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Original Article

THE EFFECT OF ANTAGONISM OF ADENOSINE A1 RECEPTOR AGAINST ISCHEMIA AND REPERFUSION INJURY OF THE LIVER

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Category: Transplantation/Immunology

Running title: KW3902 attenuates I/R injury of the liver

Key words: Liver, ischemia and reperfusion injury, adenosine, adenosine A_1 receptor antagonist, KW3902, dog.

Abstract

Background: Adenosine is known to exert protective roles in hepatic ischemia and reperfusion injury, while all adenosine receptors don't play the cytoprotective roles. We have tested our hypothesis that blockage of adenosine binding to A_1receptor by its antagonist, KW3902 [8-(noradamantan-3-yl)-1,3-dipropylxanthine] attenuates hepatic ischemia-reperfusion injury.

Methods: Adult female beagle dogs underwent a 2-hr total hepatic vascular exclusion (THVE) with a venovenous bypass. Non-treated animals that underwent THVE with a venovenous bypass alone were used as the control (Group CT, n=6). KW3902 was given to the animals by continuous intraportal infusion for 60 min. before ischemia at a dose of 1μg/kg/min (Group KW, n=6). Two-week survival, hemodynamics, hepatic tissue blood flow (HTBF), liver function, energy metabolism, cAMP concentration, and histopathological findings were studied.

Results: Two-week animal survival was significantly improved in group KW compared to that in group CT (group CT: 16.7% vs. group KW: 83.3%). HTBF, liver function, and hepatic adenine nucleotide concentration were remarkably better in group KW than group CT. In addition, cAMP concentration in group KW was maintained significantly higher than group CT. Histopathological examination revealed preservation of hepatic architecture and suppression of neutrophil infiltration into hepatic tissue in group KW.

Conclusion: Administration of adenosine A_1receptor antagonist before ischemia attenuates hepatic ischemia-reperfusion injury. To elicit the beneficial effect of adenosine against ischemia and reperfusion injury of the liver, it is important to oppose adenosine A1 receptor activation.

Introduction

Adenosine, an intermediary metabolite of the adenine triphosphate (ATP) degradation cascade, attenuates ischemia and reperfusion injury by ameliorating the microcirculatory disturbances, and preventing platelet aggregation (1,2,3). Reportedly, adenosine plays an important role in the protective effect of hepatic ischemic preconditioning (4,5,6).

Previously, we demonstrated that augmentation of endogenous adenosine improves postreperfusion hepatic blood flow and energy metabolism, attenuates liver enzyme release, augments cyclic AMP levels, and consequently improves animal survival in canine models (7, 8). In those reports, we showed the beneficial effect of endogenous adenosine against ischemia and reperfusion injury. There is, however, evidence that adenosine dose not always exert a cytoprotective effect (1, 2, 9-11).

The effects of adenosine are mediated by specific receptors that have been subdivided into groups A_1, A_2, and A_3 (12-14). It is known that adenosine mainly stimulates the A_1 and A_2 receptor subtypes. By activation of A_1 receptor which has a high affinity to adenosine, adenosine inhibits the release of neurotransmitter substances, produces negative inotropic effect in the heart (12), decrease of oxygen demand on the heart (13), and induces vasoconstriction in the lung (9). Also, by activation of A_1 receptors, adenosine promotes chemotaxis and adhesion of neutrophils (10). On the other hand, activation of A_2 receptors induces vasodilatation (15), inhibition of platelet aggregation (16), and migration of neutrophil (17), modulation of TNFα release (18), and suppression of P-selectin (19).

Taking these facts into consideration, activation of A_1 receptors oppose the beneficial effects of endogenous adenosine via activation of A_2 receptors in ischemia-reperfusion injury of the liver. To elicit the beneficial effect of adenosine against

hepatic ischemia and reperfusion injury, it may be necessary to oppose adenosine A_1 receptor activation. In this study, using a 2-hour total hepatic vascular exclusion model in dogs, we tested the effect of KW3902 [8-(noradamantan-3yl)-1,3-dipropylxanthine], a novel selective adenosine A_1 receptor antagonist derived from xanthine that has markedly strong binding affinity to adenosine A_1 receptor (20). Using the adenosine A_1 receptor antagonist, we were able to analyze the beneficial and adverse effects of adenosine and to investigate the mechanisms of its effect against ischemia and reperfusion injury of the liver.

Materials and Methods

The study was approved by the animal care and use committee, Hokkaido University School of Medicine, and carried out in accordance with the guidelines on the care and use of laboratory animals issued by Hokkaido University.

Animals:

Adult female beagle dogs, weighing 8 to 12 kg, were used. After overnight fasting, they were anesthetized with 25mg/kg thiopental sodium (Yoshitomi Pharmaceutical Co., Tokyo, Japan). The anesthesia was maintained by positive mechanical ventilation with a mixture of isoflurane (2%), nitrous oxide (2 L/min), and oxygen (2 L/min). The right carotid artery and the right jugular vein were cannulated for arterial and central venous pressure monitoring and for serial blood samplings, respectively. Electrocardiogram and esophageal temperatures were monitored throughout the operation. An electrolyte solution (lactated Ringer's solution) was continuously infused at a rate of 23 ml/kg/hr throughout the procedure to maintain hydration. Arterial blood gas and electrolytes were measured frequently and corrected if necessary with sodium bicarbonate, calcium dichloride, and/or potassium chloride.

Operative procedures:

Following midline laparotomy, the liver was completely skeletonized by dissection of the suspensory ligaments and the retrohepatic vena cava from the posterior abdominal wall. A 16-gauge venous catheter (Terumo Co., Tokyo, Japan) was inserted into the left hepatic vein through a puncture of the suprahepatic inferior vena cava (IVC), for collecting hepatic venous blood samples. An 18-gauge catheter was introduced into the portal vein to measure the pressure. Prior to total hepatic vascular exclusion, the splenic, left femoral and left jugular veins were cannulated with thoracic catheters (Nipro Co., Tokyo, Japan). Total hepatic

vascular exclusion was achieved by closs-clamping the portal vein and the hepatic artery with the hepatoduodenal ligament, and the supra- and infra-hepatic inferior vena cava. Pump-driven veno-venous bypass (Biomedics 520D, Minetonka, MN) was used to decompress the splanchnic venous bed and infra-hepatic inferior vena cava (8). The bypass circuit connected the left femoral vein and the splenic vein to the left jugular vein (Ishikawa Co., Tochigi, Japan). The animals were given heparin (50 U/kg) 5 min before the initiation of ischemia. After 2 hr of normothermic ischemia, the liver was reperfused, the bypass system was removed, and a splenectomy was performed. Cefazolin sodium (1 g) was given intraoperatively and continued for 3 postoperative days. Animals were allowed to eat and drink from the next morning. They were observed for 2 weeks after the operation.

***Experimental Groups*:**

Twelve beagle dogs were randomly divided into two groups; six were treated with KW3902 (group KW), and non-treated 6 animals were used as the control (group CT). KW3902 was dissolved in 20ml of normal saline at a dose of 60μg/kg, and it was given as a continuous infusion via portal vein for 60 minutes before ischemia in treated animals. KW3902 was a kind gift from the Kyowa Hakko Co., Ltd. (Shizuoka, Japan).

End Points

Survival: Two-week animal survival was evaluated.

Systemic hemodynamics Heart rate (HR) and mean arterial pressure (MAP) were serially measured during the operation and recorded as a percentage of the pre-ischemic value.

Hepatic tissue blood flow (HTBF): HTBF was measured with a laser Doppler flow meter (Omega flow, type FLO-N1; Omegawave Co., Tokyo, Japan) before ischemia, at 5, 60 and 120 minutes after initiation of ischemia, and at 5, 15, 30, and 60 minutes after

reperfusion. At each timepoint, a mean of three values obtained at three different lobes was calculated. HTBF was expressed as a percentage of the pre-ischemic value.

Liver function tests: The blood samples for liver function tests were serially collected before ischemia, at the end of 2-hr ischemia, and at 5, 15, 30, and 60 minutes and 3, 6, 12, and 24 hr after reperfusion. An autoanalyzer (Type 7020; Hitachi Co., Tokyo, Japan) was used to determine serum levels of alanine aminotransferase (ALT).

Tissue biochemistry: Wedge liver biopsy samples were collected before ischemia, after 2 hr of ischemia, and 15 and 60 min after reperfusion. Each was divided into 3 pieces. Two were immediately frozen in liquid nitrogen for biochemical studies and the other was placed in buffered formalin for histopathological analyses.

Measurements of adenine nucleotides: Tissue levels of adenine nucleotides **(**including adenosine triphosphate [ATP], adenosine diphosphate [ADP], and adenosine monophosphate [AMP]) were determined with single run high- performance liquid chromatography according to the modified method of Wynants (21), by using two HPLC system**s** (Model 501 pumps, Model 484 absorbance module, and Model 7700 WISP system; Shimazu, Kyoto, Japan) at 254 nm (Model 484, a tunable absorbance detector). Total adenine nucleotide (TAN) and energy charge (EC) were calculated using the following equations:

TAN = ATP + ADP + AMP

EC = (ATP + 1/2 ADP) / TAN (22).

Tissue cAMP concentration: Tissue samples for adenosine 3', 5'-cyclic monophosphate (cyclic AMP) levels were collected at the same points as for AN and PC. Tissue concentrations of cyclic AMP were measured using the direct assay method with the YAMASA RIA kit (YAMASA Shoyu, Chiba Japan). Radioactivity was measured using a γ-counter (ARC-950, Aroka, Japan).

Histopathology: Liver tissue samples were fixed in buffered formalin and stained with hematoxylin and eosin. The specimens were examined by a single pathologist without any knowledge of the groups or times of tissue sampling. Histologic assessment was performed semiquantitatively and graded according to the degree of sinusoidal congestion, sinusoidal derangement which represented the damage of structure of sinusoidal lining cells, and hepatocyte injury as follows: none = 0, mild = 1, moderate = 2, and severe = 3 (23, 24). The number of infiltrated neutrophils per 1,000 hepatocyte nuclei was counted.

Statistics:

Values are expressed as mean±SEM. Animal survival was determined using Fisher's exact probably test. Inter-group analysis was performed by multiple comparison test using Fisher's protected least significant difference tests. A two-sided *P* value less than 0.05 was considered statistically significant. The analyses were carried out with Stat View (version 4.5, Abacus Concepts, Inc. Berkeley, CA) on a Power Macintosh computer.

Results

Animal survival: During a 2-wk follow-up, 5 of 6 control dogs died of acute liver failure within 24 hr after surgery. In contrast, 5 of 6 treated dogs survived for two weeks. Two-week animal survival of group CT and KW was 16.7% and 83.3%, respectively ($p<0.05$, group KW vs. group CT).

Systemic hemodynamics: Heart rate (HR) and Mean arterial pressure (MAP) before ischemia and during vascular exclusion showed no remarkable changes in both group (Figure 1a, b). Immediately after reperfusion, group CT developed bradycadia and hypotension which decreased at the level of 88%, 43% of initial value. In contrast, administration of KW3902 kept HR and MAP as 104% and 73% of initial value, respectively, and maintained them higher than group CT after early reperfusion period.

Hepatic tissue blood flow (HTBF): The percent changes of HTBF in each group are illustrated in Figure 1c. No remarkable changes in HTBF were observed before and during administration of KW3902. Our surgical procedure produced complete liver ischemia, but there was false positive HTBF by Doppler flow measurement during ischemia, ranging from 8 to 11% of the pre-ischemic levels, reflecting physiological activity such as heart beat and respiration. Just after reperfusion, all untreated animals developed severe swelling and congestion of the liver. The HTBF of control animals was approximately 25% of the initial value throughout the observation period. In contrast, the livers of all treated animals demonstrated smooth and prompt revascularization. The HTBF of the group KW was statistically higher than that of control animals throughout the reperfusion period. In particular, at 3 hr after reperfusion, the HTBF of animals treated with KW3902 was still above 90% of the initial value.

Liver function tests: Serum ALT levels in the control group increased rapidly after

reperfusion, reaching 12,625±1,401 U/L at 6 hr after reperfusion (Fig. 2). Elevation of ALT levels in group KW was significantly inhibited by the treatment throughout 24 hr after reperfusion. The peak ALT value was 2,352±452 U/L in group KW.

Energy metabolism: Changes in adenine nucleotide and purine catabolite concentrations in liver tissue during ischemia and after reperfusion are shown in Table 1. Two hr of total hepatic vascular exclusion induced significant declines in ATP concentration and catabolism to purine catabolites. By the end of ischemia, ATP levels in the control group had fallen to almost 10% of baseline. In the treatment group, ATP degradation during hepatic ischemia was suppressed compared to control, and resynthesis of ATP after reperfusion was increased. Otherwise, concentration of adenosine in group KW did not increase after reperfusion compared with control group.

cAMP in liver tissue: The change of hepatic cAMP concentration was shown in Table 2. In group CT, warm ischemia of 120 minute caused a significant decrease in the hepatic cAMP concentration. After reperfusion, level of cAMP was lower and did not recover to normal level at 3-hr after reperfusion. On the other hand, inhibition of adenosine A_1 receptor by KW3902 inhibited decrease of cAMP concentration during ischemia and after reperfusion.

Histopathology: Figure 3 shows histological findings at 60 min after reperfusion. Two hours of ischemia did not appear to produce any important histologic alterations. After reperfusion, however, control livers developed significant structural abnormalities, such as massive hepatocyte necrosis, sinusoidal derangement, and congestion (Figure 3a). In contrast, these changes were remarkably attenuated in all treated animals (Figure 3b). The histopathological score in each category was better in group KW than in group CT (Table 4). The number of infiltrating neutrophils in control livers was 14.5±1.3 (per 1000 hepatocyte

nuclei) before ischemia and 63.6±23.6 at 60 min after reperfusion. In group KW, the number of PMNs infiltrating into the hepatic tissue were significantly lessened compared to controls, reaching to 41.17±11.01 at 60 min after reperfusion (Figure 4).

Discussion

This study showed that a 2-hr complete hepatic warm ischemia caused severe liver damage and high mortality in control animals. In contrast, administration of adenosine A_1receptor antagonist before ischemia improved animal survival, maintained systemic circulatory condition during reperfusion. In addition, it lessened microcirculatory disturbance, attenuated liver injury, improved energy metabolism in liver, and prevented neutrophil infiltration into the liver tissue.

In 1929, Drury and Szent-Gyorgy (25) first reported the physiologic effects of adenosine, describing it as a local hormone that regulates the function of organs, tissues, and cells. Since then, the catabolism of adenosine has been extensively studied. During ischemia, the degradation of ATP and adenosine diphosphate (ADP) is promoted and adenosine monophosphate (AMP) increases in ischemic tissues. Ecto- or endo-5'-nucleotidase dephosphorylates AMP to adenosine, and adenosine deaminase converts adenosine to inosine. Adenosine and inosine in the interstitium are rapidly transported into endothelial cells, red blood cells, or parenchymal cells via nucleoside transport proteins, and there, they are catabolized to hypoxanthine and xanthine with the formation of superoxide radicals during reperfusion. Upon reoxygenation, adenosine also becomes an important substrate for ATP resynthesis. Many investigators have reported on the protective role of adenosine against ischemia-reperfusion injury (2, 26). Rapid metabolism by adenosine deaminase and adenosine kinase (2,3,27) serves to reduce the cytoprotective benefits of adenosine. In earlier work, however, we have shown that use of R-75231 and dipyridamole, nucleoside transport inhibitors, augments endogenous adenosine and attenuates ischemia and reperfusion injury of the liver (7, 8).

On the other hand, it is known that adenosine is a purine nucleotide that modulates

tissue functions through receptor-mediated mechanism. Adenosine receptors have been divided into 3 major subclasses: A_1, A_2 and A_3 (13, 28). Dixon et al. (29) has reported that adenosine A 1 receptor mRNA was expressed in the brain, heart, aorta, liver, kidney, eye and bladder. A2 and A3 receptor mRNAs were expressed central nervous system and many peripheral tissues. In the heart, A1 and A3 receptor activation mediate the protective effect of ischemia preconditioning through inhibition of adrenergic stimulation and of noradrenalin release, and activation of ATP-sensitive potassium channels with reduction of calcium over load, thereby reducing myocardial oxygen demand (30, 31). Via A2 receptor activation, adenosine mediates vasodilatory effect and inhibits platelet aggregation and adherence to the endothelium, thus preventing microvascular obstruction and no-flow (32). In the kidney, A1 receptor activation mediates renal vasoconstriction of endogenous nitric oxide and angiotensine II (33). In the liver, several investigators have shown that A1 receptor was expressed in hepatocytes (34) and hepatic stellate cells which related to the mechanism of sinusoidal contraction (34). A2 receptor is expressed in hepatocytes (35) and sinusoidal endothelial cells (6). A3 is expressed in hepatocytes (34).

Adenosine concentrations as low as the nanomolar range are sufficient to activate the high-affinity A_1 receptor. Stimulation of Gi signal-transduction protein–coupled A_1 receptors induces the increase of cytosolic Ca^{2+} concentration via phospholipase C activation (36) and the decrease of cyclic AMP production (28), leading to vasoconstriction, bradycardia, decreased myocardial contractility, and renin release (1). The low-affinity A_2 receptor requires adenosine concentrations in the micromolar range, three orders of magnitude higher than that required for A_1 receptor activation. Activation of protein Gs–coupled A_2 receptors induces cyclic AMP production via adenylate cyclase stimulation, yielding enhancement of vasodilation (1) and inhibition of platelet aggregation (37),

neutrophil (38, 39) and Kupffer cell function (25, 26), and cytokine production (18, 19). In recent studies, adenosine has been shown to stimulate NO production via A_2 receptor activation (15, 40). Moreover, it is said that adenosine has a protective role in liver ischemia preconditioning, which is mediated by activation of adenosine A_2 receptors. (4, 5, 6)

In order to inhibit adenosine A_1 receptor, we used a selective A_1 receptor antagonist, KW3902. KW3902 has a highly affinity to A_1 then A_2 receptors. In rat brain binding assays, Ki value for A_1 and A_2 receptors are 0.19 nM and 170 nM, respectively. It is more 890-fold selective for A_1 than A_2 receptors (20). The dosage and route of KW3902 was based on previous report (20, 41) and our preliminary study. Preliminarily, we ourselves had previously demonstrated that KW3902 administered by peripheral intravenous infusion showed no beneficial effects due to insufficient liver concentration. Therefore, we tried to give KW3902 by portal infusion. To avoid sudden hemodynamic changes caused by a bolus injection, the drug was administered by continuous intravenous infusion for one hour. When administered 1μg/kg/min used in this study, drug concentration of KW3902 in hepatic vein was maintained above 10ng/mL (data was not shown). This was a sufficient dose, because hepatic cAMP concentration maintained high during ischemia and after reperfusion compared with group CT. In our study, bradycardia was observed just after reperfusion in group CT, while KW3902 prevented bradycardia. Actually, previous report described that KW3902 has an effect against R-PIA-induced bradycardia (42). It is an effect of KW3902 against negative inotropic effect of A_1 receptors.

In this study, the direct effect of KW3902 on the physiological response including adenine nucleotide metabolism in normal liver was not analyzed. Adenosine and adenosine agonist cause the heart rate depression via adenosine A1 receptor in normal animals, while KW3902 inhibits this action (43, 44). However, KW3902 dose not directly induce an

increase in heart rate in normal rat and dog (43). KW-3902 shows only the diuretic effect as peripheral actions in intact animal (43). From these findings, it is hypothesized that KW-3902 dose not show the physiological response via A1 receptor in intact liver, and KW3902 dose not influence on the energy balance in normal liver.

The beneficial effects of KW3902 in our study involved three mechanisms. First, KW3902 significantly increased adenine nucleotide levels in the ischemic tissues compared to the control. Warm ischemia causes the progressive degradation of adenine nucleotides. A decrease of ATP stores contributes to the accumulation of intracellular Ca^{2+} during ischemia (45). The increase in intracellular Ca^{2+} enhances calcium-dependent ATP-ase and induces further consumption of ATP stores (46). Therefore, augmentation of cyclic AMP, which produces an efflux of the Ca^{2+} accumulated during ischemia and ATP depletion, prevents the increase in intracellular Ca^{2+} and lessens the decrease in ATP, resulting in the stabilization of the hepatocellular membrane (47).

Second, inhibition of the A_1 receptor ameliorated the microcirculatory disturbance. Cyclic AMP produces an efflux of calcium in vascular smooth muscles through activation of the Ca^{2+}-activated K^+ channel (48). A decrease of intracellular calcium induces vasorelaxation. Accordingly, KW3902 contributed to the regulation of sinusoidal microcirculation, resulting in greater hepatic tissue blood flow and lessened biochemical liver damage. Moreover, we might encounter hypotension in treated animals, if we use adenosine A_2 receptor agonist instead of A_1 receptor antagonist (49). However, KW3902 did not cause hypotension in any treated animals during and after drug administration.

Third, treatment with KW3902 reduced the number of polymorphonuclear neutrophils (PMNs) infiltrated into liver tissues. PMNs play a crucial role in the progression of ischemia-reperfusion injury. Furthermore, A_1 receptors are up-regulated by oxidative

stress (50). Cronstein and associates showed that activation of A_1 receptors promotes chemotaxis of neutrophils (17). These findings suggested that A_1 receptors and PMNs further influence each other by facilitating PMN adhesion to endothelial cells and infiltration into hepatic tissues. Therefore, inhibition of A_1 receptors with KW3902 modulates chemotaxis and activation of PMNs (51). In recent studies, increases in tissue cyclic AMP concentration induced by forskolin, isoproterenol, and dibutyryl cyclic AMP caused relaxation of endothelial cells and inhibited the ability of neutrophils to damage the endothelial barrier (52, 53). Taking all of these findings together, it appears that the beneficial effect of KW3902 treatment on our experimental model results from inhibition of decreasing cyclic AMP in the liver tissue during ischemia by blocking of adenosine A_1 receptors.

In conclusion, blockage of adenosine A_1 receptor activation by KW3902 was effective against hepatic ischemia-reperfusion injury. Activation of A_1 receptors opposed the beneficial effects of endogenous adenosine via activation of A_2 receptors in ischemia-reperfusion injury of the liver. To elicit the beneficial effect of adenosine against hepatic ischemia and reperfusion injury, it is necessary to oppose adenosine A_1 receptor activation.

Figure Legends

Figure 1. a; changes of heart rate (HR);
b; change of mean arterial pressure (MAP);
c, changes of hepatic tissue blood flow (HTBF).
HR, MAP, and HTBF are expressed as a percentage of the preischemic initial value. Data are expressed as mean ± SEM. Group CT, untreated control (n=6); group KW, treated KW3902 (n=6). *$p<0.05$ versus group CT.

Figure 2. Changes of serum alanine aminotransferase (ALT) level. Data are expressed as mean ± SEM. Group CT, untreated control (n=6); group KW, treated KW3902 (n=6). *$p<0.05$ versus group CT.

Figure 3. Histopathological findings of control liver (a) and the treated liver with KW3902 (b) at 60min. after reperfusion (hematoxylin-eosin staining, x100).

Figure 4. The number of infiltrated neutrophils in liver tissue. Data are expressed as mean ±SEM. Group CT, negative control (n=6); group KW, treated KW3902 (n=6). *$p<0.05$ versus group CT.

Abbreviations

adenine nucleotides (AN)

adenosine (ADO)

adenosine 3', 5'-cyclic monophosphate (cyclic AMP)

adenosine diphosphate (ADP)

adenosine monophosphate (AMP)

adenosine triphosphate (ATP)

alanine aminotransferase (ALT)

endothelin-1 (ET-1)

energy charge (EC)

heart rate (HR)

Hepatic tissue blood flow (HTBF)

hypoxanthine (HX)

inferior vena cava (IVC)

inosine (INO)

ischemia and reperfusion (I/R)

mean arterial pressure (MAP)

polymorphonuclear neutrophils (PMNs)

purine catabolites (PC)

total adenine nucleotide (TAN)

uric acid (UA)

xanthine (X)

REFERENCES

1. Bouma MG, Van den Wildenberg FAJM, Buurman WA. The anti-inflammatory potential of adenosine ischemia-reperfusion injury: established and putative beneficial actions of a retaliatory metabolism. Shock 1997; 8: 313-320.
2. Forman MB, Velasco CE, Jackson EK. Adenosine attenuates reperfusion injury following regional myocardial ischemia. Cardiovasc Res 1993; 27: 9-17.
3. Kitakaze M, Hori M, Kameda T. Role of adenosine and its interaction with α adrenoceptor activity in ischaemic and reperfusion injury of the myocardium. Cardiovascular Research 1993; 27: 18-27
4. Peralta C, Hotter G, Closa D, Prats N, Xaus C, Gelpi E, et al. The protective role of adenosine in inducing nitric oxide synthesis in rat liver ischemia preconditioning is mediated by activation of adenosine A_2 receptors. Hepatology 1999; 29: 126-132.
5. Nakayama H, Yamamoto Y, Kume M, Yamagami K, Yamamoto H, Kimoto S, et al. Pharmacologic stimulation of adenosine A2 receptor supplants ischemic preconditioning in providing ischemic tolerance in rat livers. Surgery 1999; 126(5): 945-54
6. Arai M, Thurman RG, Lemasters JJ. Contribution of adenosine A (2) receptors and cyclic adenosine monophosphate to protective ischemic preconditioning of sinusoidal endothelial cells against Storage/Reperfusion injury in rat livers. Hepatology 2000; 32(2): 297-302
7. Taniguchi M, Magata S, Suzuki T, Shimamura T, Jin MB, Iida J, et al. S. Dipyridamole protects the liver against warm ischemia and reperfusion injury. J Am Coll Surg. 2004; 198(5):758-69.
8. Todo S, Zhu Y, Zhang S, Jin MB, Ishizaki N, Tanaka H, et al. Attenuation of ischemic liver injury by augmentation of endogenous adenosine. Transplantation 1997; 63:

217-223.

9. Neely CF, Keith IM. A_1 adenosine receptor antagonists block ischemia-reperfusion injury of the lung. Am J Physiol 1995; 268 (Lung Cell Mol Physiol 12): L1036-L1046.
10. Zahler S, Becker BF, Raschke P, E Gierlach. Stimulation of endothelial adenosine A_1 receptors enhances adhesion of neutrophils in the intact guinea pig coronary system. Cardiovascular Research 1994; 28: 1366-1372.
11. Zhang S, Jin MB, Zhu Y, Ishizaki N, Tanaka H, Subbotin VM, et al. Effect of endogenous adenosine augmentation on ischemia and reperfusion injury of the liver. Transplantation Proc 1997; 29: 1336-1337.
12. Ely SW, and Berne RM. Protective effects of adenosine in myocardial ischemia. Circulation 1992; 85: 893-904.
13. Olsson RA, Pearson JD. Cardiovascular purinoreceptors. Physiol Rev 1990; 70: 761-845.
14. Fredholm BB, Abbracchio MP, Burnstock G, Daly JW, Harden TK, Jacobson KA, et al. Nomenclature and classification of purinoceptors. Pharmacol Rev. 1994 ; 46(2):143-56.
15. Peralta C, Hotter G, Closa D, Gelpi E, Bulbena O, Rosello-Catafau J. Protective effect of preconditioning on the injury associated to hepatic ischemia-reperfusion in the rat: role of nitric oxide and adenosine. Hepatology 1997; 25: 934-937.
16. Cristalli G, Vittori S, Thompson RD, Padgett WL, Shi D, Daly JW, et al. Inhibition of platelet aggregation by adenosine receptor agonists. Arch Pharmacol. 1994; 349: 644-650.
17. Cronstein BN, Levin RI, Philips M, Hirschhorn R, Abramson SB, Weissmann G. The adenosine/neutrophil paradox resolved: human neutrophils possess both A_1 and A_2 receptor that promote chemotaxis and inhibit O_2^-generation, respectively. J Clin Invest 1990; 85: 1150-1157.

18. Prabhakar U, Brooks DP, Lipshilitz D, Esser KM. Inhibition of LPS-induced TNFα production in human monocytes by adenosine (A_2) receptor selective agonists. Int J Immunopharmacol 1995; 17: 221-224.
19. Minamino T, Kitakaze M, Asanuma H, Tomiyama Y, Shiraga M, Sato H et al. Endogenous adenosine inhibits P-selectin-dependent-formation of coronary thromboemboli during hypoperfusion in dogs. 1998; 101:1643-1653.
20. Nonaka H, Ichimura M, Takeda M, Kanda T, Shimada J, Suzuki F, et al. KW-3902, a selective high affinity antagonist for adenosine A_1 receptors. British J of Pharm 1996; 117: 1645-1652.
21. Wynants J, van Belle H. Single-run high-performance liquid chromatography of nucleotides, nucleosides, and major purine bases and its application to different tissue extracts. Anal Biochem. 1985; 144: 258-266.
22. Atkinson DE. The energy charge of the adenylate pool as a regulatory parameter, interaction with feed back modifiers. Biochemistry 1968; 7: 4030-4034.
23. Suzuki S, Pereyra T, Dodriguez FJ, Cejalvo D. Neutrophil infiltration as an important factor in liver ischemia and reperfusion injury. Transplantation 1993; 55: 1265-1272.
24. Totsuka E, Todo S, Zhu Y, Ishizaki N, Kawashima Y, Jin MB, et al. Attenuation of ischemic liver injury by prostaglandin E_1 analogue, Misoprostol, and prostaglandin I_2 analogue, OP-41483. J Am Coll Surg 1998; 187: 276-286.
25. Drury AN, Szent-Gyorgy A. The physiological activity of adenine compounds with special reference to their actions upon the mammalian heart. J Physiol 1929; 68: 213-237.
26. Ely SW, Berne RM. Protective effects of adenosine in myocardial ischemia. Circulation 1992; 85: 893-904.

27. Van Belle H, Goossens F, Wynants J. Formation and release of purine catabolites during hypoperfusion, anoxia, and ischemia. Am J Physiol 1987; 252(5 Pt 2): H886-93

28. Abbracchio MP, Cattaberi F, Fredholm BB, et al. Purinorecetor nomenclature: a status report. Drug Dev Res. 1993; 28: 207-213.

29. Dixon AK, Gubitz AK, Sirinathsinghji DJ, et al. Tissue distribution of adenosine receptor mRNAs in the rat. Br J Pharmacol. 1996 Jul; 118(6):1461-8.

30. Liu GS, Thornton J, Van Winkle D, et al. Protection against infarction afforded by preconditioning is mediated by A1 adenosine receptors in rabbit heart. Circulation 1991 84:350-356.

31. Downey JM. Ischemic preconditioning: Nature's own cardioprotective intervention. Trend Cardiovasc Med. 1992 2:170-176.

32. Collis MG, Hourani SMO. Adenosine receptor subtypes. Trends Pharmacol Sci. 1993 14:360-366.

33. Barrett RJ, Droppleman DA. Interaction of adenosine A1 receptor mediated renal vasoconstriction of endogenous nitric oxide and ANGⅡ. Am J Physiol 1993; 265:F651-659.

34. Guinzberg R, Diaz-Cruz A, Uribe S, et al. Ca2+ dependence of the response of three adenosine type receptors in rat hepatocytes. Eur J Pharmacol. 1997 Dec 11; 340(2-3):243-7.

35. Takemura S, Kawada N, Hirohashi K, et al. Nucleotide receptors in hepatic stellate cells of the rat. FEBS Lett.1994; 354:53-6.

36. Hayslett JP, Macala LJ, Smallwood JI, Kalghatgi L, Gasalla-Herraiz J, Isales C. Adenosine stimulation of Na^+ transport is mediated by an A_1 receptor and a $[Ca^{2+}]_i$ -dependent mechanism. Kidney International 1995; 47: 1576-1584.

37. Cristalli G, Vittori S, Thompson RD, Padgett WL, Shi D, Daly JW, et al. Inhibition of platelet aggregation by adenosine receptor agonists. Arch Pharmacol. 1994; 349: 644-650.

38. Wollner A, Wollner S, Smith JB. Acting via A2 receptors, adenosine inhibits the up regulation of Mac-1 (CD11b/CD18) expression of FMLP-stimulated neutrophils. Am J Respir Cell Mol Biol 1993; 9: 179

39. Harada N, Okajima K, Murakami K, Usune S, Sato C, Ohshima et al. Adenosine and selective A (2A) receptor agonists reduce ischemia/reperfusion injury of rat liver mainly by inhibiting leukocyte activation. J Pharmacol Exp Ther 2000; 294(3): 1034-42

40. Li J, Fenton RA, Wheeler HB, Powell CC, Peyton BD, Cutler BS, et al. Adenosine A_{2a} receptors increase atrial endothelial cell nitric oxide. 1998; 80: 357-364.

41. Aki Y, Tomohiro A, Nishiyama A, et al. Effective of KW3902, a selective and potent adenosine A_1 receptor antagonist, on renal hemodynamics and urine formation in anesthetized dogs. Pharmacology 1997; 55: 193-201.

42. Zhao Z-Q, Nakanishi K, McGee DS, et al. A_1 receptor mediated myocardial infarct size reduction by endogenous adenosine is exerted primarily during ischemia. Cardiovasc Res. 1994; 28: 270-279.

43. Mizumoto, H. Karasawa, A. Kubo, K. Diuretic and renal protective effects of 8-(noradamantan-3-yl)-1,3-dipropylxanthine (KW-3902), a novel adenosine A1-receptor antagonist, via pertussis toxin insensitive mechanism. J. Pharmacol Exp Ther. 1993 Jul;266(1):200-6.

44. Fredholm, BB. Jacobson, KA. Jonzon, B. Kirk, KL. Li, YO. Daly, JW. Evidence that a novel 8-phenyl-substituted xanthine derivative is a cardioselective adenosine receptor antagonist in vivo. J CardiovascPharmacol. 1987 Apr;9（4）:396-400.

45. Schanne FAX, Kane AB, Young EE, Farber JL. Calcium dependence of toxic cell death. A final common pathway. Science 1979; 206

46. Nakano H, Monden M, Umeshita K, Murata M, Miyoshi H, Kanai T, et al. Cytoprotective effect of prostaglandin I_2 analogues on superoxide-induced hepatocyte injury. Surgery 1994; 116:883.

47. Urakami A, Todo S, Zhu Y, Zhang S, Jin MB, Ishizaki N, et al. Attenuation of ischemia liver injury by monoclonal anti-endothelin antibody, AwETN40. J Am Coll Surg 1997; 185: 358-364.

48. Minami K, Fukuzawa K, Nakaya Y, Zeng XR, Inoue I. Mechanism of activation of the Ca^{2+}-activated K^+ channel by cyclic AMP in cultured porcine coronary artery smooth muscle cells. Life Sci 1993; 53: 1129-1135.

49. Nieri P, Martinotti E, Calderone V, Breschi MC. Adenosine-mediated hypotension in vivo guinea-pig: receptors involved and role of NO. Br J Pharmacol. 2001; 134(4):745-52.

50. Nie Z, Mei Y, Ford M, Rybak L, Marcuzzi A, Ren H, et al. Oxidative stress increases A_1 adenosine receptor expression by acting nuclear factor κB. Mol Pharmacol 1998; 53: 663-669.

51. Cronstein BN, Rosenstein ED, Kramer SB, Weissmann G, Hirschhorn R. Adenosine: a physiologic modulator of superoxide anion generation by human neutrophils. Adenosine acts via an A_1 receptor on human neutrophils. J. Immunol. 1985; 135: 1366-1371.

52. Adkins WK, Barnard JW, May S, Seibert AF, Haynes J, Taylor AE. Compounds that increase cyclic AMP prevent ischemia-reperfusion pulmonary capillary injury. J Appl Physiol. 1992; 72: 492-497.

53. Tomita I, Sawa M, Munakata T, Tanaka K, Kasai S. The beneficial effect of dibutyryl

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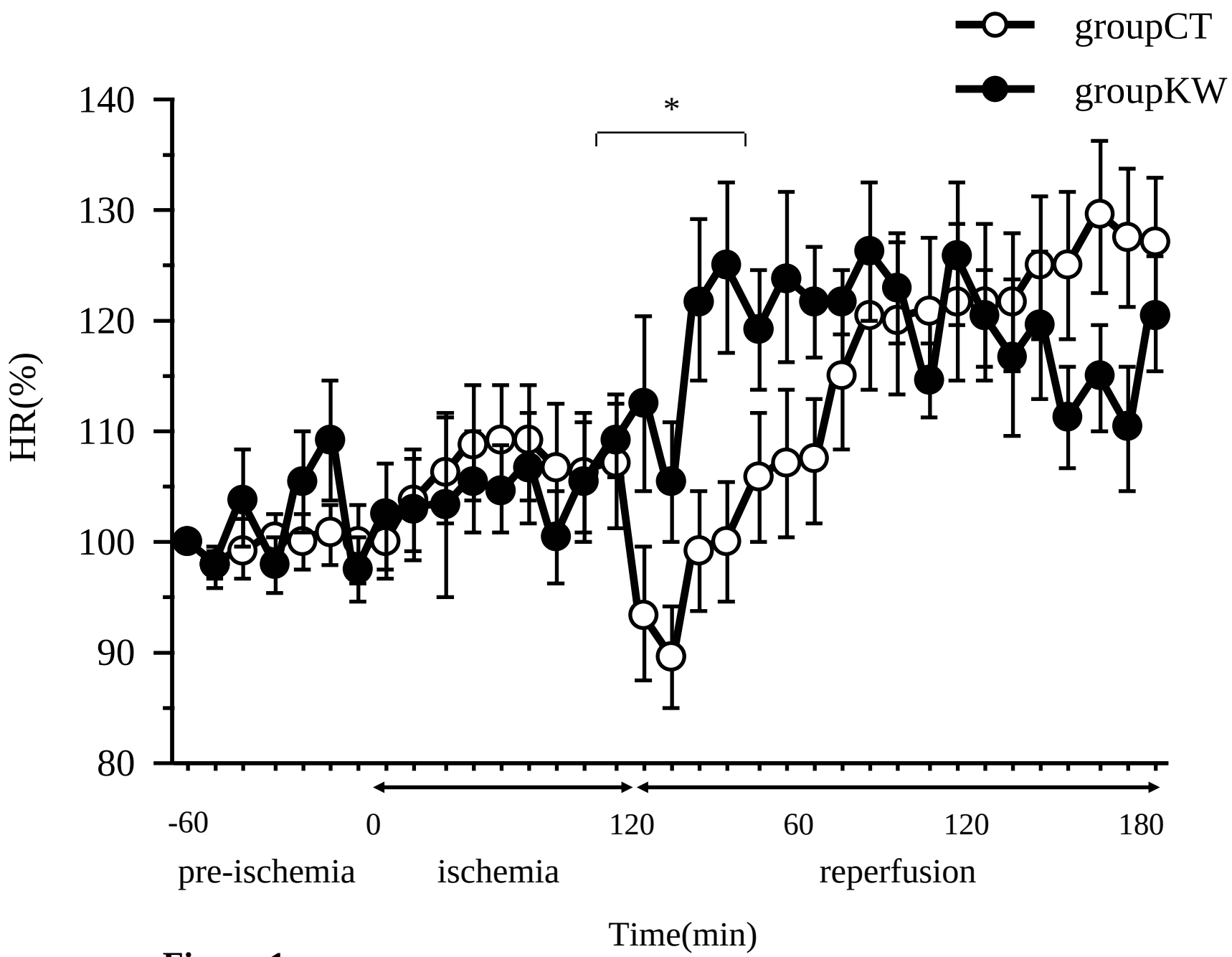
groupCT
groupKW
*
140
130
120
110
100
90
80
HR(%)
-60
0
120
60
120
180
pre-ischemia
ischemia
reperfusion
Time(min)

Figure 1a

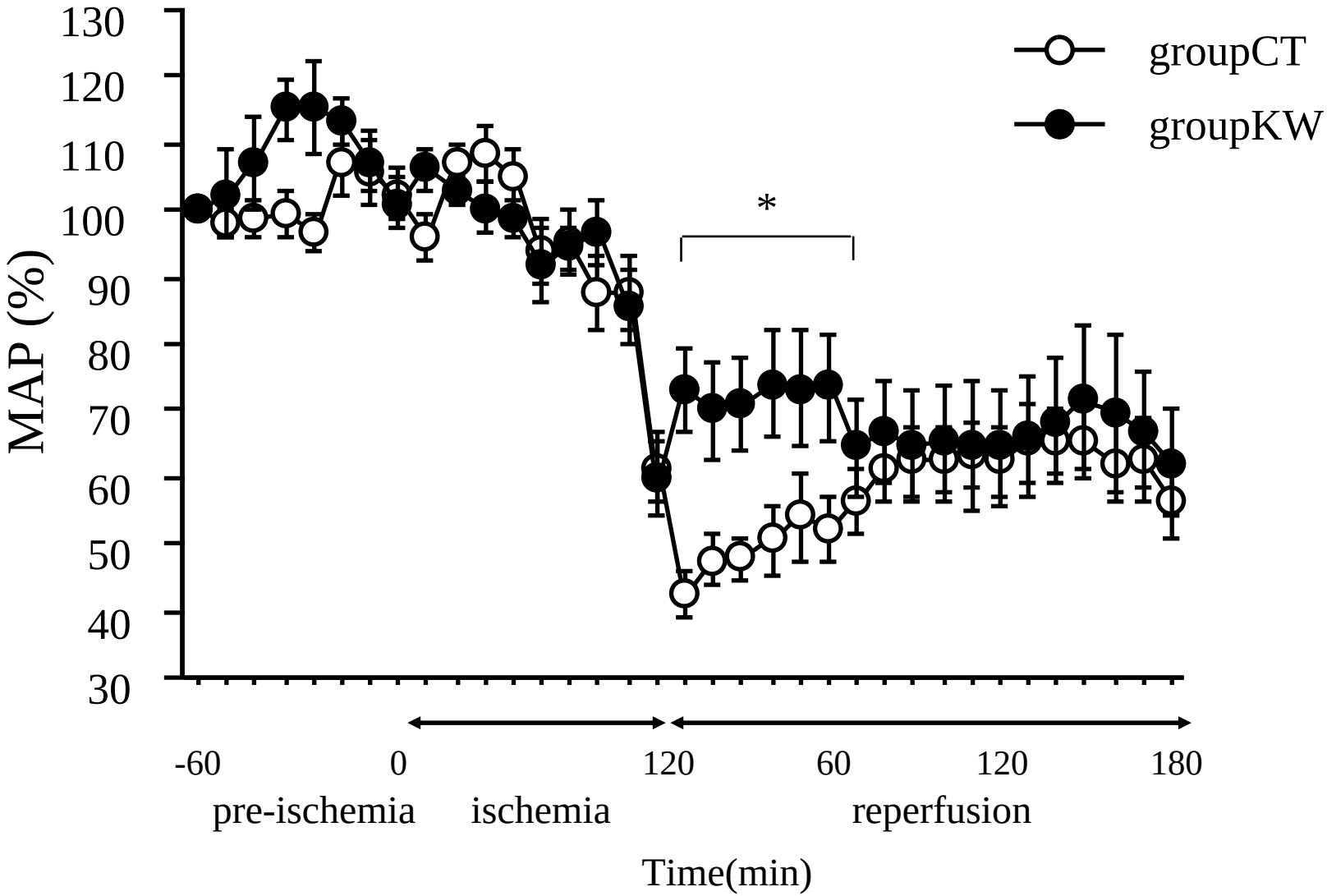
130
120
110
100
90
80
70
60
50
40
30
MAP (%)
groupCT
groupKW
*
-60
0
120
60
120
180
pre-ischemia
ischemia
reperfusion
Time(min)

Figure 1 b

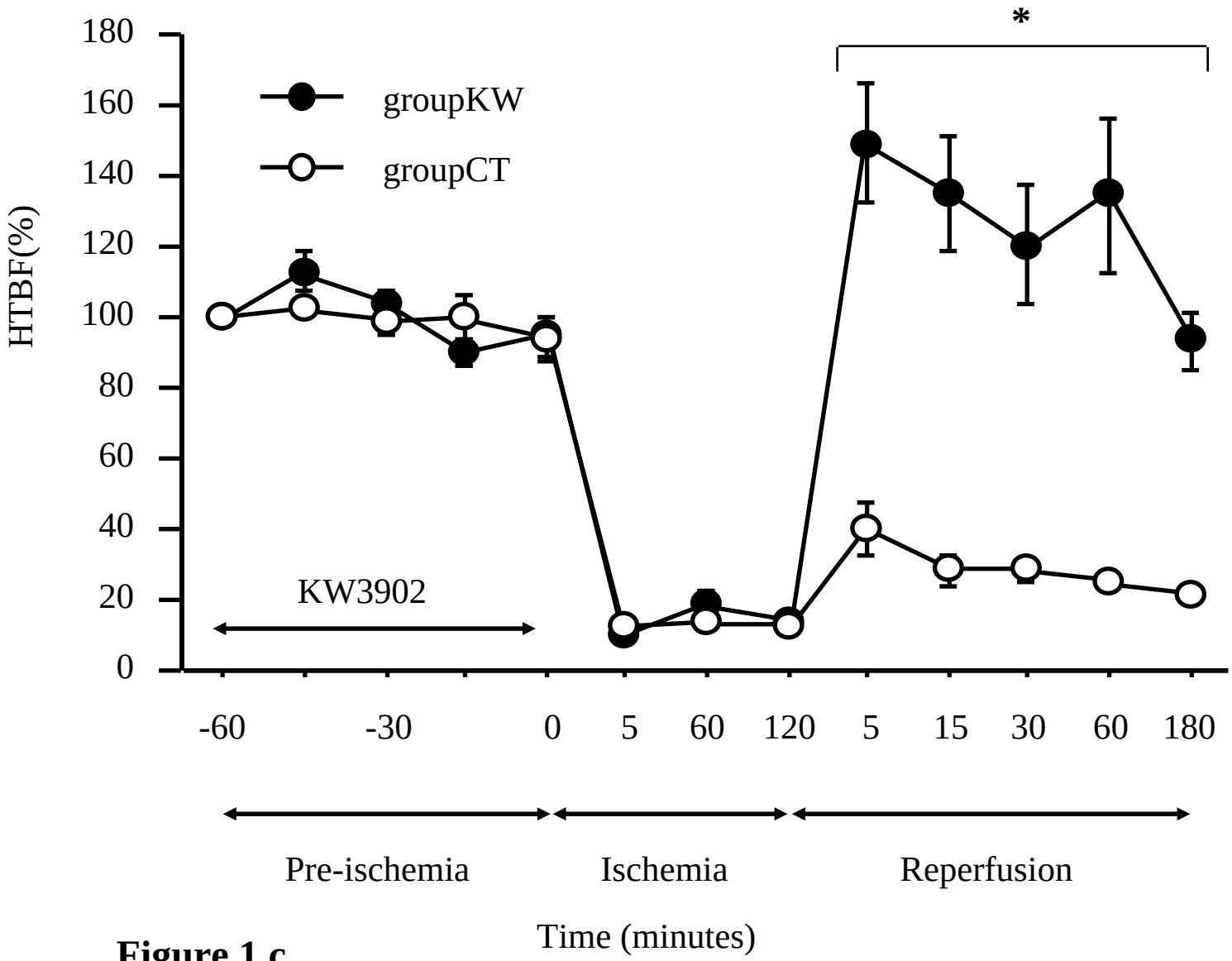
*
groupKW
groupCT
HTBF(%)
180
160
140
120
100
80
60
40
20
0
KW3902
-60
-30
0
5
60
120
5
15
30
60
180
Pre-ischemia
Ischemia
Reperfusion
Time (minutes)

Figure 1 c

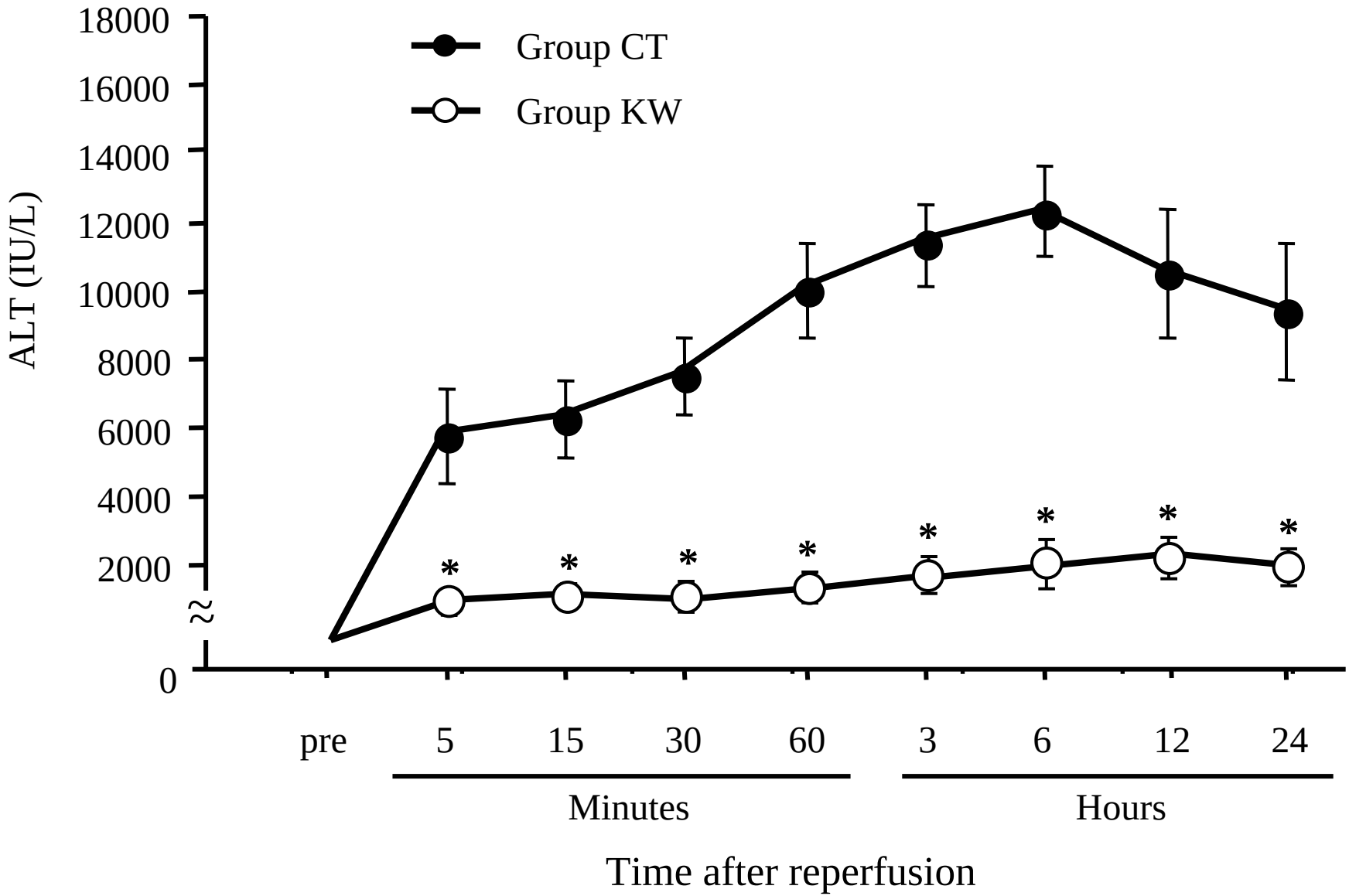
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
ALT (IU/L)
Group CT
Group KW
*
pre
5
15
30
60
3
6
12
24
Minutes
Hours
Time after reperfusion

Figure 2

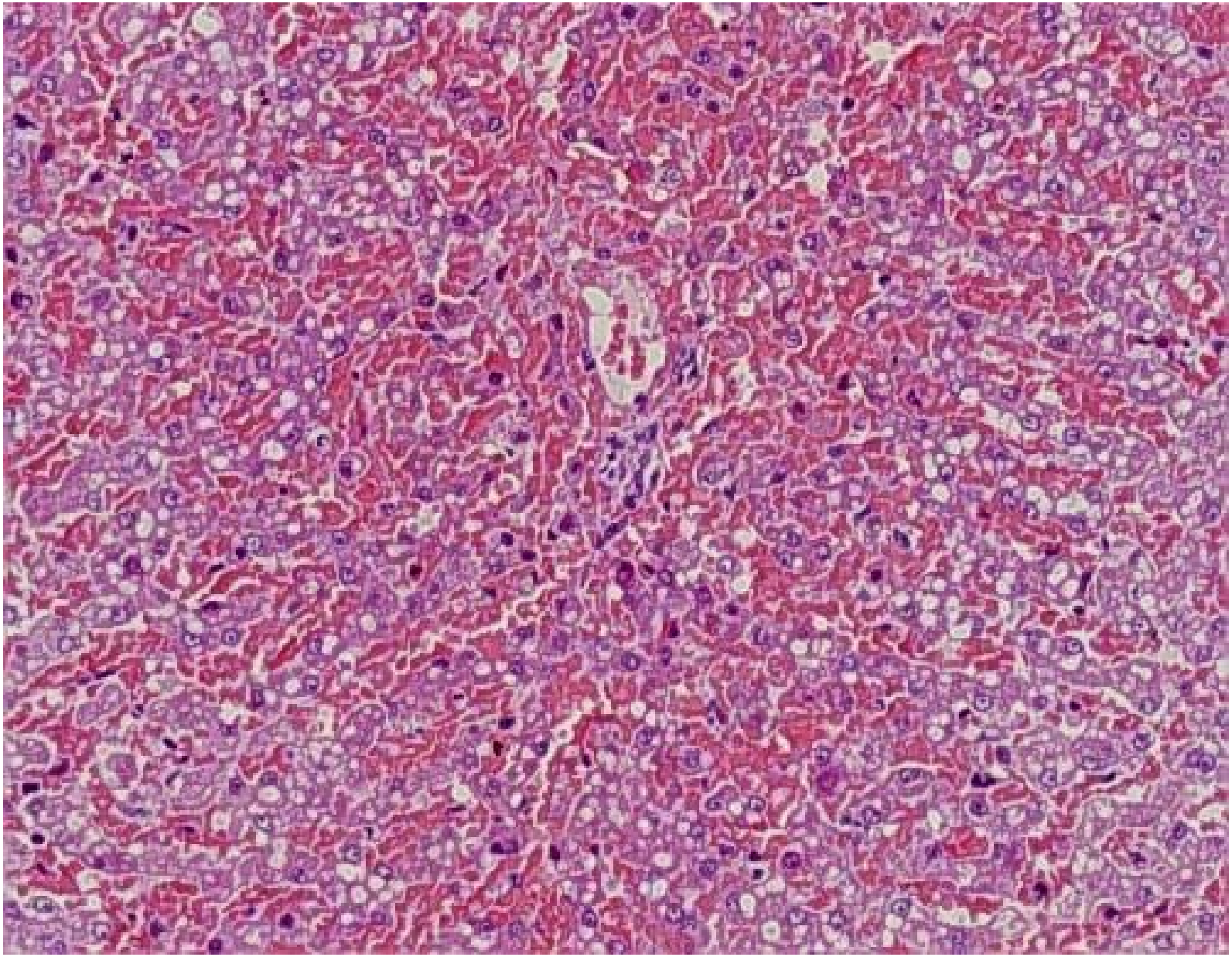

Figure 3a

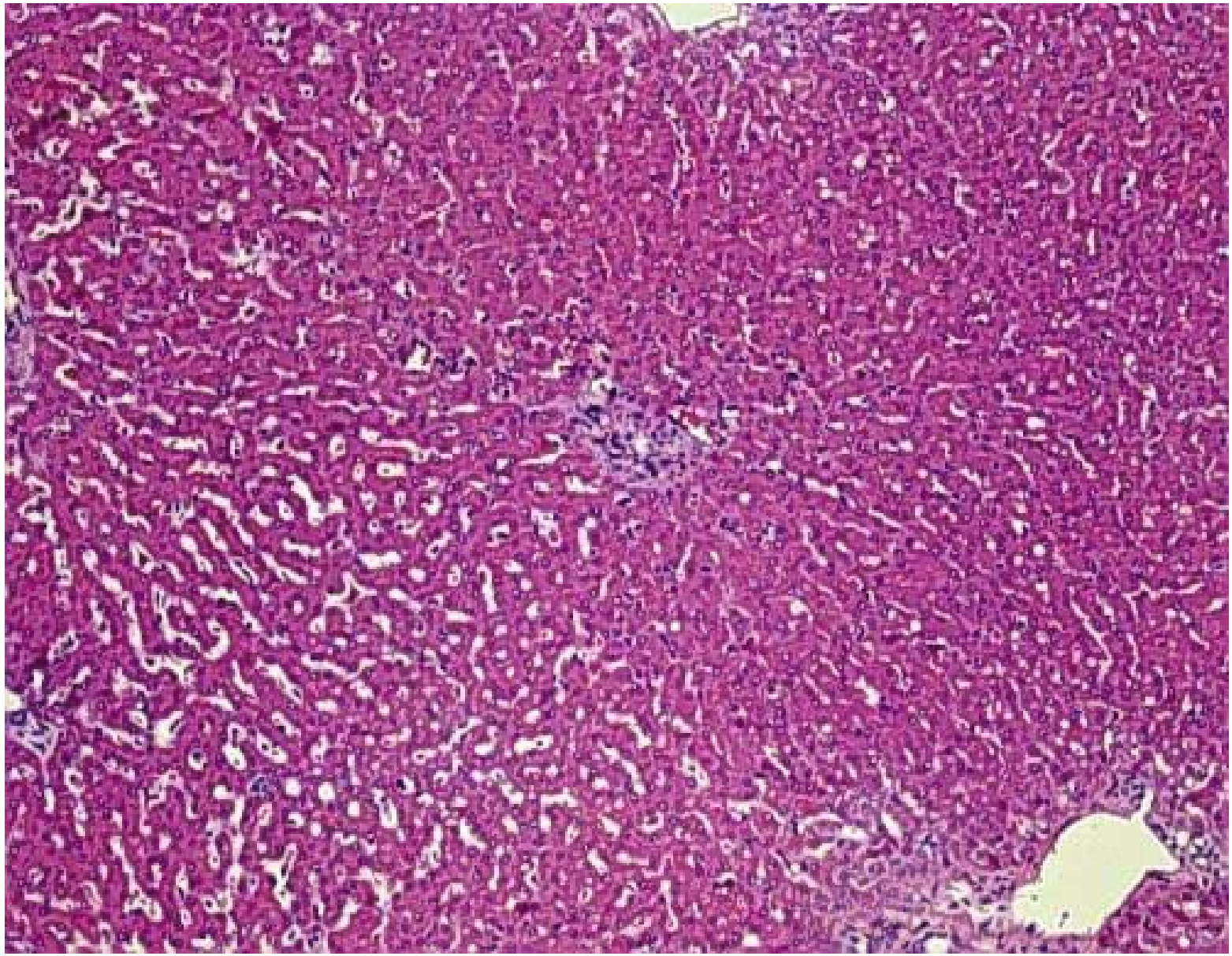

Figure 3b

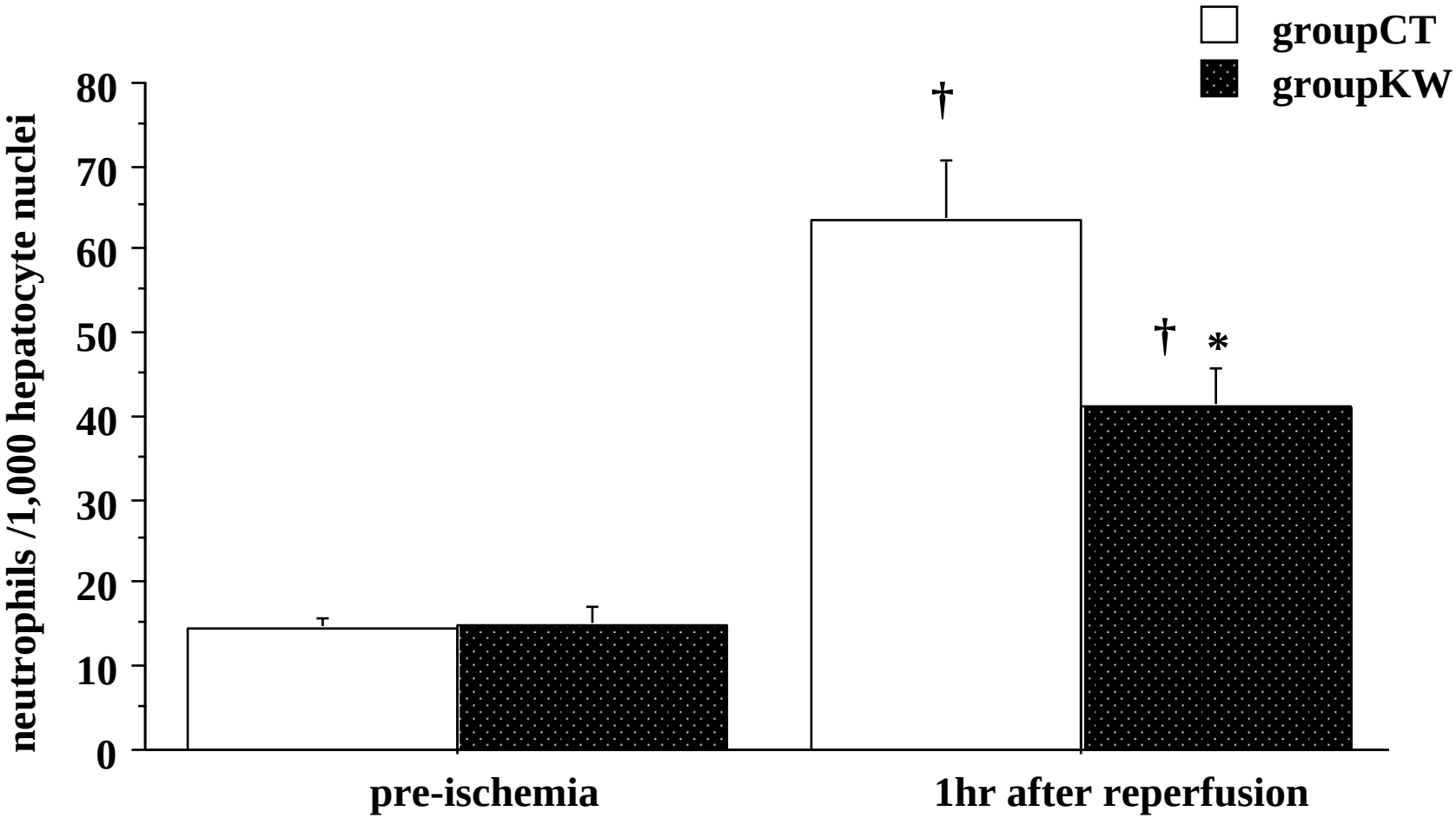
groupCT
groupKW
neutrophils /1,000 hepatocyte nuclei
80
70
60
50
40
30
20
10
0
†
†
*
pre-ischemia
1hr after reperfusion

Figure 4