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Attenuation of intestinal ischemic injury and shock by physostigmine

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ABSTRACT

Background: Recently, protection in shock (hemorrhagic or septic) by physostigmine has been demonstrated. Here, we studied the protective effect of intravenous infusion of physostigmine in a rat model of severe intestinal ischemia–reperfusion (I/R) injury and shock.

Materials and methods: Mesenteric I/R was induced in male Wistar rats by occlusion of the superior mesenteric artery (90 min) and subsequent reperfusion (120 min). Physostigmine (30 or 70 μg/kg) was administered as bolus injection before induction of I/R. One additional group received, subsequent to the bolus of 30-μg/kg physostigmine, a continuous infusion of 60-μg/kg physostigmine till the end of the experiment.

Results: Physostigmine at a dose of 70 μg/kg administered before I/R significantly decreased the macroscopically and microscopically visible intestinal damage. In addition to and presumably as a result of this local protective effect, physostigmine prevented shock induced by reperfusion of the ischemically injured intestine. Lower doses (30 μg/kg) or continuous application of physostigmine were less advantageous.

Conclusions: Physostigmine is clearly protective in intestinal I/R injury and shock. However, for this purpose, physostigmine has to be applied at a dose (70 μg/kg), that is, approximately double the amount of the presently used clinical dose.

1. Introduction

Acute mesenteric ischemia and the resulting intestinal injury is a life-threatening clinical complication with a mortality rate of 50%–70% [1]. It may be caused by embolization of the superior mesenteric artery, thrombosis of mesenteric arteries and veins, or hypoperfusion of supporting vessels, for example, during shock (low flow-ischemia [2–6]). Predisposing risk factors are cardiac insufficiency, atrial fibrillation, coronary artery disease, arterial hypertension, and peripheral arterial occlusive disease [7,8]. Mesenteric ischemia may also occur during surgical cardiovascular interventions [9–11], including abdominal aortic surgery [2,12]. The intestine, or more precisely intestinal injury, in turn is considered not only to be the gateway to systemic infection after shock but also to play an aggravating role in shock arising from several conditions, including trauma and infection (gut hypothesis of shock and multiple organ dysfunction) [13–16].

Physostigmine (also called eserine) is a reversible cholinesterase inhibitor alkaloid extracted from the seeds of Calabar bean (*Physostigma venenosum*). It is a short acting inhibitor of the enzymes acetylcholinesterase (AChE) and butyrylcholinesterase, thereby increasing acetylcholine levels. It is a lipid soluble tertiary amine and because of its structure capable of passing the blood-brain barrier. The medical use of the drug physostigmine (Anticholium, agent: Physostigmine

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Salicylate) is, among others, the treatment of central anticholinergic syndrome, shivering, alcohol withdrawal delirium, and intoxication with atropine.

In experiments with hemorrhaged rabbits and rats, the administration of physostigmine after bleeding led to an increase in mean arterial blood pressure (MAP) and an improvement of the survival rate [17–19]. Studies in a murine sepsis model showed a decrease in the inflammatory response after application of physostigmine, and concomitantly, an increase in survival [20]. Together, these findings strongly suggest that physostigmine possesses a general protective potential in ischemic diseases and thus should also be protective in intestinal ischemia–reperfusion (I/R) injury. Experimental evidence to support this assumption, however, is missing. Therefore, we here studied the effect of intravenously applied physostigmine on severe small intestine I/R injury using a rat model based on the occlusion of the superior mesenteric artery (SMAO).

2. Materials and methods

2.1. Chemicals and/or materials

Physostigmine Salicylate (Anticholium; 2.0 mg/5 mL) was obtained from Dr Franz Köhler Chemie (Bensheim, Germany). Hydrogen peroxide, Naphthol-AS-D-chloroacetate esterase kit, and o-Dianisidine were from Sigma–Aldrich (St Louis, MO). Complete protease inhibitor mixture was purchased from Roche (Mannheim, Germany), isoflurane (Florene) from Abbott (Wiesbaden, Germany), ketamine 10% from Ceva (Düsseldorf, Germany), and lidocaine (Xylocaine 1%) from AstraZeneca (Wedel, Germany). Portex catheters (inner diameter: 0.58 mm, outer diameter: 0.96 mm) were obtained from Smith Medical International (Hythe, United Kingdom). Paraffin (Paraplast Tissue Embedding Medium) was from McCormick Scientific (St Louis, MO) and medical oxygen from Air Liquide (Düsseldorf, Germany).

2.2. Animals

Male Wistar rats (400–470 g) were obtained from the central animal unit of the Essen University Hospital. Animals were kept under standardized conditions of temperature (22 ± 1°C), humidity (55 ± 5%), 12-h–12-h light–dark cycles, and had free access to water and food (ssniff-Spezialdiäten, Soest, Germany). They were not fasted before the experiments. All animals received humane care according to the standards of the Federation of European Laboratory Animal Science Association. The experimental protocol has been approved based on the local animal protection act.

2.3. Anesthesia, analgesia, and surgical procedure

Anesthesia, analgesia, and surgical procedure were basically performed as described previously [21,22], with slight modifications. Catheter insertions were carried out in the right femoral artery and vein [23]. Rats were anesthetized with isoflurane (2% in 100% medical O_2 at 4 L/min for induction of anesthesia and 1%–1.7% at 1 L/min throughout the experiment) through face masks connected to a vaporizer (Isoflurane Vet. med. Vapor, Dräger, Lübeck, Germany) and received ketamine (50 mg/kg, subcutaneously) into the right chest wall for analgesia. After local lidocaine application (5 mg/kg, subcutaneously), a skin-deep incision along the right groin was performed to place a Portex catheter within the right femoral artery and vein, respectively. Subsequently, the *A. mesenterica superior* was occluded using an atraumatic minibulldog (Aesculap, Tuttlingen, Germany). Immediately after the ischemic period (I, duration 90 min), the microvascular clamp was removed and reperfusion of the organ (R, duration 120 min) started. The complete small intestine was resected, and animals were sacrificed by cardiac incision under deep isoflurane anesthesia at the end of the reperfusion phase.

2.4. Study groups

Two successive series were performed.

- Series A (high-dose bolus administration), bolus application of 70-μg/kg physostigmine
- Series B (low-dose bolus administration), bolus application of 30-μg/kg physostigmine with or without subsequent continuous physostigmine administration (60 μg/kg × h)

In Series A, 70-μg physostigmine (corresponds to 175-μL Anticholium) was freshly dissolved in 825 μL of sterile 0.9% NaCl solution. As Anticholium is a pharmaceutical product, adjusting the pH-value and sterile filtration were not necessary. Using a tuberculin syringe, the bolus of physostigmine solution (1 mL/kg) was injected manually into the femoral vein over a period of 5 min. This was done 5 min before occluding the *A mesenterica superior* or 5 min before reopening it or at both time points consecutively. In addition, 0.9% NaCl solution (7 mL/kg × h) was given throughout the experiment, to compensate for fluid loss over surgical areas and the respiratory epithelium [21]. An I/R control and a sham-operated group, which run through all surgical procedures except the SMAO, received only 0.9% NaCl solution during the experimental period. The following experimental groups were compared ($n = 6$):

- Normoxic control group (no I/R, no physostigmine)
- I/R control group (90 min I/120 min R, no physostigmine)
- I/R highPhys bI group (90 min I/120 min R, 70-μg/kg physostigmine before I)
- I/R highPhys bR group (90 min I/120 min R, 70-μg/kg physostigmine before R)
- I/R highPhys bI + R group (90 min I/120 min R, 70-μg/kg physostigmine before I and R)

In series B, 300-μg physostigmine (corresponding to 750-μL Anticholium) was freshly dissolved in 34.25 mL of sterile 0.9% NaCl. The physostigmine solution was then infused with a syringe pump (Perfusor-Secura FT; B. Braun, Melsungen, Germany) into the femoral vein at a rate of 21 mL/kg × h over a period of 10 min to administer 30-μg/kg physostigmine either 10 min before starting ischemia or 10 min before starting ischemia plus 10 min before starting reperfusion. One group received subsequent to the 10-min bolus before ischemia a