Determination of intestinal α-glutathione S-transferase after ischemia-reperfusion injury

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Key words : glutathione S-transferase, ischemia-reperfusion, intestinal transplantation, swine model.

Introduction

The results in small bowel transplantation improved since the introduction the novel immunosuppressant tacrolimus. However, the graft survival rates are lower compared to kidney and liver transplants. The most common complication of intestinal transplantation is rejection and most of the deaths after intestinal transplantation are related to the intense use of immunosuprression to prevent graft rejection (Tzakis, 1994 ; Grant, 1996).

In solid organ transplantation, function parameters are used for monitoring graft function and early detection of graft dysfunction. Urine output, creatinine, and urea are established parameters indicating renal graft dysfunction like bile flow, bile color, transaminases, bilirubin, and factor V in liver transplantation. Unlike renal or liver transplantation, parameters for monitoring intestinal graft function are rare. Therefore, intestinal biopsies are the gold standard in small bowel transplantation (de Bruin, 1994). The risk of graft perforation and the access without cutaneous stomas have to be taken into consideration taking full thickness biopsies. Therefore, several parameters have been investigated (e.g. brush border enzymes) without replacing frequent histological examinations so far (Kunsanmäki, 1996 ; de Bruin, 1994).

Recently, α-glutathione S-transferase (α-GST) was found to be a parameter for monitoring ischemia-reperfusion injury and acute rejection in clinical liver transplantation (Trull, 1994 ; Hughes, 1997 ; Platz, 1997). GSTs are multi-functional proteins that play an important role in the detoxification of xenobiotics, exhibit peroxidase activity toward organic hydroperoxides and serve to combat oxidative stress (Beckett, 1993). Therefore, we investigated α-GST during ischemia-reperfusion injury as a marker for monitoring the intestinal function in an isolated small bowel perfusion model.

Methods

Animals and operative procedure

Twelve small bowels (5-6 m) of pigs weighing 25-30 kg were used for perfusion. After median laparotomy with lateral extension, the infrarenal aorta and vena cava were prepared in cranial direction. Thereafter, the pancreas was discontinued from the portal vein. The superior mesenteric artery was prepared proximal at the aorta. The jejunum was transsected 10-15 cm distal to the ligament of Treitz and the ileum 10-15 cm proximal of the ileocoecal valve. The colon was separated from the small bowel and removed. After clamping of the subdiaphragmatic aorta, insertion of a canula in the infrarenal aorta and placement of an arterial catheter, the intestine was perfused with cold HTK (histidin-tryptophan-ketoglutarate, Dr. Franz Koehler Chemie GmbH, Alsbach-Haehnlein, Germany). The intestine was explanted and the lumen was flushed with 0.5 l of cold HTK. The intestine was stored at 4° C in HTK until reperfusion.

Ex vivo small bowel perfusion

The intestines were reperfused with swine blood in an closed circuit perfusion chamber after cold ischemia times (CIT) of 2 (n = 4), 8 (n = 3) and 20 (n = 5) h. The closed circuit perfusion device consisted of a roller pump, a perfusion chamber, a hemo filter, a blood pressure monitor, a heater, an oxygen blender and a combined oxygenator and heat exchanger. In- and outflow was achieved through catheters in the superior mesenteric artery and portal vein. Heparinized porcine blood was used for perfusion at 37°C. Arterial blood flow and pressure were adjusted to 200-400 ml/min and 80-140 mmHg during perfusion (Braun, 1998). Perfusion ended when the arterial pressure exceeded 180 mmHg.

Determination of α-GST in blood and mucus

Venous blood samples for monitoring GST were taken at reperfusion, 2 h, 5 h and 7 h after reperfusion. Mucus was collected at the same time as blood. Blood and mucus samples were immediately stored at -80° C. A porcine α-GST enzyme immunoassay (EIA) (Biotrin HEPKIT™-Alpha-Porcine GST-Alpha, Biotrin Ltd., The Rise, Mount Merrion Co. Dublin, Ireland) was

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used for monitoring GST (Rees, 1995). The GST-EIA procedure was carried out according to manufacturer's instructions.

Histology and Immunohistochemistry

Intestinal resections were taken for histological examinations from the distal intestine before HTK-perfusion, before reperfusion, immediately after reperfusion, and every hour after reperfusion. Mucosal damage was classified according to Chiu *et al.* (Chiu, 1970) on paraffine sections. For immunohistochemistry, tissues were immediately frozen in liquid nitrogen and stored at -80°C. Frozen sections of 4 µm were cut with a Reichert Jung cryotome, fixed in aceton for 10 min, and dried by air over night. Intrinsic peroxidase activity was blocked with 70 ml methanol/700 µl H_2O_2 for 30 minutes in the dark. Thereafter, samples were washed out by 70 ml TRIS-buffered saline (TBS, pH 7.4) for 10 minutes. TBS consisted of NaCl 9 g, Tris 6 g, and 21 ml 2 M HCl added at 1 l destillated H_2O. Sections were incubated with anti-α-GST and anti-µ-GST polyclonal antibodies (1:100 in 0.1 M TBS/BSA pH 7.4) for 1½ h at room temperature. Sections were washed for 10 minutes in TBS. A goat anti-rabbit antibody (1:50 in TBS/BSA) was used for bridging while 30 minutes incubation time at room temperature. Sections were washed in TBS for 10 minutes. Reactions were detected by means of the peroxidase-anti-peroxidase (PAP ; 1:150 in TBS/BSA ; 30 minutes incubation time) method (bridge antibody goat anti-rabbit antibody and PAP complex from the rabbit were purchased from Dako, Hamburg, Germany) and developed with diaminobenzidine (Sigma, München, Germany). Nuclei were then counterstained with hematoxylin and the sections were dehydrated and cover slipped (Quandamatteo, 1998).

Results

Mean (± SD) perfusion time was 540 ± 56 min after a CIT of 2 h, 410 ± 62 min after a CIT of 8 h, and 426 ± 69 min after a CIT of 20 h. While the flow was kept stable, the arterial pressure increased over time as a consequence of the ischemia-reperfusion injury. The arterial pressure showed a sharp increase crossing 140 mmHg at 6 h after a CIT of 8 and 20 h, while the intestines with a CIT of 2 h showed this increase with a delay of 2 h. (figure 1).

Histologically, regular mucosa morphology was present at start of reperfusion after CIT of 2 and 8 h, while a CIT of 20 h already showed subepithelial spaces at the apex of the villus (grade 1). After 2 h of reperfusion the mucosal damage was staged grade 1 (CIT 2 h), grade 1-2 (CIT 8 h), and grade 2-3 (CIT 20 h). Grade 2 contained subepithelial spaces at the upper villus half's and apex denudation and grade 3 denudation of the upper villus and subepitehlial spaces in the lower villus half's. Five after reperfusion, mucosal damage of intestines with 2 h CIT reached grade 3, 8 h CIT grade 2-4, and 20 h CIT grade 4 with congested capillaries and complete villus denudation. Grade 4 was reached after 7 h of reperfusion in intestines with 2 and 8 h

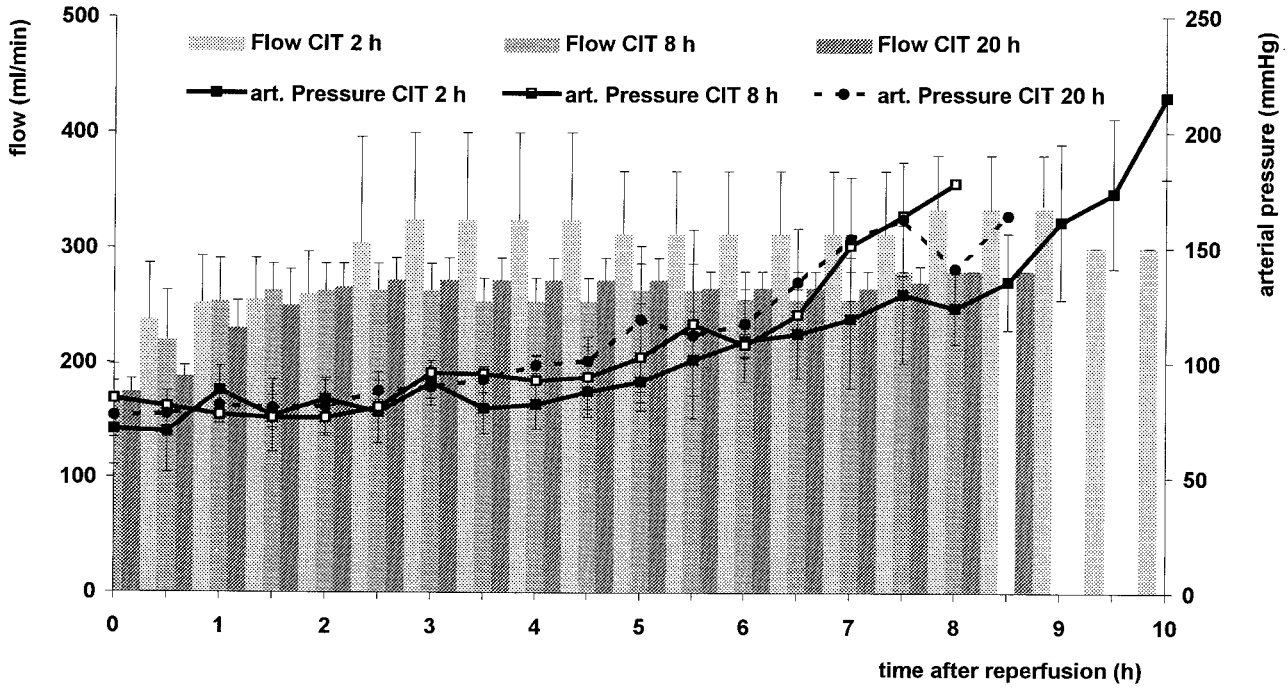

Fig. 1. — Mean arterial pressure and flow monitored in the ex vivo small perfusion model after cold ischemia times of 2, 8, and 20 h.

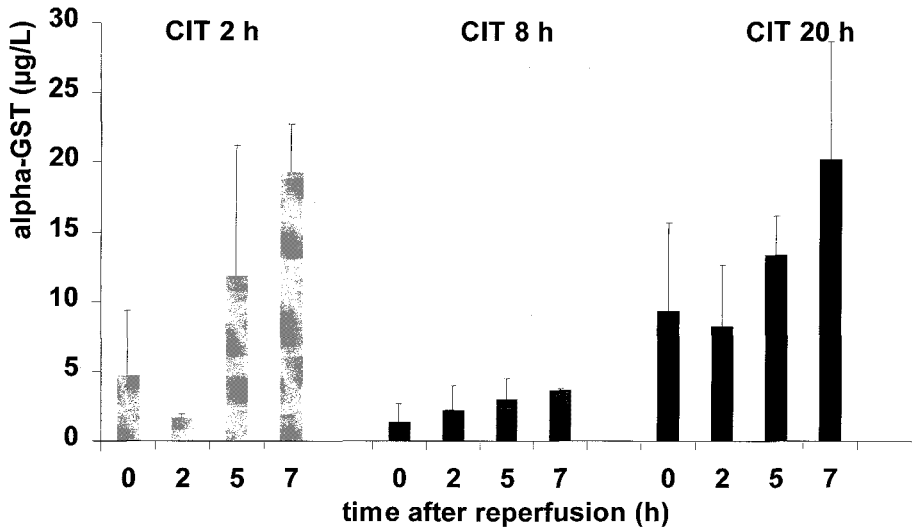

Fig. 2. — Mean (± SD) concentrations of α-GST in blood measured by α-GST-EIA at 0 h, 2 h, 5 h, and 7 h after reperfusion (CIT = cold ischemia time).

CIT, while 20 h CIT ended in mucosal damage grade 5 with severe hemorrhages and villus destruction (table I).

After a CIT of 2 h, α-GST in blood increased from 2.9 (< 0.7-12.5) µg/L immediately after reperfusion to 1.8 (1.4-55.7) µg/L at 2 h, 8.5 (3.6-26.7) µg/L at 5 h, and to 19.5 (15.0-23.4) µg/L 7 h after reperfusion, after a CIT of 8 h from 0.87 (< 0.7-3.2) µg/L to 2.7 (< 0.7-4.1) µg/L at 2 h, to 2.6 (1.5-5.0) µg/L at 5 h, to 3.7 (3.6-3.7) µg/L at 7 h, and after a CIT of 20 h from 5.2 (3.9-20.1) µg/L to 6.0 (4.9-16.6) µg/L at 2 h, to 12.9 (9.9-17.7) µg/L at 5 h, and to 21.3 (4.3-27.6) µg/L at 7 h after reperfusion (Figure 2).

Table I. — **Mucosal damage during reperfusion in hours according to the Chiu-classification after cold ischemia times of 2, 8 and 20 h**

Grade		0	1	2	3	4	5
CIT 2 h	time after reperfusion	0	2	5-7			
CIT 8 h	time after reperfusion	0	2	2-5	7	7	
CIT 20 h	time after reperfusion		0	2	2-5	5-7	7

Immunohistochemically, the goblet cells stained positive to α- and µ-GST before HTK-perfusion and after reperfusion with swine blood. Also, we found that superficial mucus stained positive to α- and µ-GST (Figure 3 and 4). Therefore, we measured α-GST in mucus by EIA. Interestingly, concentrations of α-GST were found be 54 (8-761)-fold higher in mucus ranging 275 (76.9-1068.2) µg/L (Figure 4).

Discussion

Determination of GST in blood and especially in mucus for monitoring the intestinal function is an

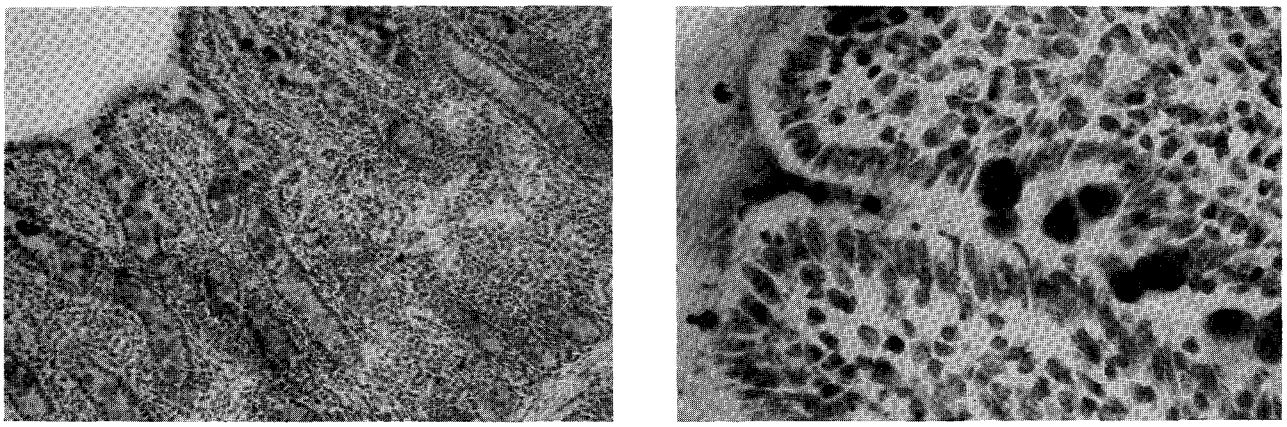

Fig. 3.

Fig. 3 and 4. — The goblet cells and the superficial mucus stained positive to anti-α- and anti-µ-GST (immunohistochemistry, peroxidas-anti-peroxidase ; 10- and 40-fold magnification). α-GST is shown on left and µ-GST on the right picture.

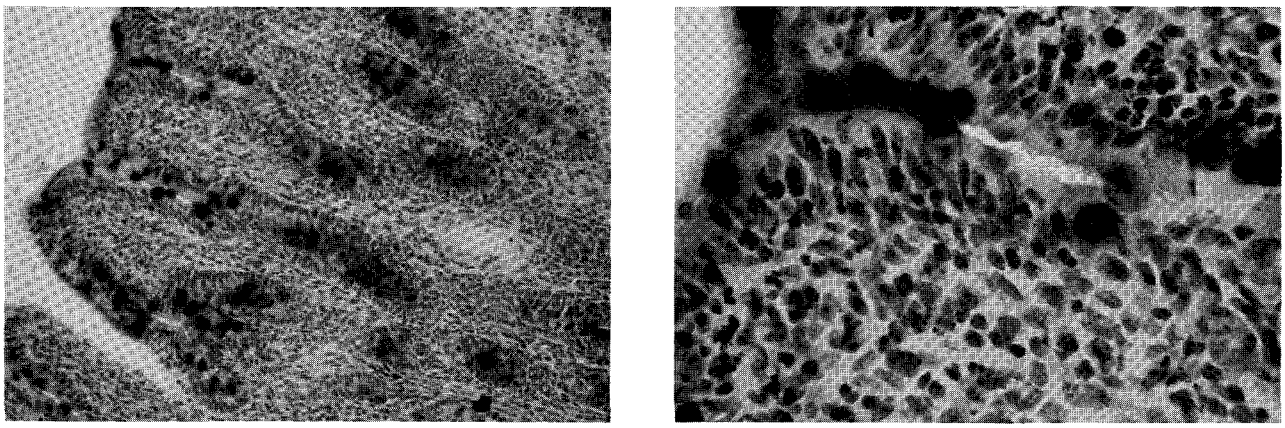

Fig. 4.

attractive approach for monitoring of mucosal damage. The assessment of mucosal injury is of high clinical relevance in small bowel transplant recipients. Even if the surgical technique is perfect, the recipient runs a high risk of bacterial or fungal infection from the intestinal graft because rejection, ischemia injury, or viral infection increases the permeability of the mucosal barrier to enteral organisms or destroys the mucosal barrier (Grant, 1991 ; Tzakis, 1994). The resulting septicemia through bacterial translocation can be fatal in the immunocompromized recipient.

In contrast to rejection episodes, the ischemia-reperfusion injury affects the early graft function. Organ and cell damage during ischemia-reperfusion injury is caused by manipulation during the explantation procedure and the exposure to cold and warm ischemia times. However, upon reperfusion of ischemic tissue, a paradoxical injury process is elicited at the microcirculatory level that can be simply characterized as an exaggerated inflammatory response. The consequences of this inflammatory process is cellular necrosis and organ dysfunction. In the setting of small bowel transplantation, the mucosa of the small bowel is very susceptible to ischemia-reperfusion injury (Parks, 1982 ; Park, 1991).

Therefore, monitoring of intestinal GST, that is located in the mucosal goblet cells, should be investigated in clinical intestinal transplantation starting early after reperfusion of the graft. Hereby, it might be possible to calculate the mucosal damage from ischemia-reperfuion injury and to follow the further graft function. The current operative procedure in small bowel transplantation contains a cutaneous ileostomy, that provides venting and allows the physicians easy access when performing endoscopies and biopsies. The stoma is closed when the graft stabilizes and frequent endoscopies are no longer necessary, usually several months after transplantation (Tzakis, 1994 ; Frezza, 1996). During the time of cutaneous ileostomy, α-GST monitoring in mucus might be become an interesting parameter for monitoring mucosal damage due to the easy access for collecting mucus.

Whether the increase of α-GST in mucus is related to excretion by the goblet cells or mucosal damage with destruction of the goblet cells remains unclear yet. However, the concentration of α-GST in mucus reflects mucosal damage in general. It is speculative if a quotient of blood α-GST and mucus α-GST might be established to differentiate immunologic from ischemic alterations. Further investigations should be undertaken to distinguish the role of intestinal α-GST.

In conclusion, α-GST should be used for monitoring intestinal function after intestinal transplantation. Especially, the determination of α-GST in mucus appears to be a new attractive approach.

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