Original Paper

Oral CPSI-2364 Treatment Prevents Postoperative Ileus in Swine without Impairment of Anastomotic Healing

Tim O. Vilz^a Nils Sommer^a Philip Kahl^b Dimitrios Pantelis^a Joerg C. Kalff^a
Sven Wehner^a

^aDepartment of Surgery, University of Bonn, Bonn, Germany; ^bDepartment of Pathology, University of Cologne, Cologne, Germany

Key Words

Postoperative ileus • Muscularis externa • Intestinal inflammation • Anastomotic healing

Abstract

Background: Postoperative ileus (POI) is an iatrogenic complication of abdominal surgery, mediated by a severe inflammation of the muscularis externa (ME). We demonstrated that orally applied CPSI-2364 prevents POI in rodents by blockade of p38 MAPK pathway and abrogation of NO production in macrophages. In the present experimental swine study we compared the effect of orally and intravenously administered CPSI-2364 on POI and examined CPSI-2364 effect on anastomotic healing. **Methods:** CPSI-2364 was administered preoperatively via oral or intravenous route. POI was induced by intestinal manipulation of the small bowel. ME specimens were examined by quantitative PCR for CCL2 chemokine gene expression and myeloperoxidase activity. Functional analyzes included measurement of ileal smooth-muscle *ex vivo* contractility, *in vivo* intestinal and colonic transit. Furthermore, anastomotic healing of a rectorectostomy after CPSI-2364 treatment was assessed by perianastomotic hydroxyproline concentration, a histochemically evaluated healing score and anastomotic bursting pressure (ABP). **Results:** CPSI-2364 abolished inflammation of the ME and improved postoperative smooth muscle contractility and intestinal transit independently of its application route. Hydroxyproline concentration and ABP measurement revealed no wound healing disturbances after oral or intravenous CPSI-2364 treatment whereas histological scoring demonstrated delayed anastomotic healing after intravenous treatment. **Conclusion:** CPSI-2364 effectively prevents POI in swine independently of its application route. Impairment of anastomotic healing could be observed after intravenous but not oral preoperative CPSI-2364 treatment. Subsumed, an oral preoperative administration of CPSI-2364 appears to be a safe and efficient strategy for prophylaxis of POI.

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Introduction

POI is a frequent complication after abdominal surgery [1]. The recovery of gastrointestinal function can last for days with accumulation of fluids and gas, abdominal distension, nausea and vomiting, leading to risk of aspiration and to infectious complications. POI is linked with a high economic burden with costs exceeding US\$ 1 billion per year [2].

Various pathophysiological mechanisms have been investigated and our current knowledge divides POI pathogenesis into an early neurogenic [3] and a late proinflammatory phase [4]. Activation of the latter occurs already during surgery by handling of the gastrointestinal tract (GI-tract) and is a key element in POI development [5, 6]. The inflammation is complexly orchestrated and facilitated by proinflammatory mediator release like IL-6 [7], CCL2 [8] and inhibitory neurotransmitters like nitric oxide (NO) [9]. A hallmark of POI is the infiltration of circulating monocytes and polymorphonuclear neutrophils (PMN) into the muscularis externa (ME) [5] contributing for manifestation of ME inflammation. In the initial phase, a network of resident ME macrophages was shown to play a key role in POI onset and elimination of these cells protect rodents from POI [10].

Previously we demonstrated that a single preoperative intravenous dose of the tetravalent guanlylhydrazone CNI-1493, also known as semapimod, prevents POI in rodents by macrophage-specific inhibition of the p38 mitogen-activated protein kinase (MAPK) pathways and reduction of NO liberation [11]. Macrophage specific inhibition of p38 MAPK pathway and NO release were also shown by others [12, 13]. Unfortunately, intravenous application does not only affect resident ME macrophage function, but also other macrophage populations throughout the whole organism suggesting a possible risk for systemic or distant site adverse effects. In a former study we demonstrated that an oral active mesylate salt of CNI-1493, named CPSI-2364, also prevents POI in rodents [14]. Importantly, oral administration resulted in a nearly exclusive enrichment within the intestinal wall and systemic distribution was not observed. These rodent data firstly revealed orally administered CPSI-2364 as a promising strategy for POI prevention.

It has long been accepted that macrophages facilitate wound healing by supplying growth factors, cytokines and NO and for several mediators crucial roles have been shown in rodent models of anastomotic healing [15, 16]. As we and others have shown that CNI-1493 and CPSI-2364 inhibit macrophage cytokine and NO release, the question arose if preoperative CPSI-2364 treatment for POI prophylaxis could be accompanied by adverse effects of anastomotic wound healing.

In the present study we investigated if orally and intravenously administered CPSI-2364 affect postoperative ME inflammation and POI in a standardized swine model of intestinal manipulation. In a subsequent model of rectorectostomy we analyzed CPSI-2364 effects on anastomotic wound healing within the rectum. Together, we provide several lines of evidence that oral preoperative CPSI-2364 application is an effective and safe prophylaxis of POI.

Materials and Methods

Animals

All experiments were performed with adult swine (German landrace) of mixed gender. Swine were maintained on a 12 hour light / dark cycle and fed with a rodent diet (ssniff) *ad libitum*. The animals were fasted for several hours prior to the operation.

All experiments were approved by the committee for animal experiments of North-Rhine Westphalia (LANUV) and performed in accordance with the federal law regarding the protection of animals.

Operation technique and treatment

Swine were fed preoperatively with 1mg/kg CPSI-2364 mixed with soft cheese or placebo (soft cheese alone) twice, at 14 h and 3 h before the operation. In the intravenous (iv) group, CPSI-2364 resolved in

Mannitol 1.5 % or placebo (Mannitol 1.5 %) was administered 60 minutes before laparotomy. The animals were sedated with ketamine, xylazine and atropine (10, 0.2 and 0.02 mg/kg respectively) intramuscularly and anesthesia was induced by intravenous application of midazolam (0.2 - 0.3 mg/kg), fentanyl (5 µg/kg) and pancuronium (0.06 mg/kg). After intubation anesthesia was maintained by isoflurane (1–1.5%) and oxygen (6 liters/min) inhalation with a ventilation rate of 16 - 18 breaths/min (Sulla 80V, Draeger, Germany). A single preoperative bolus of antibiotics (1 g Mezlocillin) was injected and a central venous catheter was inserted intraoperatively.

Intestinal Manipulation (IM)

After midline laparotomy the small bowel was everted and intestinally manipulated twice from the ligament of Treitz to the ileocecal junction (n=6 for Placebo- or CPSI-treatment, n=4 for Sham operated animals). No microvascular bleeding was observed as a result of the IM. For analysis of intestinal transit time we performed a small duodenotomy followed by a placement of 15 radio-opaque glass balls with a diameter of 5 mm (Worf, Mainz, Germany) in the pars descendens duodeni. For measurement of colonic transit 10 glass balls with a diameter of 7 mm were put via a small incision into the cecum. Both incisions were closed with Monocryl 4/0 (Johnson and Johnson, Norderstedt, Germany) followed by closure of the abdominal wall using running sutures of Mersilene 0 (Johnson and Johnson, Norderstedt, Germany). Intestinal transit time and colonic transit time were measured simultaneously in the same animal. Postoperative analgesia was performed with Carprofen (5 mg/kg) twice a day. Food and water were provided immediately after the animals had regained mobility after recovery from anesthesia. Sham operated animals were treated in the same way, but were not intestinally manipulated.

Twenty-four hours after closure of the abdominal wall anaesthesia was induced again as described above. 20 cm of the Ileum (starting 70 cm orally of the ileocecal valve) were resected and placed in 4°C PBS for further investigation. Swine were killed by exsanguination and the entire GI tract was removed and analyzed by X-Ray radiation for examination of intestinal and colonic transit time.

Rectal anastomoses

Placebo, oral or iv treatment with CPSI-2364 was performed as described above (n=8 for each group). After laparotomy the middle third of the rectum was transected followed by a rectorectostomy with one layer of continuous suture using 4/0 Monocryl (Johnson and Johnson, Norderstedt, Germany). None of these animals received IM.

For postoperative care, swine were brought to an animal intensive care unit and laboratory parameters were controlled at 6 h, 24 h, postoperative 2, postoperative day 4, postoperative day 6 after the operation. Postoperative analgesia was performed with Carprofen (5 mg/kg) twice a day. Food and water were provided immediately after the animals had regained mobility. Six days after rectorectostomy anesthesia was induced again as described above. The abdominal wall and the anastomotic region were inspected for wound healing disturbances or abscess formation followed by resection of the anastomosis including surrounding tissue. The tissue was placed in 4°C PBS for further investigation. Subsequently, Swine were killed using T-61 (Fa. Intervet, Unterschleißheim, Germany) intravenously.

Gene expression analyzes

Gene expression was analyzed in an isolated ileal ME specimen that was taken 70 cm orally of the cecum 24 h after operation (n=6 for Placebo- or CPSI-treatment, n=4 for Sham operated swine). Total RNA was isolated, reverse transcribed to cDNA and analyzed by quantitative PCR as described before [10]. Primers used for CCL2 amplification were (forward) 5'-ATT CTC CAG TCA CCT GCT CG-3' and (reverse) 5'-TCA AGG CTT CGG AGT TTG G-3'. The PCR reaction was performed in SYBR Green Mastermix with 10 ng cDNA for 40 cycles (95°C 15 sec, 60°C 1 minute) on an AbiPrism 7900HT. Data quantification was performed by the $\Delta\Delta$ CT method. All reagents were obtained from Applied Biosystems, Darmstadt, Germany.

Myeloperoxidase (MPO) detection

An MPO - assay of small bowel ME specimen was performed as described before (n=6 for Placebo- or CPSI-treatment, n=4 for Sham operated swine) [17]. MPO activity was calculated as µU/mg protein.

In vitro contractility

In vitro contractility of circular ME strips (harvested 70 cm orally of the ileocecal valve) was measured 24 h after IM as described before [18, 19]. Briefly, mucosa-free ME strips (n=6 muscle strips per animal) were equilibrated in a standard Krebs-Henseleit buffer perfused organ chambers at 37°C and underwent alternating stimulation with increasing doses of bethanechol (1–100 mol/L) and wash periods with buffer (group size was n=6 for placebo- or CPSI-treatment and n=4 for Sham-operated swine). The contractile response was recorded and analyzed with ChartPro Software (ADInstruments, Heidelberg, Germany) and calculated as g/mm²/s.

Intestinal and colonic transit

Intestinal and colonic transit were analyzed simultaneously 24h after IM by X-Ray detection of intraoperatively administered 5 or 7 mm radio-opaque glass beads, respectively. The 5 mm markers were counted in 22 equally sized segments (stomach, 10 small bowel segments, cecum and 10 large bowel segments). Colonic transit was measured by distribution of the 7mm beads within in the cecum and 10 large bowel segments. In both transit examinations we investigated n=6 animals using Placebo- or CPSI-treatment and n=4 in the Sham group. The geometric center (GC) was calculated using the following formula: $GC = \Sigma (\% \text{ of total glass balls per segment} \times \text{segment number}) / 100$. The data were expressed as the percentage of glass balls per segment and plotted in a median histogram

Hydroxyproline concentration in anastomotic tissue

For analysis of hydroxyproline concentration (HPC) a 2 x 2 cm measuring piece of anastomotic tissue was chosen from each swine (n=8 for each group). HPC was quantified as described previously [20, 21] and calculated using the following formula: [absorption of sample / coefficient of the standard curve] x 666.7 = µg hydroxyproline / g bowel.

Histochemical analysis

Anastomotic segments were dissected 6 days after surgery, fixed in 4% paraformaldehyde, embedded in paraffin and 5µm sections were stained with hematoxylin–eosin (n=8 in each group). Microscopical analysis was conducted by a blinded pathologist and anastomotic healing score was calculated using a method modified from Biert et al. [22]. A score from 1 to 5 was assigned for mucosal continuity, muscular continuity, re-epithelisation, and granular tissue formation (1 = none, 2 = poor, 3 = moderate, 4 = good, 5 = excellent and completely). The score was calculated from the sum of the individual scores assigned to each of the parameters assessed.

Anastomotic bursting pressure (ABP)

ABP of the rectorectostomy was measured immediately after the animals (n=8 in each group) were killed. A 15 cm segment of the colon including the anastomosis was resected and carefully cleared of fecal contamination. The proximal end was ligated twice using a Mersilene 0 suture (Johnson and Johnson, Norderstedt, Germany). An endotracheal tube (10 CH) was inserted intraluminally within the rectum at the distal end and 2 stay sutures (Mersilene 0) were tied to prevent leakage. The other end of the tube was connected to an oxygen port in the wall and placed in a water bath. Oxygen was infused at a constant rate of 6 l/min. The intraluminal pressure (mmHg) was measured and recorded using a pressure transducer with an amplifier and analyzed using the Biopac A/D systems (Acknowledge_ Software; Biopac Systems, Santa Barbara, CA). ABP was indicated as a sudden loss of pressure or sudden outlet of bubbles and defined as the maximum intraluminal pressure prior to leakage.

Drugs and Solutions

PBS was purchased from Lonza (Verviers, Belgium). All other chemicals used for this study were purchased from Sigma Aldrich (Taufkirchen, Germany). CPSI-2364 was kindly provided by Cytokine PharmaSciences, Inc. (King of Prussia, USA).

Data analysis

Statistical analysis was performed with Prism software (GraphPad, CA, USA) using 1-way ANOVA followed by a Bonferroni's posttest or an unpaired t-test (analysis of smooth muscle contractility). Box and whiskers plots indicate mean ± SD and the range from minimum to maximum.

Fig. 1. IM-induced increase in CCL2 gene expression and MPO activity is abolished by CPSI-2364 treatment. (A) Preoperative oral and iv CPSI-2364 application resulted in a significant reduction of CCL2 transcripts within the ME compared to the placebo-treated group 24 h after IM (** $p < 0.01$, *** $p < 0.001$, $n = 4$ for sham, $n = 6-8$ for IM groups). (B) MPO activity within the ME is significantly increased 24 h after IM in placebo-treated but not in orally or intravenously CPSI-2364 treated swine (** $p < 0.01$, $n = 4$ for sham, $n = 6$ for IM groups).

Results

In the present study we analyzed the influence of CPSI-2364 on the development of POI in a swine model. In a subsequent study CPSI-2364 effects on anastomotic healing were analyzed after partial rectal resection and rectorectostomy.

POI study

CPSI-2364 ameliorates chemokine transcription in swine ME. As shown in rodent models before, POI is based on extravasation of circulating monocytes in a CCL2 dependent manner [10]. In our swine model, CCL2 transcripts were upregulated in placebo-treated (8.8 fold $\pm$ 3.3) swine 24 h after IM. After CPSI-2364 treatment, CCL2 mRNA levels (oral: 2.8 fold $\pm$ 1.1; iv: 3.1 fold $\pm$ 1.9) were comparable to sham-operated controls (1.8 fold $\pm$ 1.6) (Fig. 1A).

CPSI-2364 reduces PMN infiltration. Next, we investigated whether CPSI-2364 affects IM-induced leukocyte extravasation into small bowel ME by indirect measurement of MPO activity in the ileum. In sham-operated swine, a basal MPO activity of 102.3 $\pm$ 48.6 μ U/mg tissue was measured 24 h after laparotomy (Fig. 1B). Oral (102.9 $\pm$ 38.3 μ U/mg) or intravenous (123.8 $\pm$ 41.5 μ U/mg) CPSI-2364 treatment significantly prevented IM-induced MPO activity increase compared to placebo treated swine (254.2 $\pm$ 87.7 μ U/mg).

CPSI-2364 diminishes IM-induced smooth muscle dysfunction. In a set of functional experiments we next measured contractile activity of isolated ileal circular smooth muscle strips in an *in vitro* organ bath setting and GI motility *in vivo*. Ileal muscle strips demonstrated an impaired contractility to increasing bethanechol between IM and sham-operated swine after IM. (Fig. 2) (1 μ M: 0.04 $\pm$ 0.01 vs. 0.18 $\pm$ 0.07 g/mm²/s; 3 μ M: 0.15 $\pm$ 0.03 vs. 0.53 $\pm$ 0.18 g/mm²/s; 10 μ M: 0.42 $\pm$ 0.09 vs. 0.92 $\pm$ 0.19; 30 μ M: 1.1 $\pm$ 0.16 vs. 2.3 $\pm$ 0.51; 100 μ M:

Fig. 2. *In vitro* measurement of spontaneous and bethanechol-induced contractility of ileal circular ME strips 24h after operation. Bethanechol-induced contractility was significantly reduced in the IM+Placebo group at concentrations from 1 μ M to 100 μ M compared to sham operated animals. ME strips from CPSI-2364-treated animals demonstrated an improved contractility after IM at 10 μ M to 100 μ M compared to IM+Placebo. Data are expressed as mean $\pm$ SEM. Sham vs IM+Placebo: * p <0.05 and ** p <0.01; IM+CPSI po vs. IM+Placebo; + p <0.05 and ++ p <0.01; IM + CPSI iv vs IM+Placebo x p <0.05. n = 4 for sham and n =6 for IM groups.

1.55 $\pm$ 0.18 vs. 3.16 $\pm$ 0.73). Spontaneous contractions did not differ between all groups. Oral CPSI-2364 treatment abrogated IM-induced contractility disturbances at 10 μ M (1.33 $\pm$ 0.21 g/mm²/s), 30 μ M (2.14 $\pm$ 0.43) and 100 μ M (2.67 $\pm$ 0.49) bethanechol. Intravenous application of CPSI-2364 led to an improved contractility compared to placebo treated swine at 10 μ M (1.16 $\pm$ 0.22), 30 μ M (1.76 $\pm$ 0.17) and 100 μ M (2.29 $\pm$ 0.22). Differences between orally or iv treated CPSI-2364 groups were not detected.

In vivo intestinal transit and colonic transit were analyzed simultaneously within the same swine via X-ray 24 h after surgery. In sham-operated placebo treated swine, all intestinal transit markers were distributed within the cecum and the proximal and middle colon (Fig. 3A+E; GC: 11.6 $\pm$ 1.0). IM showed a trend to delayed intestinal transit (GC: 7.7 $\pm$ 4.3) with most of the markers located within the distal small bowel (Fig. 3B+E) compared to sham-operated controls. Oral (GC 13.0 $\pm$ 0.7) and iv (GC 14.0 $\pm$ 1.1) CPSI-2364 treatment resulted in an enhanced GIT (Fig. 3C-E) compared to sham-operated and placebo-treated swine with most markers localized in the proximal colon. Clinical observation demonstrated a distended small bowel (which is a typical clinical sign for manifest POI) in placebo but not CPSI-2364 treated swine (Fig. 4 A+B, arrows). Distribution of the colonic markers (Fig. 3A-D; 3F) demonstrated no significant differences between the placebo-treated IM group (GC: 1.9 $\pm$ 0.8) and sham-operated controls (GC: 3.5 $\pm$ 1.7). Colonic transit in orally CPSI-2364 treated swine showed a trend to accelerated colonic transit (GC: 2.9 $\pm$ 0.6) while intravenous treatment resulted in significantly enhanced colonic transit time (GC 3.6 $\pm$ 1.4).

Anastomotic healing study

Presence and function of macrophages is critical in anastomotic healing processes [16]. As we demonstrated before that CPSI-2364 affects postoperative macrophage cytokine and NO production in a rodent model of POI [14] we consequently analyzed intestinal

Fig. 3. Effects of CPSI-2364 on intestinal transit and colonic transit in swine. Swine underwent preoperative placebo or oral or iv treatment with CPSI-2364 before IM. At the end of IM, radio-opaque markers of different diameter were administered intraduodenally (5mm, thin arrows) or into the cecum (7mm, thick arrows) and X-ray analyses of the GI-tract was performed 24h after surgery. Representative X-ray analysis from (A) sham-operated animals and intestinally manipulated swine 24h after (B) placebo or (C) oral or (D) intravenous CPSI2364 treatment. (E) Calculated geometric centers of intraduodenally applied marker distribution along the GI tract. $**p < 0.01$ vs. IM + Placebo (F) Calculated geometric centers of intracecally administered colonic transit markers. $*p < 0.05$ vs. IM + Placebo, $n = 4$ for sham, $n = 6$ for IM groups.

anastomotic healing after oral and iv administration of CPSI-2364 in swine. We decided to investigate anastomoses after a partial colon resection and rectorectostomy because leakage could be observed more often in the rectum than in the small bowel or colon. Additionally we considered postoperative day 6 for analyzes because anastomotic insufficiency is clinically often visible between postoperative days 5 to 7.

Fig. 4. CPSI-2364 prevents postoperative ileus in swine. Swine received preoperative placebo (A) or CPSI-2364 po or iv (B) and underwent IM procedure. After 24h an abdominal inspection was performed. Pictures are representative images from n=6 animals per groups.

Fig. 5. Preoperative treatment with CPSI-2364 does not influence perianastomotic hydroxyproline concentration and anastomotic bursting pressure. Swine underwent partial colon resection and rectorectostomy after placebo or oral or iv CPSI2364 treatment. (A) Hydroxyproline concentration (n=8 per group) and (B) anastomotic bursting pressure (n=8 per group) were analyzed 6 days after surgery. n=8 per group.

Clinical observation and intraoperative examination

All pigs completed the study with no major signs of illness. On postoperative day 6 swine underwent a relaparotomy. The abdominal wall, the abdominal cavity and the rectorectostomy were examined for wound healing disturbances (abscess formation, peritonitis or anastomotic leakage). Ultimately, none of the pigs showed any abnormalities in healing of the abdominal wall or the anastomoses, no adhesions were observed.

Anastomotic healing

It is well known that unaltered anastomotic healing strongly depends on regular macrophage function. As we could describe before CPSI-2364 temporarily suppresses macrophage activity in the small bowel ME. Therefore we analyzed submucosal collagen content which is crucial for anastomotic strength [23] by hydroxyproline measurement. HPC did not differ between sham-operated animals and operated swine treated with placebo (2794 ± 529 µg/g tissue), oral (2981 ± 396 µg/g tissue) or intravenous CPSI-2364 (2518 ± 276 µg/g tissue) (Fig. 5A).

Additionally, mechanical strength of anastomoses (measured as ABP) was analyzed *ex vivo*. ABP was indicated as a sudden loss of pressure and defined as the maximum intraluminal

Fig. 6. Intravenous CPSI-2364 treatment impairs anastomotic healing. (A) Histopathological changes of the anastomotic region were graded 6 days after partial colon resection and rectorectostomy in CPSI-2364- and placebo-treated swine (** $p < 0.01$, $n = 8$ per group). (B-D). Representative HE sections of paraffin-embedded swine rectorectostomies on postoperative day 6. Swine underwent preoperative (B) placebo, (C) CPSI-2364 per os or (D) CPSI-2364 iv. Mu: Mucosa, M: Muscularis, Gr: granulation tissue, R: re-epithelisation zone.

pressure prior to leakage. Preoperative oral or iv administration of CPSI-2364 did not impair ABP compared to placebo-treated swine (Fig. 5B: Placebo: 86 ± 20 mmHg, CPSI 2364 oral: 94 ± 18 , CPSI 2364 iv: 113 ± 22).

Finally, histopathological analyses were performed to determine whether CPSI-2364 treatment affects anastomotic wound healing (Fig. 6A). On postoperative day 6 no differences were observed between the healing scores of oral CPSI-2364 (13.9 ± 2.5) or placebo (13.7 ± 2.6) treatment. However, a delayed healing with incomplete re-epithelisation of the mucosa was observed within the intravenously treated CPSI-2364 group (Fig. 6B-D) leading to a significantly reduced healing score (9.5 ± 2).

Complications after CPSI-2364 treatment

In the oral CPSI-2364 treated group no adverse events could be observed. After intravenous administration of CPSI-2364 an anaphylactic shock with rash, hypotension and bronchospasm occurred once. Using iv application of corticosteroids and histamine-receptor blockers the symptoms disappeared rapidly.

Laboratory parameters (hemogram, electrolytes, liver and pancreatic enzymes, kidney values, coagulation factors) were checked at postoperative time points (6 h, 24 h, postoperative day 2, 4 and 6) without any conspicuities (data not shown).

Discussion

It is widely accepted that POI originates from a post-traumatic inflammation of the ME, initiated by surgical manipulation of the intestine [5-8, 18]. Currently, there is no prevention or evidence-based treatment of postoperative motility disorders. In the present swine study we demonstrate that the tetravalent guanyldiazide CPSI-2364 prevents postoperative ME inflammation and subsequent POI after preoperative intravenous and oral application. Furthermore, we provide several lines of evidence that oral CPSI-2364 does not disturb

anastomotic wound healing after a rectorectostomy facing it out as a promising and safe option for prophylaxis of POI also in humans.

The clinically relevant prolonged form of POI originates from an inflammatory response within the ME induced by the surgical handling of the GI - tract during abdominal operation. Resident macrophages of the ME [10] have been shown to be the cellular key players in the onset of this inflammation. Once the local inflammation is induced it can spread along the entire GI tract [24] resulting in a panenteric dysmotility. In previous studies we have shown that elimination of the resident ME macrophages prevents POI in rodents [10]. As macrophage elimination is clinically contraindicated we investigated if a macrophage specific temporarily inactivation also prevents POI in rodents. Successfully, the tetravalent guanylylhydrazone semapimod was identified to be effective in POI prevention. In this former study we used a chloride salt of semapimod, known as CNI-1493 which effectively prevented POI in mice partially via inactivation of peripheral macrophages and abrogation of macrophage-derived NO production [11]. Intravenous CNI-1493 was shown to reach brain levels high enough to centrally activate vagus nerve signaling which is known to fulfill antiinflammatory response in peripheral tissues, like the peritoneal cavity [25] and the GI tract [11]. The et al. further demonstrated that intracerebroventricular application of CNI-1493 also ameliorates POI [26] confirming its central mode of action next to the peripheral macrophage-inhibiting capacity. Importantly, effectiveness in POI prevention does not depend on simultaneous action of both, central vagus activating and peripheral macrophage inhibition, because intravenous application of CNI-1493 also prevents POI in vagotomized mice [14]. Although intravenous CNI-1493 was effective in POI prevention, systemic distribution and inactivation of resident macrophage population in other organs could be critical in a clinical setting.

CPSI-2364 is a mesylate salt form that, in contrast to CNI-1493, demonstrates oral bioactivity. In a previous study we demonstrated that oral CPSI-2364 prevents POI in mice. Importantly, oral administration leads to a near inclusive enrichment within the gastrointestinal wall and systemic distribution was not observed [14]. This indicates a lower risk of CPSI-2364 induced side effects. However, there is growing awareness that a disparity between mouse models of inflammatory diseases and their human counterparts exist [27]. Therefore and with regard to a future clinical use, we tested our previous findings in the present large animal model. We chose swine because of its similarity in wound healing processes and POI development compared to human [19, 28, 29]. Indeed, oral CPSI-2364 reduced postoperative inflammation in swine. This was shown by transcript reduction of the chemokine CCL2 that is crucial for leukocyte infiltration into the ME during POI [10] and a decrease of MPO activity (as a marker of PMN migration into the ME) after CPSI-2364 treatment. This reduction coincides with the improvement of smooth muscle contractility and an acceleration of intestinal transit.

Previous rodent studies demonstrated that surgical manipulation of the small bowel leads to dissemination of the inflammation to unmanipulated part of GI-tract followed by decreased motility. [24] Interestingly, the transit time of the unmanipulated colon shows a clear trend but no significant reduction in manipulated swine compared to sham operated animals. The missing statistical significance is probably based on both, a distinct degree of inflammation in sham-operated animals (who also received a laparotomy with two enterotomies for transit marker application) and the smaller group size in the sham-operated group. Nevertheless, intravenous CPSI-2364 application demonstrated significant improvement of colonic transit, indicating that CPSI-2364 also prevents dissemination of the ME inflammation. Subsumed, our former rodent and the present swine data revealed the preoperative prevention of macrophage activation by CPSI-2364 as a promising strategy for POI prophylaxis.

However, it is well known that immunocytes, particularly macrophages, facilitate wound healing processes in the GI-tract [16, 30, 31] and express many mediators like NO [15] and cytokines [32] which are important for the anastomotic repair process. Wound healing disturbances may lead to leakage of intestinal anastomoses resulting in high perioperative

morbidity and mortality. As CPSI-2364 acts via reduction of macrophage p38-MAPK-dependent cytokine and NO liberation, we analyzed its effect on anastomotic wound healing in a rectorectostomy swine model. We chose anastomoses of the rectum because a higher incidence of leakages can be observed after rectorectostomy than in small bowel or colonic anastomoses. Although hydroxyproline concentration, a component of collagen that in turn mediates tensile strength of the anastomosis [23] and ABP did not differ among application route and presence of CPSI-2364, scoring of paraffin-embedded anastomoses linked the intravenous application with a significant reduced anastomotic healing. However, neither wound healing disturbances nor abscess formation were observed during relaparotomy. The delayed wound healing in the iv group may be explained by the systemic distribution of CPSI-2364 leading to an inactivation of circulating monocytes/macrophages that extravasate into the anastomosis and beneficially affect the healing process. As oral CPSI-2364 is nearly exclusively found within the GI wall, but not in the circulation, inactivation of circulating monocytes/macrophages is prevented [14].

In conclusion, this study demonstrates that preoperative oral and intravenous application of CPSI-2364 prevents POI in swine. Intravenous, but not oral application led to a delayed anastomotic healing after rectorectostomy. These findings point out oral CPSI-2364 treatment as a safe and promising strategy in POI prophylaxis.

Abbreviations

ME (muscularis externa); KHB (krebs-henseleit-buffer); GI (gastrointestinal); POI (postoperative ileus); PMN (polymorphonuclear neutrophils); p38-MAPK (p38 mitogen activated protein kinase); NO (nitric oxide); IM (intestinal manipulation).

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