The use of wireless capsule enteroscopy in a preclinical study: a novel diagnostic tool for indomethacin-induced gastrointestinal injury in experimental pigs

Jaroslav Květina [1], Martin Kuneš [1], Jan Bureš [2], Marcela Kopáčová [2], Ilja Tachecí [2], Stanislav Špelda [1], Vladimír Herout [1], Stanislav Rejchrt [2]

1. Institute of Experimental Biopharmaceutics, Joint Research Centre of the Czech Academy of Science and PRO.MED.CS Praha a.s., Hradec Kralove, Czech Republic
2. 2nd Department of Medicine, Charles University in Prague, Faculty of Medicine in Hradec Králové, University Teaching Hospital, Hradec Kralove, Czech Republic

Correspondence to: Prof. M.A. Jaroslav Květina, D.Sc.
Institute of Experimental Biopharmaceutics, Joint Research Centre of PRO.MED.CS Praha a.s. and the Czech Academy of Sciences,
Heyrovskeho 1207, Hradec Kralove, Czech Republic
TEL.: +420 495 514 771, FAX: +420 495 512 719
E-MAIL: kvetina@uebf.cas.cz

Submitted: 2008-07-08 Accepted: 2008-09-09

Key words: **indomethacin; gastrointestinal lesions; experimental pig; microcamera; optical-microscopy; confocal laser microscopy**

Neuroendocrinol Lett 2008; **29**(5):763–769 **PMID:** 18987574 NEL290508A05

Abstract

OBJECTIVES: The aim was the utilization of capsule microscopy and other diagnostic techniques for prospective pre-clinical research of absorption and biotransformation mechanisms of xenobiotics in the intestinal wall after induction of gastrointestinal dysfunction. Consequently, there is a demonstration of the extrems of gastrointestinal lesions development induced with indomethacin as a representative of non-steroidal anti-inflammatory drug.

METHODS: The experimental animal species were small adult pigs (n=10; body weight 30–35 kg; 4–5 months old) used for their relative physiological and metabolic resemblance to man. The following experimental methods were used for diagnostic verification of gastrointestinal lesions (damage scale: 1 – erosions, red spots, inflammatory infiltration, 2 – single ulcers, 3 – strings of ulcers): endoscopic image for the diagnostics of gastro-duodenal segment (*in vivo* conditions), confocal laser microscopy (*ex vivo* imaging) and optical light microscopy (*in vitro*), small intestinal imaging by means of wireless capsule enteroscopy (*in vivo*), macroscopic findings and optical light microscopy (after animal sacrifice).

RESULTS: The mutual confrontation of used methodological approaches proved the conformity in the frequency and extent of damage in the gastric wall and caecum, partly in the duodenal wall and terminal ileum. The signs of first-degree damage were discovered in the jejunal-ileal segment.

CONCLUSIONS: The scale of lesions in particular gastrointestinal segments was verified using the combination of five diagnostic techniques for prospective utilisation of non-invasive capsule enteroscopy for the through-control of mucosal state.

ORIGINAL ARTICLE

INTRODUCTION

Gastrointestinal permeability changes, inflammation and ulceration are frequently associated with ingestion of non-steroidal anti-inflammatory drugs (NSAIDs) (Vane & Botting, 1995). The lesions induced this way (ulceration and haemorrhage in concrete) occurs namely in the gastroduodenal region. Conventional NSAIDs have been reported to change permeability and cause inflammation also in the intestinal segments (Halter *et al*. 1996). This view is supported by observations of NSAIDs caused mucosal lesions in the jejunoileal region of rats (Anthony *et al*. 1994; Billingham & Tucker, 1979; Nygard *et al*. 1994; Rainsford, 1988). This fact may influence the intestinal absorption and the enterohepatic circulation of orally administered drugs. There are not many reports about the influence of injured intestinal wall on the transport of xenobiotics. For our experiments pigs were chosen since they closely resemble to humans in respect to anatomy and physiological functions in the gastrointestinal (GI) tract (Rainsford *et al*. 2003). The next open question is whether different degrees of GI damage are expressed uniformly on the quality of transintestinal transport of drugs or differently in case of drugs transported by mechanism of lipoid diffusion or active carrier mechanism. For example, the papers published previously (Hradil *et al*. 1978; Fendrich *et al*. 1970; Fendrich & Květina, 1979; Květina *et al*. 1984) demonstrated that malabsorption syndrome (induced with whole-body irradiation or with methotrexate administration) increased the transport in case of diffusion in the rat. On the other hand, in active carrier transport the absorption was decreased. There was also a decrease in cellularity of the intestinal mucosa in rats altered in such a way (Květina & Pařízek, 1966). Further a systematic study should elucidate the influences of intestinal dysfunctions on biotransformation activity of the intestinal wall.

The set designed pre-clinical studies (directed on pig as an animal representative metabolically close to man) is aimed on the prediction of changes in transintestinal transport of xenobiotics with different absorption mechanisms and with different metabolism in the intestine. The first experimental phase (this paper) is standardisation of induced GI pathological changes using combined morphological techniques (Rey *et al*. 2006; Goetz *et al*. 2007; Goetz *et al*. 2008).

MATERIAL AND METHODS

Animals

Ten mature female pigs (Sus scrofa f. domestica), hybrids of Czech White and Landrace breeds, weighing 30–35 kg of body weight (4–5 months old), were used in the study. They were kept in air-conditioned rooms (22±2 °C and 50±10% relative humidity, with lights from 7 a.m. to 7 p.m.), fed twice a day (standard food A1; Cerea a.s., Czech Republic) and with access to water *ad libitum*.

Gastrointestinal lesions induction

Gastrointestinal lesions were induced by the utilisation of "indomethacin adverse effect" (resulting in blockade of the oxygenous transformation of arachidonic acid on prostanoids):

10-day indomethacin administration (10 mg/kg/day) as one-shot dietary bolus to greedy pigs.

Diagnostic conditions

Eight transmission antennae were fixed on the skin in abdominal parts of animals kept in general anaesthesia using intramuscular injection of 20 mg ketamine (Narkamon, Spofa, Praha, Czech Republic) and 20 mg azaperone (Stresnil, Janssen-Pharmaceutica, Beerse, Belgium) as an introduction, continued by infusion of 1% thiopental (Thiopental Valeant, Valeant Czech Pharma, Czech Republic). Infusions of 0.9% saline solu-

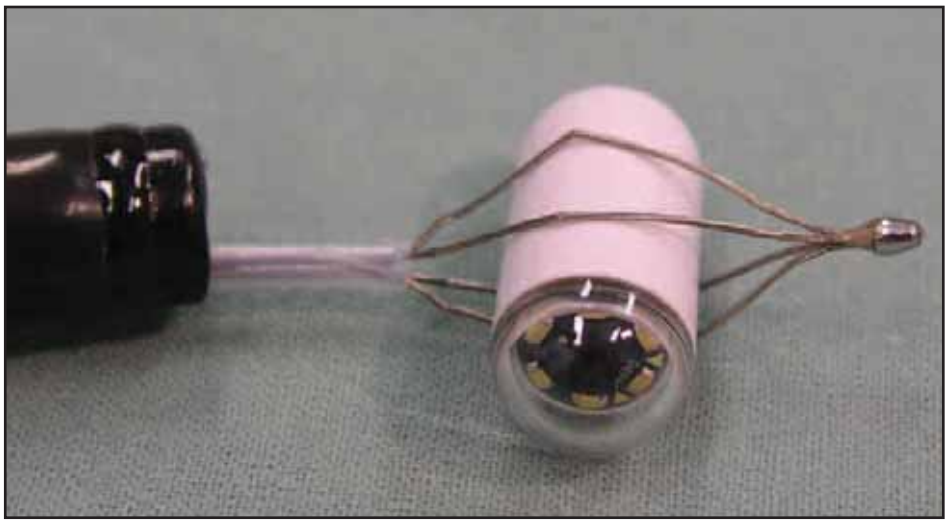

Figure1. Endoscopy capsule grasped into a basket. The basket is inserted through the endoscopic working channel.

tion were given to secure basal hydration (1000 mL per 8 hours). All animals were covered by blankets to prevent hypothermia during capsule enteroscopy.

Diagnostic techniques

A) Video capsule enteroscopy (Keuchel et al. 2006):
The wireless capsule endoscope is a disposable capsule with an outer diameter of 11 mm, length of 26 mm and weighing 3.8 g (Figure 1). It moves through the small bowel, propelled by peristalsis and transmits data to a portable data recorder. It provides direct colour video images of the intestinal mucosa at a rate of 2 images per second for approximately 8 hours. Wireless capsule endoscopes were introduced into the duodenum by means of standard video-gastroscope. The system of EndoCapsule (Olympus Optical Co, Tokyo, Japan) was used for video-capsule enteroscopies in all animals. The system provides direct colour video images of the GI mucosa and consists of four components: (a) capsule endoscope, (b) recorder unit, (c) real time viewer, and (d) integrated workstation containing the proprietary application software. The capsule is naturally passed through the intestine and subsequently is excreted in faeces.

B) Endoscopic scanning of the gastric surface through the endoscope during the microcamera introducing. Endoscopy procedures were performed using video-gastroscope GIF-Q130 (Olympus Optical Co, Tokyo, Japan) designed for animal use only. All endoscopies were video-recorded.

C) Confocal laser microscopy of the gastric wall samples collected at the end of the microcamera scanning; 10 min. after the intravenous injection of the diagnostic fluoresceine (500 mg/animal) and following pharmacological euthanasia (an intravenous injection of embutramide, mebezonium iodide and tetracaine hydrochloride in mixture; T61, Intervet Int. BV, Boxmeer, the Netherlands; dose of 2 mL per kg) and exsanguination. Confocal laser endomicroscopy on an *ex vivo in vitro* basis was carried out within 20 minutes after the tissue samples collection. Methods of this imaging were described elsewhere (Kopáčová *et al*. in press), briefly: video endomicroscopy was performed by means of Confocal laser endomicroscopy system Pentax (Tokyo, Japan)/Optiscan (Notting Hill, Australia). It consists of a standard video colonoscope (EC3870K) with miniaturised confocal microscope on its tip, processor (EPK-100) and laser system (ISC OU1000). To prevent direct contact of the tip of video-endoscope and confocal microscope with porcine tissue we used a semipermeable membrane Dialysis tubing visking (Carl Roth, Karlsruhe, Germany). Both tissue and membrane were flushed by a saline solution during investigation.

D) Gross observation of the gastrointestinal wall during tissue collection and preparation. The photos were taken using a camera (Konica Minolta, Dimage Z5, Tokyo, Japan) and saved as ".jpg" file in the computer.

E) Optical light microscopy of the gastrointestinal wall samples collected post-mortem. The specimens were then fixed in 10% neutral buffered formaldehyde, embedded in paraffin, sectioned (5μm) and stained with hematoxylin eosin and periodic acid Schiff. The histological preparations were evaluated using a calibrated micrometer (optical zoom 010 x M10).

Evaluative criteria (scale of damage)

I – erosions, red spots, inflammatory infiltration;
II – single ulcers;
III – strings of ulcers

Ethics

The study was approved by the Institutional Review Board of Animal Care Committee of the Institute of Experimental Biopharmaceutics, the Czech Academy of Sciences. Animals were held and treated in accordance with the European Convention for The Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Council of Europe 1986).

RESULTS

After 10-day indomethacin administration differently graded – morphologically detected – damage was observed in different segments of the gastrointestinal tract in all animals . The findings using the diagnostic methods described are summarised and documented in Table 1 and in Figures 2–6.

A)
Small intestinal imaging by means of wireless capsule enteroscopy show the small erosions, erythema and petechie in the jejunum and small ulcer in the pylorus [Figures 2A), 2B), 2C), 2D)].

B)
Erosions and ulcers observed with endoscope in the stomach [Figures 3A), 3B)]

C)
The confocal laser microscopy display the ulcerative tissue in the stomach in comparison to normal gastric wall [Figures 4A), 4B)].

D)
Erosions and ulcers in stomach and ulceration in caecum after gross tissue observations [Figures 5A), 5B), 5C), 5D)].

E)
Mucosal abnormalities, especially in the stomach and in distal parts of the small intestine using optical light microscopy [Figs. 6A), 6B), 6C), 6D)].

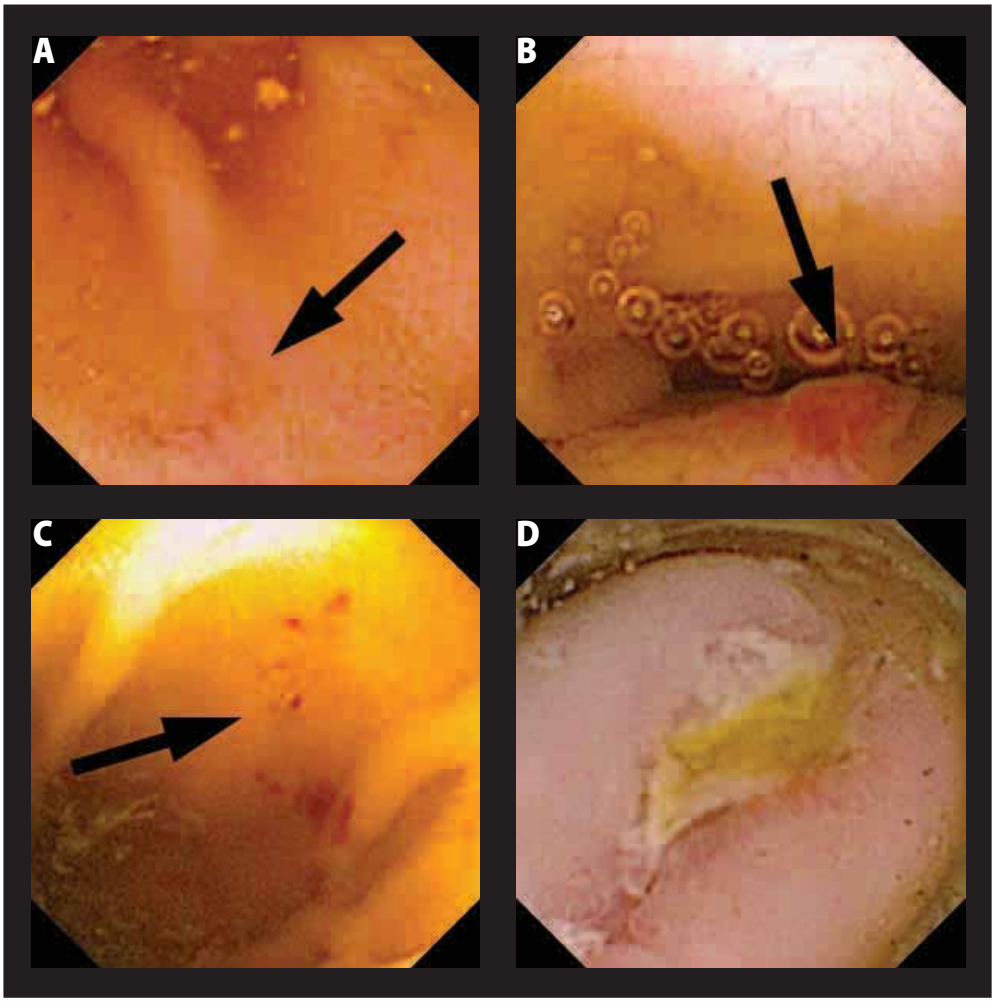

Figure 2. A) Small erosion in the proximal jejunum – arrow. Capsule endoscopy. (Diameter of image 2 cm).
B) Focal mucosal erythema in the jejunum-arrow. Capsule endoscopy. (Diameter of image 2 cm).
C) Multiple petechie of the jejunum - arrow. Capsule endoscopy. (Diameter of image 2 cm).
D) Small ulcer of the pylorus, base is covered with fibrin. Capsule endoscopy. (Diameter of image 2 cm).

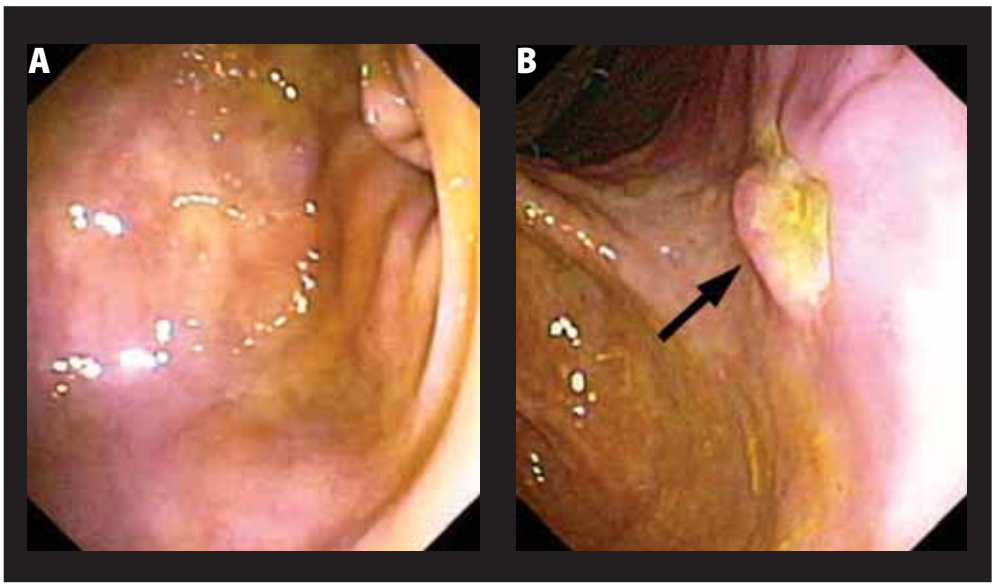

Figure 3. A) Normal gastric mucosa in the antrum. Gastroscopy.
B) Deep ulcer in the distal part of gastric body, base is covered with fibrin Gastroscopy.

The most pronounced pathological findings were observed in the gastric wall and caecum. The gastric mucosal lesions were demonstrated in all animals, covering the range from erosions to ulcer string. The erosions in approximately 50% of animals and single ulcers in approximately 20–30% of animals were found in the duodenal segment, while the variously fusing erosions in the proximal parts of the jejunum. No changes were found in the jejunal-ileal part, the erosions and solitary ulcers were manifested in several animals in the terminal ileum; the changes (erosions or ulcers, even a perforating ulcer – in one pig) were observed in all animals.

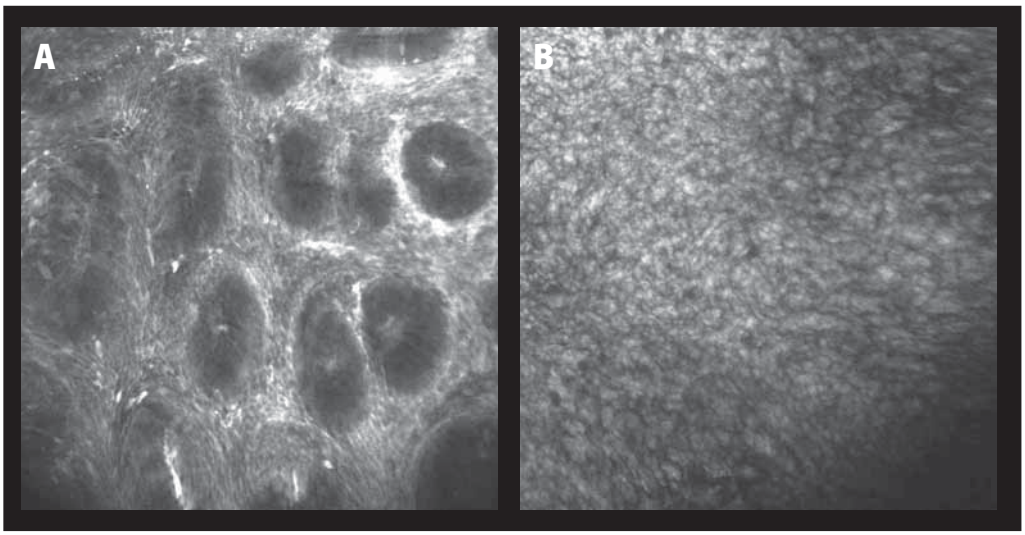

Figure 4. A) Confocal laser endomicroscopy of the normal gastric mucosa. Foveolae gastricae are clearly visible. (Magnification 1000×). **B)** Confocal laser endomicroscopy of the gastric ulcer. (Magnification 1000×).

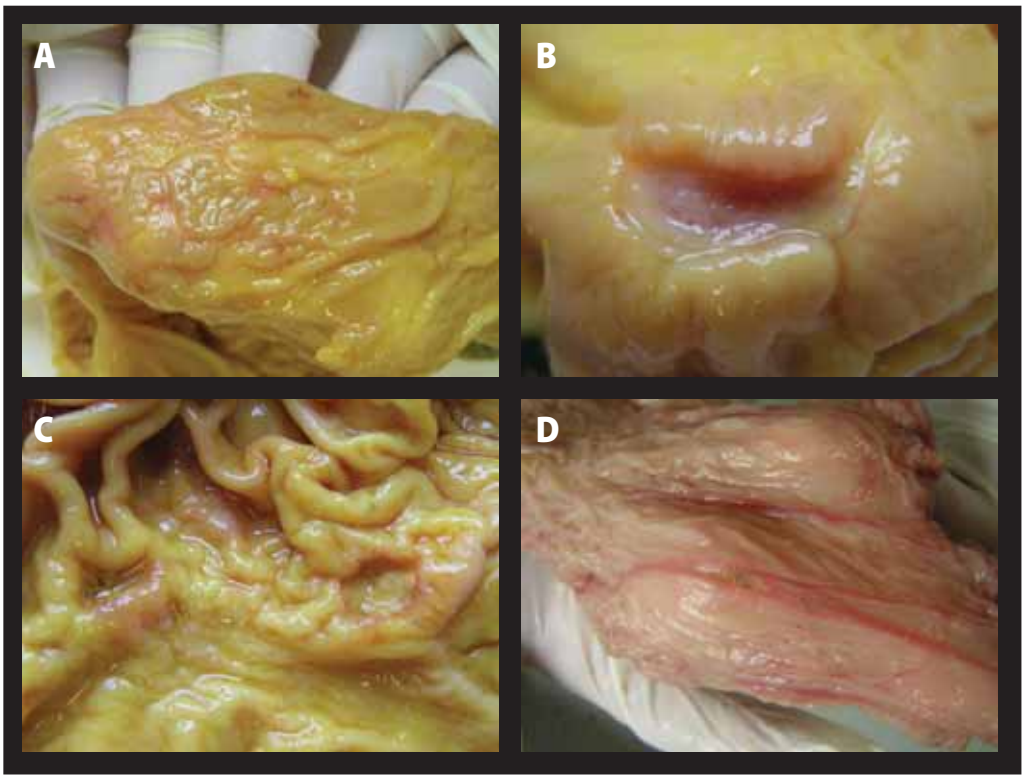

Figure 5. A) Erosions in the stomach. Gross observation. **B)** Large and deep ulcer in the stomach. Gross observation. **C)** Several ulcers. Gross observation. **D)** Filiform ulceration in the caecum. Gross observation.

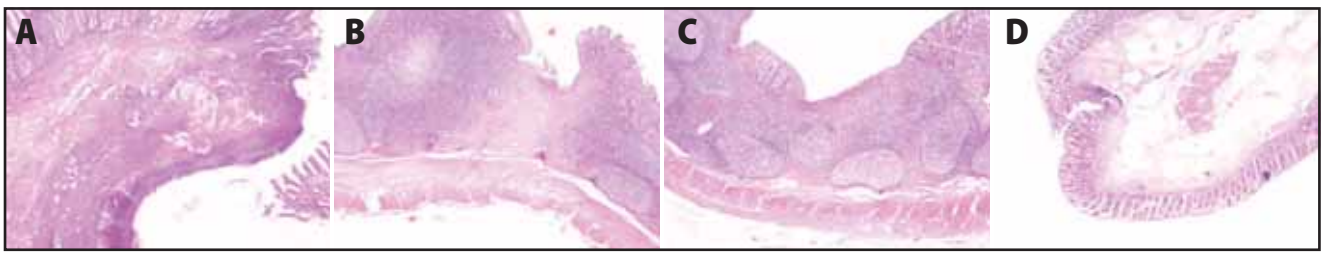

Figure 6. A) Ulcer in the stomach. **B)** Ulcer in the ileum. **C)** Erosion in the caecum. **D)** Ulcer in the caecum. Optical light microscopy. (Magnification 100×).

Table 1. The occurence and severity of indomethacin-induced lesions

Organ	Grade	Gross observation	Gastroscopy	Capsule enteroscopy	Microscopy
Stomach	I	8/10	6/7	–	10/10
	II	10/10	7/7	–	8/10
	III	6/10	5/7	–	3/10
Duodenum	I	5/10	–	5/7	5/10
	II	3/10	–	1/7	2/10
	III	0/10	–	0/7	0/10
Jejunum	I	5/10	–	5/7	8/10
	II	0/10	–	0/7	0/10
	III	0/10	–	0/7	0/10
Ileum	I	2/10	–	3/7	5/10
	II	2/10	–	0/7	0/10
	III	0/10	–	0/7	0/10
Caecum	I	8/10	–	2/7	5/10
	II	3/10	–	2/7	2/10
	III	2/10	–	0/7	1/10

(Optical microscopy and Gros observation, n=10; Gastroscopy and Capsule enteroscopy, n=7)
Data are numbers of animals with lesions / animals investigated.
Severity of lesions: Grade I – erosions, red spots, inflammatory infiltration; Grade II – individual ulcers; Grade III – strings of ulcers.

DISCUSSION

Despite numerous clinical reports about GI lesions induced with NSAIDs, there are rare similar findings in experimental species (especially in pigs). The minipig or pig (without overweight) as an omnivorous representative has been pharmacologically declared as a suitable experimental animal species to obtain this piece of knowledge relevant for man. The results of the presented work can be confronted with similar experimental design of the paper published by Rainsford *et al.* (2003). The aim of their research was to compare the gastrointestinal impact after three NSAIDs representatives medication (oxygenase non-selective inhibitors: aspirine, naproxen, indomethacin). The macroscopic and optical-macroscopic image of the presence and size of GI lesions and of myeloperoxidase activity in the damaged areas were the main criteria evaluated. Results of this cited paper showed that relatively the most significant GI-toxic effects were found after indomethacin medication. Similar findings from experiments performed in rats (Nygard *et al.* 1994), confirmed the justification of using indomethacin as an inducer of GI lesions with the most frequent and thus the most reliable GI injury. Ten-day exposure with NSAID was used in our paper similarly as in that of Rainsford *et al.* (2003). In the case of indomethacin, the doses of 5 mg/kg/day (n=3) and 10 mg/kg/day (n=6) were used. The body weight of pigs in the paper cited above was 13–20 kg only, while the pigs used in our experiments were in the period of new-maturity without excessive content of subcutaneous adipose tissue. The body weight range (30–35 kg) of pigs in our experiments represented approximately 50% average body weight of an adult man. Thus, the daily dose of indomethacin (10 mg/kg) was closer to the daily recommended therapeutic dose in man (3 mg/kg) than the dose in the study of Rainsford *et al.* (2003) (although is still more than threefold higher). The findings of the most important morphological changes in the stomach, pyloric area, duodenal part and in caecum are in accordance with both experimental studies.

The methodological preparation, as the declared purpose of the presented research phase, consisted in the induction of reproducible GI tract dysfunction, usable in further experimental studies aimed at systematic survey of lesions influence affecting pharmacokinetics of xenobiotics after their oral administration. For this purpose, various morphological techniques were used to characterise frequency, intensity, depth of pathological findings and their localisation. The described results and their mutual comparison helped to create the diagnostic scale for changes in particular GI segments. The application of this scale in the perspective research phase will make it possible to determine the stage of lesions according to the particular GI segments in conditions *in vivo* by means of wireless capsule enteroscopy as a relatively least invasive technique. Wireless capsule enteroscopy can be carried out simultaneously

with the tested xenobiotics without mutual interference. The confrontation between the extent of pathological findings in the particular sections of digestion tract and the intensity of absorption of model drugs can be helpful towards the character of absorption, exsorption and biotransformation changes revealed. At the same time, it can contribute to the identification of "absorption window" or "absorptive deaf sections" in GI tract for various model drugs (according to their physical-chemical and structural characteristics).

ACKNOWLEDEGMENTS

The experimental part of the study was supported by research project GAČR 305/08/0535. The endoscopic part of the study was supported by research project MZO 00179906 from the Ministry of Health, Czech Republic.

REFERENCES

1 Anthony A, Dhillon A P, Nygard G, Pounder RE, Wakefield AJ (1994). Ulceration, fibrosis and diaphragmalike lesions in the caecum of rats treated with indomethacin. Aliment Pharmacol Ther. **8**: 417–424.
2 Billingham MEJ, Tucker MJ (1979). Correlations between the rise in acute phase proteins and histological evidence of ulceration in the rat following indometacin treatment. Br J Pharmacol. **65**: 450P–451P.
3 Fendrich Z, Květina J (1979). Transfer of water and drugs by the isolated jejunum of irradiated rats. Folia Pharm Univ Carol. **1**: 73–81.
4 Fendrich Z, Květina J, Deml F, Vávrová E (1970). Intestinal transport of ^{14}C-labeled para-amino-benzoic acid in irradiated rats (serosa-mucosa direction). Sbor. prací VLVDÚ, Hradec Králové. **55**:47–53.
5 Goetz M, Memadathil B, Biesterfeld S, Schneider C, Gregor S, Galle PR, Neurath MF, Kiesslich R (2007). *In vivo* subsurface morphological and functional cellular and subcellular imaging of the gastrointestinal tract with confocal mini-miscroscopy. World J Gastroenterol. **13**: 2160–2165.
6 Goetz M, Vieth M, Kanzler S, Galle PR, Delaney P, Neurath MF, Kiesslich R (2008). *In vivo* confocal laser laparoscopy allows real time subsurface microscopy in animal models of liver disease. J Hepatol. **48**: 91–97.
7 Halter F, Gut A, Ruchlti C (1996). Intestinal pathology from NSAIDs. Inflammopharmacology. **4**: 44–60.
8 Hradil J, Fendrich Z, Senius KEO, Květina J. (1978). A new method for pharmacokinetic analysis of gastrointestinal drug absorption. Arzneim-Forsch/Drug Res. **28**:2127–2134.
9 Keuchel M, Hagenmüller F, Fleischer DE, editors (2006). Atlas of video capsule endoscopy. Springer, Heidelberg.
10 Kopáčová M, Bureš J, Österreicher J, Květina J, Pejchal J, Tachecí I, Kuneš M, Špelda S, Rejchrt S. Confocal laser endomicroscopy in experimental pigs. Methods of *ex vivo* imaging. In press.
11 Květina J, Fendrich Z, Lázníček M, Šrámek B (1984). The effect of altered function of the several pharmacokinetic parameters. Folia Pharm Univ Carol. **6**: 87–101.
12 Květina J, Pařízek J (1966). Die Änderungen einiger biochemischer Darm-eigenshaften im Versuchere der akuten Strahlenkrankenheit. (Changes of some biochemical intestinal properties in the course of acute radiation injury). Sbor. prací LF UK, Hradec Králové. **9**: 4–5.
13 Nygard G, Anthony A, Piasecki C, Trevethick MA, Hudson M, Dhillon AG *et al*. (1994). Acute indometacin induced jejunal injury in the rat: early morphological and biochemical changes. Gastroenterology. **106**: 567–575.
14 Rainsford KD (1988). Mucosal lesions induced in rat intestinal tract by the antiinflammatory drug, WY 41, 770, a weak inhibitor of prostaglandin synthesis, contrasted with those from the potent prostaglandin synthesis inhibitor, indometacin. Toxicol Pathol. **16**: 366–376.
15 Rainsford KD, Quadir M (1995). Gastrointestinal damage and bleeding from non-steroidal anti-inflammatory drugs. Clinical and epidemiological aspects. Inflammopharmacology. **3**: 169–190.
16 Rainsford KD, Stetsko PI, Sirko SP, Debski S (2003). Gastrointestinal mucosal injury following repeated daily oral administration of conventional formulations of indometacin and other non-steroidal anti-inflammatory drugs to pigs: a model for human gastrointestinal disease. J Pharm Pharmacology. **55** : 661–668.
17 Rey JF, Ladas S, Alhassani A, Kuznetsov K (2006). European Society of Gastrointestinal Endoscopy (ESGE). Video capsule endoscopy: update to guidelines (May 2006). Endoscopy. **38**:1047–1053.
18 Vane JR, Botting RM (1995). New insights into the mode of action of antiinflammatory drugs. Inflamm Res. **44**: 1–10.