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ORIGINAL ARTICLE

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Intestinal ischemia-reperfusion and plasma enzyme levels

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Abstract Determination of blood levels of intracellular enzymes is an appropriate method to evaluate tissue and organ damage. To show systemic tissue damage resulting from intestinal ischemia-reperfusion, New Zealand rabbits underwent 60 min intestinal ischemia and 60 min reperfusion. Plasma samples were obtained before and at 55, 70, and 120 min after operation and enzyme levels were determined. Plasma aspartate aminotransferase (AST) showed a significant increase during reperfusion while lactate dehydrogenase (LDH) and creatine kinase (CK) levels were significantly increased at the end of ischemia and continued to be so throughout reperfusion. It is difficult to claim that enzymes arise from the intestine, but an increase of CK, LDH, and later of AST without any increase in alanine aminotransferase levels during ischemia suggests that their primary source is the injured intestine. Increased levels of plasma enzymes do not provide exact information about the location, but do reveal the presence of an injury.

Keywords Intestinal ischemia-reperfusion · Plasma enzyme levels

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Introduction

Physiologically, reactive oxygen metabolites (ROM) are formed as a result of various processes. These harmful molecules are detoxified by endogenous antioxidant systems. Any pathology that results in increased ROM or decreased antioxidant capacity leads to harmful developments. Ischemia is such a process, which both decreases antioxidant capacity and triggers mechanisms that result in increased free-radical production in case of reoxygenation. Reperfusion resulting from ischemia causes very high levels of free radicals.

Ischemia triggers a sequence of chemical reactions in tissues, and may ultimately result in cellular injury and death. Cellular energy depletion and accumulation of toxic metabolites are responsible for these adverse effects. Reperfusion can lead to more severe injury than ischemia: reperfusion of ischemic tissue exacerbates the injury after only short periods of ischemia. ROM and activated polymorphonuclear leukocytes (PMN) play a key role in this process. Free radicals derived from the xanthine oxidase pathway activate the PMNs. These cells adhere to the capillary wall, emigrate into the tissue, and then produce and release proinflammatory agents and proteases. They also disrupt microvascular integrity [14].

An important pathologic process of ischemia and reperfusion (I/R) in the gastrointestinal system is injury of the intestinal-mucosal barrier [1, 7]: catalytic enzymes in the lumen and bacteria and their products pass through the intestinal wall and into the systemic circulation, after which the pathological process begins. Activation of phospholipase A_2 and neutrophils, ROM, and agents arising from the bowel lumen are responsible for the damage to the affected intestine. They also adversely affect other organs, especially the heart, liver, and lungs [6, 8, 13].

Determination of blood levels of intracellular enzymes is an appropriate method to evaluate tissue and organ damage. In the present study, plasma levels of various

intracellular enzymes were investigated in rabbits after 1 h intestinal ischemia and 1 h reperfusion to determine alterations resulting from tissue damage after I/R.

Materials and methods

A total of 18 New Zealand white rabbits weighing 1,500 to 3,000 g were included in the study; 12 were chosen as the I/R group and 6 as controls randomly. After fasting overnight, the animals were anesthetized with 25 mg/kg ketamine hydrochloride i.m. and maintained by i.v. infusion of 25 mg/kg sodium pentobarbital via an ear vein.

A midline laparotomy was performed after preparation of the abdominal wall with 10% povidone-iodine solution. All the following procedures except I/R were performed on the sham group. The small intestine was reflected to the left of the abdominal incision and the mesenteric artery that perfused a 30-cm segment of distal ileum was exposed. An atraumatic microvascular clamp was placed across the artery, avoiding occlusion of the related vein. The marginal vessels at both ends of the segment were divided and ligated, and the intramural collateral blood flow was stopped with atraumatic intestinal clamps. Mesenteric ischemia was confirmed when the mesenteric pulsations were lost and the intestinal segment became pale. The bowel was returned to the abdominal cavity and the incision was closed with interrupted 2/0 silk sutures. After 60 min ischemia, a relaparotomy was performed and the microvascular clamp on the artery was removed for 60 min reperfusion.

Heparinized blood samples were obtained from the inferior vena cava at 0 min (before ischemia), 55 (end of ischemia), 70 (beginning of reperfusion), and 120 (end of reperfusion) min. Plasma was separated immediately and stored at –20 °C until analyses were performed. Plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatine kinase (CK), and lactate dehydrogenase (LDH) levels were measured in a Technicon RA-XT autoanalyzer using commercially available spectrophometric kits (Biotrol, France).

The results were evaluated by SPSS for Windows 6.0. The differences between samples were analyzed by Friedman two-way ANOVA, and a P value below 0.01 was accepted as significant. The Wilcoxon matched-pairs signed-ranks test was used to show whether differences between the blood samples were present. Because six comparisons were made between blood samples, Bonferroni's adjustment was applied to the results, so that P values of less than 0.017 were accepted as significant.

Results

There was no significant difference between the enzyme levels of any of the four sequential blood samples in the control group (data not presented). Plasma ALT and ALP in the I/R group did not change throughout the study, while there was a significant increase in plasma AST during reperfusion. Plasma LDH and CK levels were significantly increased at the end of ischemia; this increase continued throughout reperfusion. LDH and CK levels had risen to twice their original levels at the end of reperfusion (Table 1).

Discussion

The use of noninvasive, inexpensive, and practical methods to determine the tissue injury induced by I/R would be helpful. These methods facilitate the diagnosis of ischemia and follow-up of the reperfusion injury. When the degree of injury is determined, it can be decided that whether any additional therapeutic measures are necessary, avoiding unnecessary procedures and ensuring that required interventions are performed promptly.

Blood levels of intracellular enzymes are a way of estimating tissue damage: tissue- or organ-specific enzyme levels provide valuable information about related tissues. Both ischemia and reperfusion damage the entire intestine, especially the mucosal layer. In our previous study [5], mucosal damage was assessed by the standard of Chiu et al. [2]. It was shown that mucosal injury was more prominent after reperfusion: mucosal injury scores were 2.8 ± 0.8 and 4.2 ± 0.8 after ischemia and reperfusion, respectively [5]. The difference was statistically significant.

In the other studies serum CK, LDH, ALP, and AST levels have been investigated in order to determine intestinal necrosis resulting from ischemia. Significant increases in serum levels have been found after periods of ischemia [3, 4, 10]. In those studies, enzyme levels were generally found to be increased 3 h or more after ischemia. In this study, we found the same increase in CK and LDH levels just 1 h after ischemia. Although ALP is a diagnostic parameter of ischemic bowel, CK was found to increase earlier and more than ALP. Also, CK-BB has been reported to be a more sensitive indicator of bowel necrosis than the intestinal izoenzyme of ALP [4]. ALP was not increased at any stage in our study. We also found no significant effects of ischemia on AST and ALT levels.

In humans, the changes in blood enzyme activity observed during intestinal ischemia were reported to be related to the pathology caused by the ischemia [9].

Table 1 Plasma enzyme levels and results of statistical analysis (mean ± SD) (*AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *ALP* alkaline phosphatase, *CK* creatine kinase, *LDH* lactate dehydrogenase)

Sample	1	2	3	4	F	*P*	1–2	1–3	1–4	2–3	2–4	3–4
AST (IU/l)	27.1 ± 14.6	27.8 ± 16.6	33.9 ± 16.3	34.8 ± 19.5	11.8	0.008		*		*	*	
ALT (IU/l)	43.2 ± 25.5	42.5 ± 26.9	46.5 ± 28.8	50.3 ± 29.3	8.78	0.032						
ALP (IU/l)	74.4 ± 41.2	74.6 ± 43.5	75.7 ± 43.6	78.0 ± 45.4	1.125	0.771						
CK (IU/l)	754 ± 340	1,309 ± 775	1,392 ± 766	1,529 ± 1014	27.12	0.000	*	*	*		*	
LDH (IU/l)	118 ± 33	174 ± 29	193 ± 33	242 ± 52	14.02	0.003	*	*	*		*	*

* $P < 0.017$

Complete or incomplete ischemia and the presence of venous or lymphatic drainage from the damaged tissue may affect blood enzyme levels from damaged tissue during ischemia. In addition, the kind of experimental animal used in studies may play a role.

Apart from ischemia of other organs, the injury caused by intestinal ischemia is not limited to the affected organ. Hepatic hypoperfusion and acute hepatic damage after I/R was reported, and after 2 h intestinal ischemia an increase of up to four times the normal ALT level was shown [13]. In our study, plasma ALT levels remained unchanged during ischemia and increased slightly but not significantly after reperfusion. Our model thus did not result in significant damage to the liver during this period of study.

The enzymes released from damaged tissue during ischemia enter the circulation when reperfusion starts, and their blood levels increase. The addition of a reperfusion injury and damage to distant organs will further increase blood enzyme levels in this period. As a result, a more marked increase than that during ischemia can be seen. In our study, the increase in CK and LDH levels continued during reperfusion. Also, significant elevation of AST was observed during that period.

Injuries to other organs caused by intestinal I/R have been shown in many studies [6, 8, 13]. In our study, the increase in CK, LDH, and later AST without any increase in ALT levels during ischemia suggests that the primary source was the intestinal segment. Thompson et al. [12] found plasma CK levels increased after 4 h, while AST, LDH, and ALP levels were increased after 24 h after I/R. We could not show such a late increase, since the duration of our experiment was only 2 h.

The increase in plasma enzyme levels during ischemia depends on the model used, and this increase continues during reperfusion. Increased enzyme levels do not provide any information about the extent and reversibility of the injury [11, 12], but only show the presence of an injury. It may be possible to learn which organ or tissue has been affected by investigating other tissue-specific parameters.

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