport inhibitor attenuates postreperfusion liver injury, due to the apparent positive effect of augmented levels of endogenous adenosine (Todo et al., 1997). The present data suggest that the beneficial effect of ATP–$MgCl_2$ is related to augmented endogenous adenosine.

Accumulating evidence indicates that oxygen-derived free radicals play a major role in producing the microvascular and parenchymal cell damage associated with reperfusion of ischemic tissues (Drugas et al., 1991). The attack of free radicals on biological membranes, such as the plasma membrane, mitochondria and endoplasmic reticulum, can lead to the oxidative destruction of the polyunsaturated fatty acids of the membranes through lipid peroxidation. Once cellular membranes are damaged by free radicals during ischemia, they become leaky to constituents, such as adenine nucleotides. The adenine nucleotides are converted to nucleosides in the interstitial space and are taken up by endothelial cells (Rounds et al., 1994), where they are catabolized to hypoxanthine. Since sinusoidal endothelial cells are abundant in xanthine oxidase (Bruder et al., 1982), they may be more vulnerable to oxygen radicals generated by the xanthine oxidase system when the blood supply is restored. Evidence indicates that treatment with ATP–$MgCl_2$ improved energy metabolism during reperfusion in ischemic liver by protecting against the functional damage of cellular and subcellular membranes during lipid peroxidation. This is supported by the observation that ATP–$MgCl_2$ administration suppressed the increase in hepatic lipid peroxide after 1 and 5 h of reperfusion (Fig. 5).

In conclusion, treatment with ATP–$MgCl_2$ infusion, by improving ischemia-induced mitochondria energy metabolism, ameliorates liver function in ischemia and reperfusion.

Acknowledgements

This work was supported by grant 981-0714-110-2 from the Basic Research program of the Korea Science and Engineering Foundation.

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