ORIGINAL ARTICLE

Type and distribution of indomethacin-induced lesions in the gastrointestinal tract of rat

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Abstract

OBJECTIVES: The therapy with non-steroidal anti-inflammatory drugs (e.g. indomethacin) is often accompanied with adverse effects in gastrointestinal tract. Aim of this experimental study was to define the time range of the creation of indomethacin-induced gastrointestinal lesions in rat (for prospective study of potential probiotic therapy). The paper follows our previous experiments where the different gastrointestinal lesions were described in the pig (Kvetina *et al.* 2008)
METHODS: Indomethacin (25mg/kg) was administered orally by a single application to rat (Wistar Han II, 200-250g). Six, 24, 48 and 72 hours after the indomethacin administration all parts of the gastrointestinal tract of six rats in each time interval were macroscopically and histologically examined.
RESULTS AND CONCLUSION: The gradual development of lesions was observed 6 hours in stomach and 24-72 hours in the intestine after the indomethacin administration. Not only the gradual development of pathophysiological alterations was observed but also the reparative phase (in stomach). 24 hours seem to be advisable time suitable for the evaluation of the probiotics effect as a potential therapy) on the indomethacin-induced gastrointestinal lesions in rats. Sensitivity of the gastrointestinal tract to the pathological lesions development seems to be higher in rats in comparison to findings described in our previous experiments in pig (Kvetina *et al.* 2008). This adverts to interspecies differences in the manifestation and in the dynamics of the development of gastrointestinal lesions.

INTRODUCTION

One of the causes of the gastrointestinal lesions genesis may be adverse reactions during the therapy with non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs treatment is the second most common aetiological factor for peptic ulcer disease and a major factor for peptic ulcer complications in human beings (Voutilainen *et al.* 2001). Conventional NSAIDs have been reported to change permeability and cause inflammation in the intestinal segments (Halter *et al.* 1996). This view is supported by observations of NSAIDs' caused mucosal lesions in the jejuno-ileal region of rats and dogs (Anthony *et al.* 1994; Billingham

& Tucker, 1979; Nygard *et al.* 1994; Rainsford, 1988). In our previous experiments, experimentally indomethacin-induced gastrointestinal lesions were also found in pig in the stomach, in terminal ileum and in the caecum (Kvetina *et al.* 2008). Aim of this experimental study was to define the dynamics of the development of the gastrointestinal lesion in rat, its distribution along the digestion tract and to compare the findings in rats and pigs. Subsequently, the study was aimed to determine the time range for the evaluation of the potential influence of probiotics (as a potential therapy) on the gastrointestinal lesions formation (in the prospective experimental studies).

MATERIAL AND METHODS

Animals and gastrointestinal lesions induction. Male Wistar Han II rats weighing 280±39g (conventional breed Biotest Ltd., Konárovice nad Labem) kept in air-conditioned rooms in plastic boxes with free access to water. Indomethacin was administered orally in one dose of 25 mg/kg to four groups of six animals. The animals were not given solid food for 12 hours before euthanasia and organ samples collection. The samples from all parts of gastrointestinal tract were macroscopically evaluated and collected for histological examination after 6 (group 1), 24 (group 2), 48 (group 3) and 72 (group 4) hours after indomethacin application. The animals were exposed to halothane overdosing, sacrificed and exsanguinated.

Diagnostic techniques:

Macroscopic examination. The images were taken using a digital camera (Konica Minolta, Dimage Z5, Tokyo, Japan) and saved as .jpg files in the computer. The type and the number of lesions distributed along the whole small and large intestine were noted.

Light microscopy. Samples were fixed in 10% neutral buffered formalin, material was processed by the common paraffin technique and histological sections 5μm-thick were stained with haematoxylin and eosin and with PAS reaction.

Evaluative criteria (the scale of damage):

I. gastritis, hyperaemia, haemorrhages, erosion II. ulceration, 0. normal (without pathological lesions).

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

The formation of lesions was found in the stomach already after 6 hours (***Fig. 1 and 8***) but not in the intestine. In the intestine, the lesions were found within 24–72 hours (***Fig. 3, 4, 5 and 10***) while they became extinct during this time in stomach (***Fig. 2***). The very strong ulcerations were found in the small intestine after the 72 hours (***Fig. 6***). The damage was observed in the whole small intestine, especially in the terminal segments (jejunum, ileum) and in caecum (***Fig. 12***). Colon was not damaged by indomethacin. The microscopic evaluation of lesions was compared with tissue samples of control animals (***Figs. 7, 9, 11***). The frequency and severity of indomethacin-induced lesions are showed in the ***Table 1***.

DISCUSSION

NSAIDs are one of the most commonly used agents in the clinical practice today. All these drugs are known to produce gastro-intestinal lesions (Misra *et al.* 1990). NSAIDs cause dyspeptic complaints and lesions in the upper gastrointestinal tract (Hollenz *et al.* 2006). These effects are attributed to mechanisms such as drug-induced cyclooxygenase inhibition, oxidative stress, mitochondrial dysfunction, changes in cell membrane lipids

Table 1. The frequency any severity of indomethacin-induced lessions in rat (numbers of animals with lesions/animals investigated).
The scale of damage:
I gastritis, hyperaemia, haemorrhages, erosions
II ulceration 0 normal

Tissue	Lesion	6h	24h	48h	72h
Stomach	I	6/6	5/6	2/6	1/6
	II	6/6	3/6	1/6	1/6
	0	0/6	0/6	3/6	4/6
Duodenum	I	0/6	1/6	2/6	3/6
	II	0/6	0/6	0/6	3/6
	0	6/6	5/6	4/6	0/6
Jejunum	I	1/6	3/6	6/6	6/6
	II	0/6	3/6	6/6	6/6
	0	5/6	0/6	0/6	0/6
Ileum	I	1/6	4/6	6/6	6/6
	II	0/6	5/6	6/6	6/6
	0	5/6	0/6	0/6	0/6
Caecum	I	0/6	1/6	4/6	6/6
	II	0/6	0/6	3/6	3/6
	0	6/6	5/6	1/6	0/6
Colon	I	0/6	0/6	0/6	1/6
	II	0/6	0/6	0/6	0/6
	0	6/6	6/6	6/6	5/6

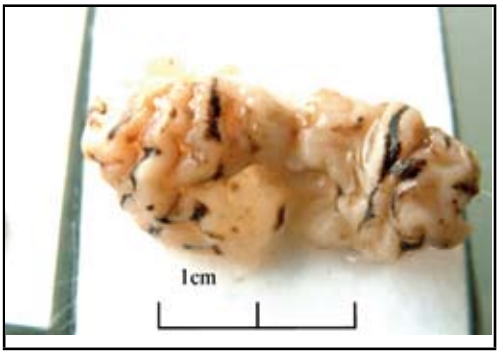

Fig. 1. Stomach-ulcers (black spots). 6 hours after indomethacin administration.

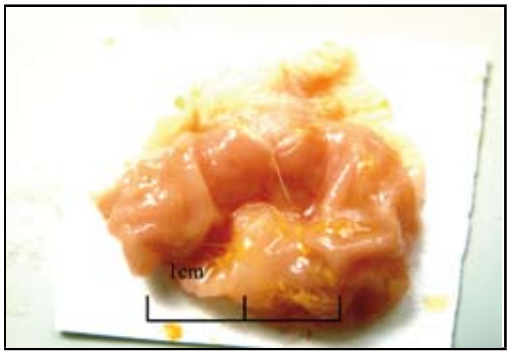

Fig. 2. Stomach without pathological lesions. 48 hours after indomethacin administration.

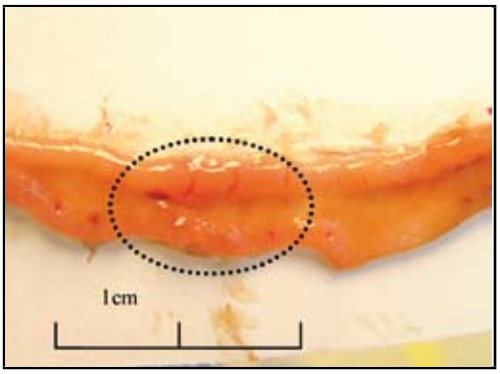

Fig. 3. Jejunum-erosions and ulcers. 24 hours after indomethacin administration.

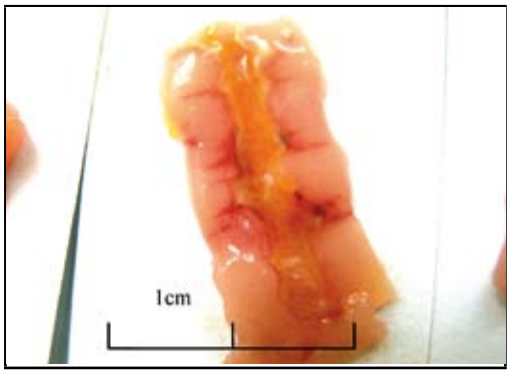

Fig. 4. Jejunum-extensive ulceration. 72 hours after indomethacin administration.

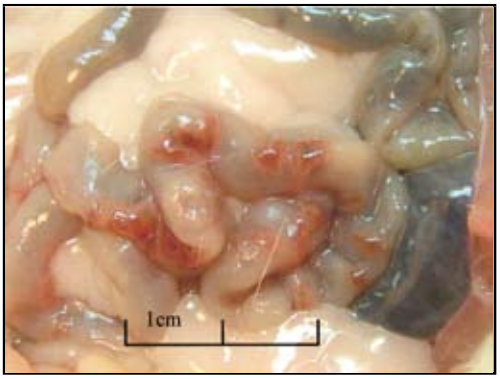

Fig. 5. Ileum-ulceration. The view from the serosal side. 48h after indomethacin administration.

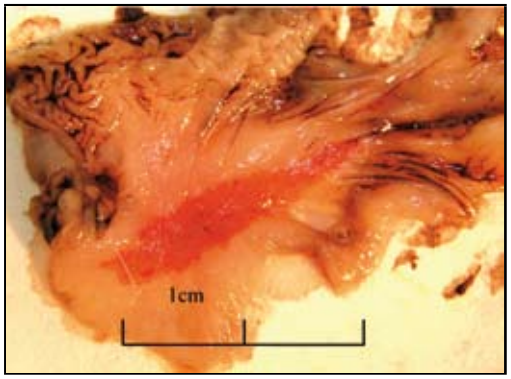

Fig. 6. Caecum-mucosal erythema. 72h after indomethacin administration.

and by an increased production of pro-inflammatory cytokines (Sivalingam *et al.* 2009; Maes, 2008). We used indomethacin (commonly used NSAIDs drug) as an experimental inducer of gastrointestinal lesions according to Nygard *et al.* (1994). Other authors also used the model of indomethacin to induce the gastroenteropathy in the rat (Suleyman *et al.* 2009; Odabasoglu *et al.* 2006; Mehrabani *et al.* 2009). We have found certain dynamics in the formation of the indomethacin-induced lesions in the gastrointestinal tract of rat. While the lesions were observed in stomach already 6 hours after the administration of indomethacin, no lesions were found in the intestine after that time. It is interesting that although the large-scale pathological lesions were found in the whole small intestine after 48-72h, stomach was practically without any pathological lesions at those times. Thus, we observed not only the progressive time-dependent genesis of gastrointestinal lesions but also reparative phase in stomach. Mehrabani *et al.* (2009) described the occurrence of multiple ulcers in stomach 24 hours after the administration of the same (25mg/kg) dose of indomethacin to rat.

We can see inter-species differences while comparing the promotion of the indomethacin-induced lesions between rat (this paper) and pig (Rainsford *et al.* 2003). In our previous paper, the most significant findings were found in stomach (petechia, erosions, single ulcers and ulcers chain) and in caecal segment (erosions, filiform ulceration, enlarged lymph-node) after the 10 consecutive days of the indomethacin medica-

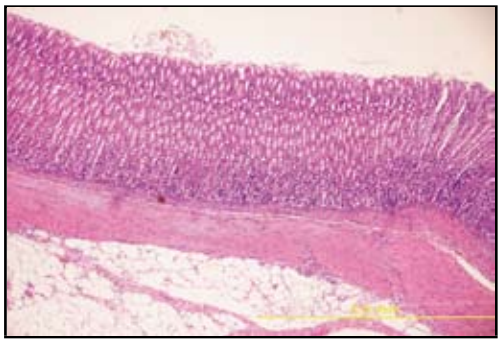

Fig. 7. Stomach – control. Magnification 100x. Staining HE/PAS. Normal gastric mucosa without pathological lesions.

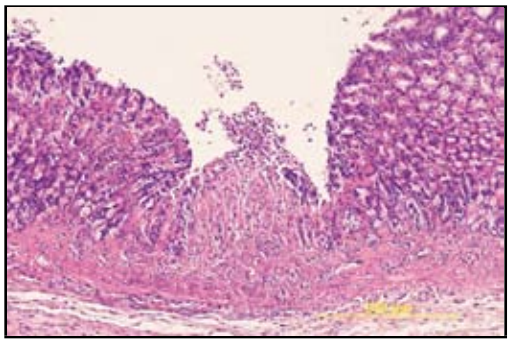

Fig. 8. Stomach – erosion. Magnification 100x. Staining HE/PAS. Erosion no exceed the layer of muscularis mucosae.

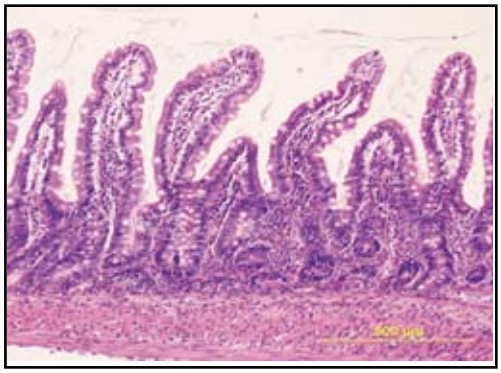

Fig. 9. Jejunum – control. Magnification 100x. Staining HE/PAS. Normal intestinal mucosa, slight and high villi.

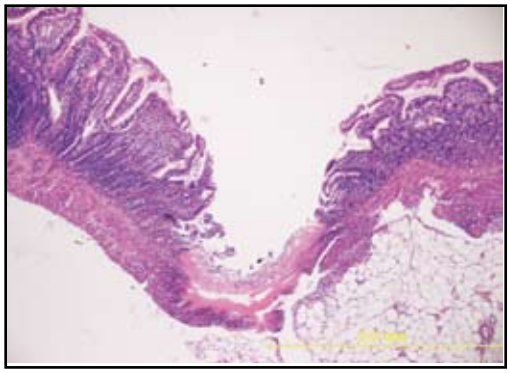

Fig. 10. Jejunum – ulcer. Magnification 100x. Staining HE/PAS. Deep ulcer, base is covered with fibrin.

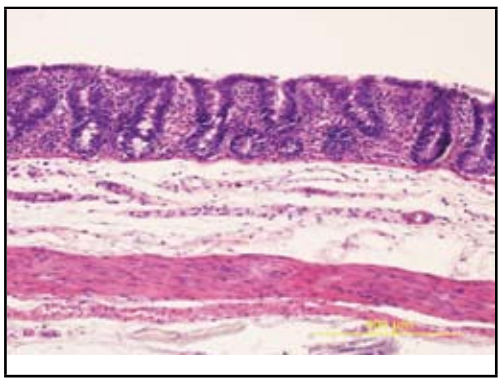

Fig. 11. Caecum – control. Magnification 100x. Staining HE/PAS. Normal mucosa in caecum.

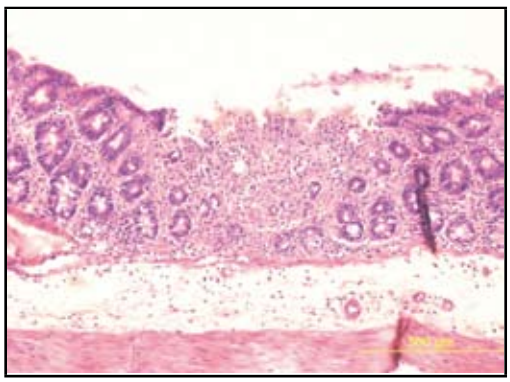

Fig. 12. Caecum – erosion. Magnification 100x. Staining HE/PAS. Erosion of the mucosa surface.

tion to pig (Kvetina *et al.* 2008). Although we can not compare the dynamics of the origin of the lesions in pig and rat, we can conclude that the sensitivity of gastrointestinal tract in pig seems to be lower in comparison to rat in the course of relatively comparable dose of orally administered indomethacin.

We also found out that the time of 24 hours after administration (erosions and the beginning formation of ulcers) seems to be suitable for the evaluation of the influence of the probiotics on the creation of the indomethacin-induced gastrointestinal lesions in the rat. It is known that probiotics supply has beneficial effect on the gastrointestinal epithelium (Sullivan & Nord, 2005). Our further experiments will be aimed on the evaluation of the effect of nonpathogenic strain of Escherichia coli (Nissle, 1917) on indomethacin-induced small intestinal injury in rat. Watanabe *et al.* (2009) already performed similar investigation with the Lactobacillus casei strain Shirota (LcS) using the same model of induced enteropathy. Inflammatory responses triggered by activation of the lipopolysaccharide (LPS)/Toll-like receptor (TLR) 4 signalling pathway are a key mechanism in non-steroidal anti-inflammatory drug-induced enteropathy. Their findings suggest that LcS exhibits a prophylactic effect on indomethacin-induced enteropathy by suppressing the LPS/TLR4 signaling pathway, and that this probiotic effect of LcS may be mediated by L-lactic acid.

The results of our experiments documented significant degree of toxicity (already after one single dose administration) of the commonly used drug from the class of the non-steroidal anti-inflammatory acting substances. It is important to emphasize that these drugs belong among over-the-counter medicinal products.

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