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Effect of ischemic preconditioning on hepatic tolerance to cold ischemia in the rat

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Abstract A short warm ischemia before reperfusion has been shown to improve the tolerance of the heart and the liver to a prolonged warm ischaemia. The present experimental study was conducted to evaluate the effect of such preconditioning on hepatic tolerance to an extended cold ischemia. In a model of isolated perfused liver, livers from Wistar rats (250–350 g) were stored for 24 h in UW (4 °C) immediately after harvesting and reperfused for 3 h at 37 °C. Control livers subjected to a 24-h cold ischemia were compared to livers subjected to preconditioning (defined as a 5- or 10-min clamping of the hepatic pedicle followed by a 10-min reperfusion before liver harvesting) prior to the definitive 24-h cold ischemia. While there was no difference in bile production between the preconditioned groups and the controls, transaminases and LDH release was significantly increased, vascular resistance was enhanced, and preservation injury was more extensive in both preconditioned groups as compared to controls. In contrast to the beneficial effect reported on prolonged warm ischaemia, preconditioning has a deleterious effect on hepatic tolerance to an extended cold ischemia.

Key words Liver ischemia · Preconditioning · Preservation

Introduction

Ischemic preconditioning is defined as a short period of ischemia followed by reperfusion prior to a long ischemic episode. This preconditioning has been reported to attenuate the tissue damage observed after ischemia and reperfusion of both the heart [1] and the liver [2]. The protective effect of preconditioning has been demonstrated for normothermic ischemia but, to date, no data are available for cold ischemia. A protection for cold ischemia similar to that observed for normothermic ischemia may facilitate application on organ transplantation. The aim of this study was, therefore, to evaluate whether the effect of ischemic preconditioning can benefit hepatic tolerance to a 24-h cold ischemia in the isolated perfused rat liver.

Materials and methods

Wistar rats weighing 250–350 g were used. Donor hepatectomy was performed by the method of Kamada [3]. Animals were randomly allocated to ischemic preconditioning (study group) or no preconditioning (control group). Ischemic preconditioning was defined as a 5- or 10-min occlusion of the hepatic pedicly by clamping ($n = 5$ and $n = 7$, respectively), followed by a 10-min reperfusion, before donor liver harvesting and cold storage. Livers were flushed with 10 ml cold UW solution (4 °C) before being harvested, and were preserved for 24 h in UW solution at 4 °C until reperfusion. The perfusate consisted of Krebs-Henseleit bicarbonate buffer solution (pH 7.4) enriched with 3 % human albumin and 15 % human red blood cells and oxygenated with a 95 % O_2–5 % CO_2 mixture. Livers were reperfused for 180 min at 37 °C in a temperature-controlled box. The portal vein was perfused by gravity at 9–10 cm pressure. Bile was collected during the whole period of perfusion. Blood samples were taken for liver function tests, and vascular resistance was measured at 60, 120, and 180 min after the liver was put into the circuit.

Table 1 Effect of ischemic preconditioning followed by 24 h of cold preservation in the isolated rat liver (mean ± SD). *P* values are 5-min preconditioning compared with control/10-min preconditioning compared with control (Mann-Whitney test)

Groups	Times of perfusion (min)	Controls	Five-min preconditioning	Ten-min preconditioning	*P* value
N		7	4	7	
AST (IU/l)	60	55 ± 23	80 ± 35	114 ± 50	NS/0.02
	120	111 ± 44	156 ± 24	161 ± 47	NS/NS
	180	160 ± 57	161 ± 47	231 ± 45	0.007/0.02
ALT (IU/l)	60	51 ± 20	88 ± 33	131 ± 56	0.04/0.004
	120	78 ± 20	153 ± 30	182 ± 54	0.001/0.001
	180	120 ± 29	294 ± 68	254 ± 51	0.002/0.001
LDH (IU/l)	60	1271 ± 542	2104 ± 811	1944 ± 522	0.02/0.04
	120	1892 ± 689	3886 ± 873	2769 ± 570	0.002/0.02
	180	2754 ± 980	7888 ± 2267	3907 ± 949	0.001/0.04
Vascular resistance (ml/min)	60	0.178 ± 0.069	0.216 ± 0.040	0.268 ± 0.138	NS/NS
	120	0.152 ± 0.042	0.187 ± 0.022	0.254 ± 0.121	NS/0.06
	180	0.15 ± 0.04	0.189 ± 0.015	0.262 ± 0.098	NS/0.02
Cumulative bile production (ml/liver)	60	16.6 ± 12.3	12.5 ± 7.1	16.8 ± 10.1	NS/NS
	120	55.7 ± 25.05	46.1 ± 16.5	56.7 ± 14.3	NS/NS
	180	26.5 ± 34.7	72.3 ± 23.9	82.5 ± 17.4	NS/NS

Results

The results of transaminase (AST, ALT) and LDH release as well as the production of bile and the level of vascular resistance are summarized in Table 1. While there was no difference in bile production between both the 5- or 10-min preconditioning groups and the controls, transaminase and LDH release was significantly increased in both preconditioned groups as compared to controls. Vascular resistance was also enhanced by ischemic preconditioning. The severity of preservation injury, assessed by liver cell necrosis, vascular congestion, and peliosis on pathological examination of the liver at the end of the 180-min reperfusion, was also more extensive in the preconditioned groups.

Discussion

Since its first description by Pringle in 1908 [4], clamping of the hepatic pedicle is frequently used in liver surgery to control bleeding. With this procedure, a continuous warm ischemia can be tolerated for periods of up to 60 min [5], but it induces hepatic damage. Intermittent vascular occlusion of 15- to 30-min periods followed by 5 min revascularization, has been shown to diminish the hepatic injury induced by prolonged vascular occlusion [6]. Ischemic preconditioning, i.e., a preparatory brief ischemia before a subsequent long ischemia is another way of improving the tolerance of the liver to prolonged ischemia. In animal models, it has been demonstrated to protect the liver from warm ischemia reperfusion injury, as reflected by both lower transaminase and LDH release [2, 7], and to improve survival [2]. However, no data were available concerning the effect of preconditioning on prolonged cold ischemia. The results of our study show that transaminase and LDH release following a 24-h cold ischemia is significantly increased after preconditioning as compared to control livers and that hepatic vascular resistance is increased along with the duration of preconditioning. Therefore, no benefit from preconditioning exists for prolonged cold ischemia and the results indicate the opposite, a deleterious effect resulting in the additive injuries of warm and cold ischemia. These data are in accordance with the clinical experience of transplanted livers from non-heart-beating donors, for which it is usually reported that the previous warm ischemia related to cardiac arrest cumulated its effects to that of cold ischemia [8]. Also in the liver transplant rat model, it is our experience that postoperative mortality correlates with the period of warm ischemia needed for liver harvesting and that temperatures of storage over 4 °C are deleterious [9]. Conversely, it has been demonstrated that a brief rinse of liver grafts with warm buffer after cold preservation markedly improves the hepatic microcirculation, leading to dramatic improvements in graft survival [10, 11]. Therefore, a progressive rewarming of the liver graft following cold storage could be beneficial while a warm ischemic period before cold preservation is definitively deleterious.

The reason why we failed to identify a protective effect of preconditioning on cold ischemia, in contrast to its beneficial effect on warm ischemia, is unclear. It has been recently suggested that the mechanism by which preconditioning is protective for warm ischemia involves the release of adenosine, a compound released by the cellular consumption of adenosine triphosphate and that is believed to confer cytoprotection to the is-

chemic tissue [12]. In addition, adenosine would stimulate the production of nitric oxide, a product able by itself to simulate the protective effect of preconditioning. It has also been shown that cells exposed to a potentially harmful environment may react by inducing the expression of several stress-related genes (antioxidative enzyme, catalase, and heat shock protein genes) associated with tissue cell protection [13–16]. As it is reported that in liver allografts the main site of injury of warm ischemia is the hepacytes while cold ischemia is associated with endothelial cell damage [17], one may assume that the protective compounds released after warm ischemia may not be efficient for a long cold ischemia because of different cellular targets. In conclusion, in contrast to its beneficial effect on warm ischemia, ischemic preconditioning has a deleterious effect on hepatic tolerance to prolonged cold ischemia, suggesting different mechanisms of protection between warm and cold ischemia in the rat liver.

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