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DSC, as a new method to verify the exact warm and cold ischemic injury during small bowel surgery

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ABSTRACT

The fact that small bowel is extremely sensitive to ischemia/reperfusion injury had encouraged us to compare the influences of warm and cold ischemia on the intestinal structural changes by differential scanning calorimetry (DSC) method. Warm and cold ischemia groups were established on Wistar rats with 1, 3 and 6 h ischemic times. Intestinal biopsies were collected after laparotomy and at the end of the ischemia periods. DSC measurement was performed on mucosa, on muscular layer and on the total intestinal wall. Our DSC data confirmed that longer warm ischemia period caused more severe damage in the structure of mucosa and muscular layers. According to the results of transition temperature and calorimetric enthalpy suggest that these changes reduced by cold ischemic procedure in University of Wisconsin solution. However, the thermal destruction of each layers following cold preservation injury revealed significant differences compared to normal bowel structure.

1. Introduction

Intestinal warm ischemia/reperfusion (I/R) is an important factor associated with a high morbidity and mortality in some critical clinical settings including hemorrhagic shock, strangulation obstruction, cardiovascular surgery, and severe trauma or combustion. These warm intestinal ischemic states through the mesenteric infarction cause abdominal emergency. The mortality of patients who experience mesenteric infarction continues to be high, with up to 80% of such patients dying. In the background of this severe clinical condition stand several molecular processes, including production of oxygen free radicals (OFRs), oxidative stress, inflammatory processes, and irreversible cell death. An intact intestinal mucosa is of vital importance for efficient assimilation of ingested nutrients, but it also serves as a barrier that limits access of enteric bacteria and other noxious stimuli to the systemic circulation. Disruption of the mucosal barrier results in the absorption of nutrients and if the lesion is severe, it can lead to sepsis, to multi organ failure and finally to death [1,2]. Moreover, few study in the literature showed that, I/R significantly alters intestinal motility function. The generation of OFRs and disruptions in the calcium homeostasis also play an important role in the pathogenesis of muscular layer's damage [3].

During small bowel transplantation to minimize warm ischemic damage of intestine cold preservation has been applied in the clinical practice. This procedure can reduce cellular damages, but it is inevitably accompanied by cold ischemic injury. Although tissue injury is evident throughout the period of cold storage, this damage is exacerbated on the reintroduction of oxygen and with reperfusion of the organ in the recipients. To prevent oxidative damage, the University of Wisconsin (UW) solution is the first choice for small intestinal cold preservation, which contains among others OFRs scavengers (glutathione and allopurinol), and energy source (adenosine) [4–8].

Several experiments demonstrated that ischemia can be evaluated by the detection of various products resulting from injury, using laboratorial and histopathological methods. Numerous different histological grading systems have been described, namely the Park's, the Chiu's, the Parks's and the Sonnino's systems. From these, the Park's and Chiu's methods for grading injury are the most suitable as a standard scoring scale for histological evaluation of intestinal damage. Advantages these scoring system are, that they grades the progression of morphologic injury from mild to severe, showing well-correlation with clinical outcome. However, the disadvantages of them are firstly, they do not describe the delicious details in some tissue structures; secondly, as in a qualitative methods the diagnosis (degree of injury) are closely depend on the person of the pathologists, thus not free from the examiner-based differences; thirdly, these classifications alone are not able to take the exact tissue injury into account [9]. Unfortunately, there is no consensus exists on how the injury should be graded.

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Differential scanning calorimetry (DSC) is a thermoanalytical technique which monitors small heat changes between a sample and a reference. DSC examination is a validly efficient method for the demonstration of structural changes not only in the physical sciences, but also in numerous biological systems. It allows demonstrating the thermal consequences of local and global conformation changes in the structure of different tissue elements [10–14]. The goal of this study was to evaluate and to compare the exact and quantitative morphological changes in each layer of the intestinal tissue by DSC technique after 1, 3 and 6 h warm and cold ischemic periods.

2. Materials and methods

2.1. Animal preparation and anaesthesia

Adult male Wistar rats (250–300 g, $n = 30$) were purchased from the Laboratory Animal Centre of University of Pécs, housed under pathogen-free conditions and were fasted for 24 h preoperatively, but had free access to water. Rats were anaesthetized with intramuscular ketamine hydrochloride (0.01 mg g^{-1} of body mass) and diazepam (0.01 mg g^{-1} of body mass) (Richter Gedeon, Budapest, Hungary). All procedures were performed in accordance with the ethical guidelines of NIH and guidelines approved by the University of Pécs (BA02/2000-9/2008) to minimize pain and suffering of the animals.

2.2. Warm and cold intestinal ischemia models

After median laparotomy warm ischemia groups were established. In these groups ischemia was induced by clamping the superior mesenteric artery for 1, 3 and 6 h. In cold ischemia groups median laparotomy was followed by small bowel resection from the ligament of Treitz to the ileocoecal part. Grafts were perfused and stored in 4 °C UW solution (Viaspan, Bristol-Myers Squibb GesmbH, Vienna, Austria) for 1, 3 and 6 h. Small bowel biopsies were collected after laparotomy (control) and at the end of the warm and cold ischemia periods.

2.3. DSC measurements

The thermal unfolding of the total intestinal wall, its mucosa and muscle components were monitored by SETARAM Micro DSC-II calorimeter. We have listed our samples in Table 1. In our case "sample" means separate mucosa, muscle and total intestinal wall. We have used for 6 h ischemia 5-5, in other cases 4-4 samples. We have chosen a relatively longer bowel; half of it was separated into mucosa and muscle, and a remaining one was the total intestinal wall from the same rat. The total intestinal wall is the "intact" bowel. It is not the mixture of mucosa and muscle. In case of mucosa we have invaginated the bowel, and scraped off from its inner surface the mucosa (other way it is impossible to separate it because of its very thin layer thickness), and the remaining part was the muscle sample. The fashion of the DSC curves was reproducible. The onset and the end point of denaturation were determined by the change of the slope of scans comparing with the native (onset) and the denatured state (end point) slopes (in the figures it cannot be seen). The precision of enthalpy determination was between 5% and 10%.

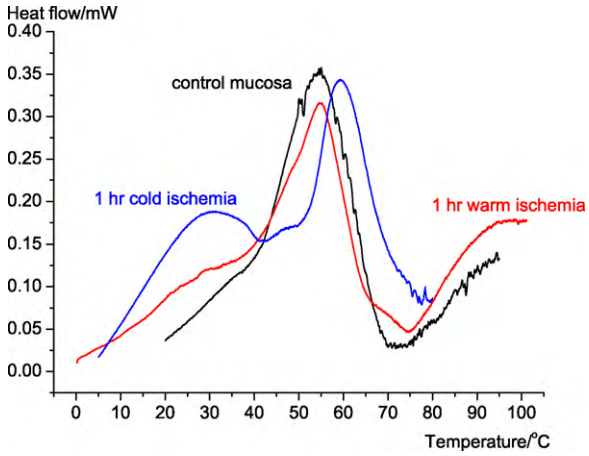

Fig. 1. DSC analysis of mucosa following 1 h intestinal warm ischemia and cold preservation. The upward deflection of DSC scans means an exotherm process, indicating the coagulation of tissue elements.

All experiments were conducted between 0 and 100 °C. The heating rate was 0.3 K/min in all cases. Conventional Hastelloy batch vessels were used during the denaturation experiments with 850 μL sample volume (samples plus buffer) in average. Typical sample wet masses for calorimetric experiments were between 100 and 150 mg. Reference sample was normal saline (0.09% NaCl) at the measurement of warm ischemia tissue samples. At cold ischemia groups tissue samples were stored in UW solution, and this solution was used as a reference sample. The sample and reference samples were equilibrated with a precision of ±0.1 mg. There was no need to do any correction from the point of view of heat capacity between sample and reference samples. The repeated scan of denatured sample was used as baseline reference, which was subtracted from the original DSC curve. Calorimetric enthalpy was calculated from the area under the heat absorption curve by using two-point setting SETARAM peak integration.

3. Results

Our DSC results are presented according to the all parts of the intestinal wall and the duration of ischemia times. As it can be seen in Figs. 1–3 the mucosa has undergone significant changes in a time-dependent manner during different types and durations of ischemia. The control (untreated) sample has an exotherm with T_ms

Table 1
DSC data of mucosa, muscle and total intestinal wall after 1, 3 and 6 h of small bowel warm ischemia and cold preservation (transition temperature: T_m (°C); calorimetric enthalpy: ΔH (J/g), average ± s.e.).

	Mucosa		Muscle		Total intestinal wall	
	T_m (°C)	ΔH (J/g)	T_m (°C)	ΔH (J/g)	T_m (°C)	ΔH (J/g)
Control	55.6 ± 0.2	−4.1 ± 0.22	52.8; 58.1; 59.9 ± 0.2	0.59 ± 0.03	17.5; 49.5 ± 0.2	0.46; 0.45 ± 0.03
1 h warm ischemia	55.6 ± 0.4	−3.41 ± 0.3	49.5; 62 ± 0.5	1.4 ± 0.2	23; 50.5; 60.2 ± 0.4	1.1 ± 0.07 0.28 ± 0.02
3 h warm ischemia	48.7 ± 0.3	−1.7 ± 0.2	60.7 ± 0.4	0.8 ± 0.06	59.8 ± 0.3	0.28 ± 0.02
6 h warm ischemia	41.1; 46.3 ± 0.2	−5.2 ± 0.3	52; 59 ± 0.5	3.1 ± 0.2	58 ± 0.3	0.58 ± 0.03
1 h cold ischemia	30.4; 59.3 ± 0.2	−5.94 ± 0.4	53.5; 56 ± 0.2	2.21 ± 0.2	56 ± 0.2	0.38 ± 0.02
3 h cold ischemia	32.6; 59.7 ± 0.2	−2.67 ± 0.2	53.6; 58 ± 0.2	3.4 ± 0.1	53; 55; 59.6 ± 0.2	0.76 ± 0.06
6 h cold ischemia	47.9; 58.8 ± 0.2	−1.96 ± 0.2	53.6; 57.9 ± 0.2	3.8 ± 0.1	41; 57.9; 58.6 ± 0.2	0.4 ± 0.02