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The liver protective effect of ischemic preconditioning may be mediated by adenosine

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Abstract We investigated the involvement of adenosine in ischemic preconditioning (IPC) by the unspecific antagonist, 8-phenyltheophylline (8-PT). Anesthetized Wistar rats were treated as follows: 1. non-ischemic controls, 2. ischemic controls: 60 min of clamping of the common hepatic artery followed by 60 min reperfusion, 3. IPC: 10 min ischemia followed by 15 min reperfusion, prior to the identical ischemia-reperfusion (IR) period as in group 2, 4. 8-PT + IPC: 8-PT 10 mg/kg i.v. was given 10 min prior to the identical procedure as in group 3. The peripheral liver blood flow was monitored by laser-Doppler flowmetry. Blood alanine aminotransferase (ALT) was analyzed once every 60 min. IPC significantly reduced impairment of liver blood flow, as well as ALT increase during reperfusion. This effect was abolished by pretreatment with 8-PT. Adenosine appears to be a crucial effector in IPC. Clinical studies need to be undertaken to explore a possible effect of IPC in liver transplantation.

Key words Adenosine · Adenosine receptors · Ischemic preconditioning · 8-phenyltheophylline

Introduction

Ischemia-reperfusion (IR) injury is a common and serious complication following organ transplantation [1, 2]. A tissue exposed to a brief period of vascular occlusion prior to a subsequent longer episode of ischemia will suffer less harmful effects of IR than tissues not subjected to such ischemic preconditioning (IPC). This has been demonstrated in a number of organs, including liver [3]. The mechanism for IPC may be the release of adenosine from the tissue, which in turn causes the induction of nitric oxide (NO) synthesis [4]. In a previous study, utilizing a model of IPC of the liver with a long-lasting total vascular occlusion of the perfusion of the right lobe, Peralta and coworkers have demonstrated that the protective effect of IPC on liver cell damage (as monitored by liver transaminases and histology findings) is abolished by a selective antagonist of adenosine A_2 receptors [5]. The present study was undertaken in a different model of IR of rat liver, with occlusion of the common hepatic artery (CHA) for 60 min only, leaving the portal venous circulation intact. Also, with this fairly mild procedure a beneficial effect of IPC on hepatic peripheral blood flow and liver cell damage has previously been demonstrated by us [7]. We have also demonstrated an involvement of adenosine and NO in IPC [6, 7]. A role for adenosine was further analyzed in this study. The aim was to investigate whether the methylxanthine compound, 8-phenyltheophylline (8-PT), an adenosine receptor blocker, would affect the protective action of IPC. At the dose used (10 mg/kg i.v.), this compound, in all likelihood, does not discriminate between subtypes of adenosine receptors [cf. 8].

Materials and methods

Animals

The study design was approved by the animal ethics committee of Göteborg University, and the *Principles of laboratory animal care* (NIH publication No. 86–23, revised 1985) were followed. The experiments were conducted with Wistar rats of either sex weighing 215–255 g (B&K Universal, Sollentuna, Sweden). The day-night cycle was constant at 12 h of light and dark, and the animals had free access to tap water and pelletted chow. The animals were deprived of food for 12 h before surgery, but were allowed water ad lib.

Surgical procedures

The rats were anesthetized with pentobarbitone (30 mg/kg i.p., supplemented when needed by repeated administrations of 10 mg/kg i.v. during the course of the experiment). The right carotid artery and jugular vein were cannulated for blood pressure recording and drug administration, respectively. The liver was exposed by a midline incision, and the CHA was dissected and arranged for subsequent temporary clamping by a thin rubber band. A laser-Doppler miniprobe (407; fiber separation = 0.25 mm) with an adhesive miniholder connected to a Periflux 4001 Master flowmeter (Perimed AB, Järfälla, Sweden) was gently placed on the liver surface for monitoring peripheral liver blood flow. The incision was closed with clips. Body temperature was maintained at 37 °C by radiant heat.

Evaluation of peripheral liver blood flow and liver cell injury

In all groups, the peripheral liver blood flow was monitored by laser-Doppler flowmetry during the study period (3 × 60 min). This method has been used for estimations of relative changes in several organs including liver [9]. The signal was continuously recorded and analyzed by a computer program (Perisoft, Perimed AB, Sweden). Minimum and maximum blood flow was recorded per time unit. Venous blood samples were collected on three occasions in each animal (see below), and serum alanine aminotransferase (ALT) was analyzed as an index of liver cell injury.

Experimental protocol

Upon the termination of surgery the animals were assigned to one of four groups. Group 1 were non-ischemic controls ($n = 10$) in which the CHA was dissected, but not subsequently clamped. These animals were monitored for a total of 180 min, and peripheral liver blood flow was estimated during the last 30 min of each 60-min period (these last 30 min denoted basal, sham-clamp and post-sham-clamp). Blood samples for ALT determination were drawn at the end of each 60-min period.

Group 2 were ischemic controls ($n = 10$). After a 60-min equilibration period, the CHA was clamped for 60 min, and, thereafter, the liver was reperfused for 60 min. Peripheral liver blood flow was estimated during the last 30 min of each 60-min period (these last 30 min denoted basal, clamp and post-clamp). Blood samples for ALT determination were drawn at the end of each 60-min period.

Group 3 was called ischemic preconditioning ($n = 10$). After 35 min of equilibration, the liver was subjected to 10 min clamping of the CHA, followed by 15 min reperfusion. Thereafter, the artery was clamped for 60 min, and the liver was then finally reperfused for 60 min. Peripheral liver blood flow was estimated during the 30 min immediately prior to IPC (denoted basal) and then during the last 30 min of the ischemic and reperfusion periods (these last 30 min denoted clamp and post-clamp, respectively). Blood samples for ALT determination were drawn at the end of each 60-min period.

Group 4 was called 8-PT + IPC ($n = 10$). After 35 min of equilibration, 8-PT 10 mg/kg i.v. was administered and IPC (identical to group 3) was performed. After another 25 min, the CHA was clamped for 60 min, and the liver was then finally reperfused for 60 min. Peripheral liver blood flow was estimated during the 30 min immediately prior to 8-PT administration and IPC (denoted basal) and then during the last 30 min of the ischemic and reperfusion periods (these last 30 min denoted clamp and post-clamp, respectively). Blood samples for ALT determination were drawn at the end of each 60-min period.

Bioactive substances and solutions

We purchased (8-PT) from Sigma (USA) and pentobarbitone (pentobarbital-sodium), from Apoteksbolaget (Umeå, Sweden). A commercially available kit from Boehringer Mannheim (Munich, Germany) was used for the determination of serum ALT.

Statistical analyses and presentation of data

Peripheral liver blood flow and ALT were expressed as the percentage change from the basal, and pre-clamp value, respectively, in each animal, or as arbitrary units (μkat/l) when relevant. All data are presented as means ± SE. Statistical evaluations were performed by analyses of variance (ANOVA), followed by Scheffe's F test for multiple comparisons, using a computer program (Statview 5, Abacus Concepts, Berkeley, Calif.). A P value of less than 0.05 was considered statistically significant.

Results

Peripheral liver blood flow

In the non-ischemic controls (group 1), peripheral liver blood flow did not change significantly during the course of the experiment (Fig. 1). In groups 2, 3 and 4 clamping of the CHA resulted in a significant decrease in peripheral liver blood flow compared with the basal period and also compared with sham-clamp. During the post-clamp period, blood flow returned to the basal value in the IPC group 3, but not in groups 2 and 4 (Fig. 1). In keeping with our previous report [6], in the IPC group, clamping of the CHA resulted in a decrease in peripheral liver blood flow to the same extent as in group 2 (Fig. 1).

Ischemia-reperfusion injury

The pre-clamp values of ALT (μkat/l) were 0.51 ± 0.03 (group 1), 0.48 ± 0.03 (group 2), 0.65 ± 0.04 (group 3) and 0.52 ± 0.03 (group 4). These did not differ signifi-

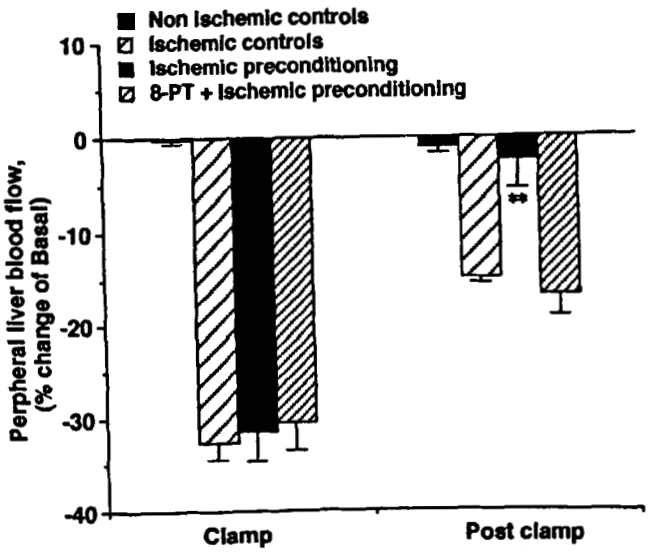

Fig.1 Peripheral liver blood flow, expressed as the percetage change in the blood flow during basal. During post-clamp, group 3 (IPC) differed significantly from groups 2 (ischemic control) and 4 (8 PT + IPC); $**P < 0.01$. Data are presented as means ± standard error (SE). For statistics, see Methods

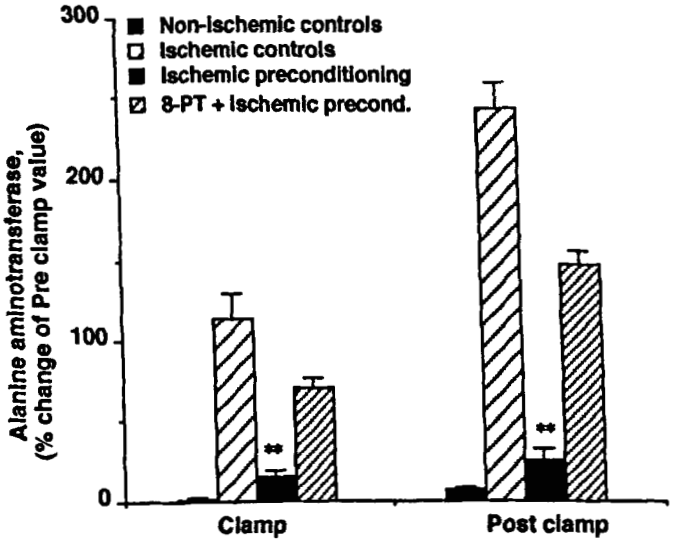

Fig.2 Blood concentration of alanine aminotransferase (ALT), expressed as the percentage in the pre-clamp value. IPC (group 3) prevented a rise in ALT, as seen in groups 2 and 4 after unclamping and after reperfusion, respectively. $**P < 0.01$

cantly. Thus, neither the preconditioning intervention as undertaken in group 3 nor the combination of 8-PT and IPC (group 4) elicited liver cell damage *per se*. There was no significant alteration in ALT during the course of the experiment in the non-ischemic controls. In the ischemic controls (group 2), clamping caused a significant increase in ALT both immediately after clamping and after the reperfusion period when compared with its own basal ($P < 0.01$ for both) and also with corresponding values in group 1 ($P < 0.01$ clamp vs. sham-clamp; post-clamp vs. post-sham clamp; Fig.2). The injury monitored as a rise in ALT was, however, completely prevented by IPC, while 8-PT abolished such a beneficial effect of IPC. It should be pointed out that the beneficial effect of IPC was during the clamping period, during which peripheral liver blood flow was seemingly unchanged by IPC. This procedure, therefore, appeared to elicit a *cytoprotective response* of the hepatocytes in one way or another.

Discussion

Our method of warm IR of rat liver has been presented briefly in a previous report [6]. In our view, this model offers a great advantage to the model utilized in most other laboratories in this species (usually implying interruption of both arterial and portal blood flow), as the insult to the liver appears to be far less advanced and thereby less definite. Since the portal circulation was spared, secondary effects due to venous congestion in the gut were eliminated. Histopathologically, the acute, as well as long-term effects of 60-min clamping of the CHA resulted in mild or no changes (unpublished findings). Moreover, biochemically (as monitored by serum levels of ALT), the extent of liver cell damage appears to be fairly low (but significantly different from controls), and, finally, peripheral liver blood flow, reflecting post-ischemic microvascular integrity, is only modestly impaired (but significantly different from controls) upon unclamping [6; present study]. Of importance is our finding that the deranged variables caused by clamping-unclamping of the CHA may be circumvented by ischemic or pharmacological preconditioning procedures [6, 7].

Both adenosine and NO have been proposed to function as mediators for IPC of the liver [10]. Peralta et al. [3] have suggested that IPC of the liver may be mediated by the inhibitory action of NO on the vasoconstrictor property of endothelin. Subsequently, these authors have suggested that NO may be the final mediator of IPC in rat liver, and that adenosine, in fact, causes the induction of NO synthesis [4, 5, 11]. In the liver, IPC is seemingly mediated by activation of A_2 adenosine receptors [5]. We have recently reported that pharmacological preconditioning by either the nucleoside transport inhibitor, dipyridamole, or the substrate for NO synthase, L-arginine, is equally efficient as IPC in preventing disturbances in blood flow and cell damage [6, 7]. The exact relationship between adenosine and NO with regard to IPC in rat liver is not yet settled. Some authors have reported that blockade of NO synthase attenuates [12] or abolishes [4] the protective effect of IPC. Likewise,

blockade of adenosine receptors abolishes the beneficial effects of IPC [13]. These findings, when taken together, suggest that adenosine and NO act in series. The brief period of ischemia could lead to a temporary accumulation of adenosine in the liver tissue, which, in turn, could cause the induction of NO, possibly in vascular endothelial cells [5, 11]. Adenosine, either by itself or via the generation of NO [cf. ref 14], could possibly protect the liver by vasodilation, inhibition of neutrophil infiltration, or preservation of the liver microvasculature [15]. Alternatively, Todo and coworkers [15] have proposed that an interstitial accumulation of adenosine could lead to beneficially enhanced high energy phosphate (i.e. ATP) resynthesis in the hepatocytes. Interestingly, recent findings strongly suggest that prostaglandins could confer protection against IR injury of the liver [16]. NO appears to induce the production of prostaglandins [12]. Whether or not NO may influence the tissue levels of adenosine is not known, but ATP could cause the release of NO from the vascular endothelium [14], as well as stimulate the production of prostaglandins [17].

Whatever the mechanism responsible for IPC, our experimental model seems to be well suited to its demonstration and further analysis. Of particular interest is the finding that IPC seemingly prevented the rise in ALT during the ischemic period. The mechanism for such a cytoprotective action of IPC as early as *during* the ischemic period is obscure; it seems unlikely that this is vascular in origin since the peripheral liver blood flow during ischemia was reduced as much as in the controls by IPC. In analogy, it may be suggested that the liver cell damage as caused by 60-min ischemia is primarily not related to reperfusion.

In our model of ischemia and reperfusion injury of rat liver, we found a protective effect of IPC. Pretreatment with the adenosine receptor antagonist, 8-PT, blocked this effect. Adenosine may, therefore, be a crucial effector in IPC. The results indicated a possible beneficial effect of IPC in, e.g., liver transplantation, in order to minimize graft injury. It is important to explore the possible mechanisms, as well as start clinical trials.

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