CONTRIBUTION TO SAFE ANTI-INFLAMMATORY THERAPY WITH INDOMETHACIN

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SUMMARY

Non-steroidal anti-inflammatory drugs possess not only therapeutic but also adverse effects, mainly on the gastrointestinal tract. The aim of this pilot study was to establish the ulcerogenic dose caused by daily administration of indomethacin to male Lewis rats. Further, the model of rat adjuvant arthritis (AA) was used to evaluate the protective effect of stobadine dipalmitate against indomethacin-induced gastroenteropathy. Indomethacin was administered subcutaneously in the daily dose of 5, 7, 10, 20 and 30 mg/kg b.w. Survival of the animals and damage of gastric and intestinal mucosa were monitored, and some biochemical parameters were determined. In AA rats stobadine dipalmitate was administered orally in the daily dose of 15 mg/kg.

For the chronical experiments on AA rats the subcutaneous indomethacin dose of 5 mg/kg was selected as the therapeutic dose and the dose of 7 mg/kg was chosen as the adequate dose for gastropathy induction. The additive adverse effect of arthritis induction and indomethacin administration was demonstrated on the basis of gastric mucosa damage observations. The supposed stobadine gastro-protection was not confirmed.

Key words: non-steroidal anti-inflammatory drugs, indomethacin, gastro-protection, stobadine, adjuvant arthritis, Lewis rats

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INTRODUCTION

The use of non-steroidal anti-inflammatory drugs (NSAIDs) is quite common in current medical practice and many arthritis patients are taking these drugs on a long-term basis. Unfortunately, NSAIDs possess not only therapeutic but also adverse effects exerted mainly on the gastrointestinal tract - GIT (1). A commonly used model to study GIT protection is damage induced by indomethacin in rats (2,3). The data in the literature differ concerning the way of administration (mostly p.o. or s.c.), the design of the experiment (single or repeated doses), the animals used (different rat strains, as Wistar, Dark Agouti, Sprague Dawley, etc.), and of course there are differences in the doses that caused GIT damage (2-7). The aim of this pilot study was to establish the ulcerogenic dose of indomethacin during its daily administration to male Lewis rats. Adjuvant-induced arthritis in rats has been widely used as a model of rodent polyarthritis and represents inflammatory arthritis following an infective process (8). Changes in ulcerogenic response to NSAIDs were reported in AA rats (5-7). In the presented study, we evaluated the changes in gastroenteropathic response to indomethacin related to the arthritis process. The possibility to protect GIT with administration of stobadine – a pyridoindole derivative with antioxidant properties – was also taken into consideration.

MATERIAL AND METHODS

Indomethacin was administered s.c. in the daily dose of 30 (A), 20 (B), 10 (C), 7 (D) and 5 (E) mg/kg b.w. Survival of the animals and macroscopic damage of gastric and intestinal mucosa were monitored, and for the doses D and E also biochemical parameters were determined: activity of γ-glutamyl transpeptidase (GGTP; nmols of p-nitroanilide were measured) in homogenates of spleen, gastric mucosa and intestine - ileum (9) and the level of reduced glutathione (GSH) in intestinal homogenate (10). AA was induced in Lewis rats with Freund adjuvans (8). In AA rats the daily dose of 15 mg/kg of stobadine dipalmitate was administered orally. The animals were sacrificed on experimental day 28 for biochemical assessment. The data were expressed as mean and standard error of mean. The statistical evaluation was performed using unpaired Student´s t-test. For declaration of significance the two-tailed p value was taken in consideration (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). The arthritis group was compared to control animals (+). Treated arthritis groups were compared to untreated arthritis animals (*).

RESULTS AND DISCUSSION

In the experimental groups A and B, all animals died up to experimental day 8. Similarly for the dose C, the animals survived only until experimental day 9. Autopsy showed petechial, small haemorrhagic lesions of the gastric mucosa, adhesions of intestinal loops and abdominal exudate, with the intestinal damage being more pronounced than the gastric injury. For the dose D, survival was delayed for one third of animals until experimental day 21 and for the remaining animals until experimental day 28. The gastroenteropathic response was very close to that seen in animals with dose

Table 1. *Effect of indomethacin and stobadine co-administration in rats with adjuvant arthritis monitored by different parameters*

Group of animals	Length of gastric lesion (mm) mean ± SEM	Survival till exp. day 28 (%)	Spleen capsulation (%)	Spleen weight/body weight (g) mean ± SEM
Controls + In 7	0.88 ± 0.43	57	0	1.70 ± 0.03
Arthritis	0.89 ± 0.56	100	42	2.43 ± 0.14^{+++}
Arthritis + In 7	4.00 ± 1.59*	50	80	3.34 ± 0.29**
Arthritis + St + In 7	4.33 ± 1.28*	70	43	2.85 ± 0.19

C, but the extent of the damage was lower. For the last dose tested, i.e. E, all animals survived until the end of the experiment - day 28. Macroscopic damage of the GIT was not observed. Based upon these results, for chronical experiments on adjuvant arthritis rats the subcutaneous dose indomethacin of 5 mg/kg was selected as the therapeutic standard and the dose of 7 mg/kg was selected as the adequate dose for gastroenteropathy induction in Lewis rat model. In the AA rats administered indomethacin in the dose of 7 mg/kg s.c., the mean length of the gastric lesions was 5 times higher than in the arthritic group without indomethacin administration or in the control group without arthritis and only indomethacin administration in the same dose. A variety of miscellaneous compounds, including antioxidants, have been reported to possess gastroentero-protective activity (11). Stobadine was shown to be a molecule with excellent antioxidant properties in vivo and in vitro (12). Thus in this study we investigated the gastroentero-protective effect of this substance. Yet stobadine failed to exert any protective effect; moreover, the value of the mean gastric lesion length was slightly increased after administration of indomethacin in the combination with stobadine (Table 1).

The failure of stobadine to protect against gastric and intestinal damage caused by indomethacin administration to rats with AA was shown also in the changes of GGTP activity assessed in the gastric mucosa (Fig.1) or in the ileum (Fig.2). Stobadine in combination with indomethacin did not prevent the increase of GGTP activity induced by the arthritic process. The level of reduced glutathione decreased in both groups of treated arthritic animals (indomethacin and indomethacin+stobadin) to respective 3.81 ± 0.26 and 3.51 ± 0.18 μg GSH/ mg of proteins in comparison to 5.2 ± 0.66 μg GSH/ mg of proteins in the arthritic control (Fig. 3). Completely different was the effect of stobadine on the relative weight of the spleen, the incidence of spleen fibrous capsulation, spleen GGTP activity or animal survival (Table 1, Fig. 4). In all these parameters stobadine proved therapeutically effective in combination with indomethacin: stobadine decreased the values of morphological as well as biochemical parameters of the spleen close to the untreated arthritic control and markedly prolonged the survival of animals.

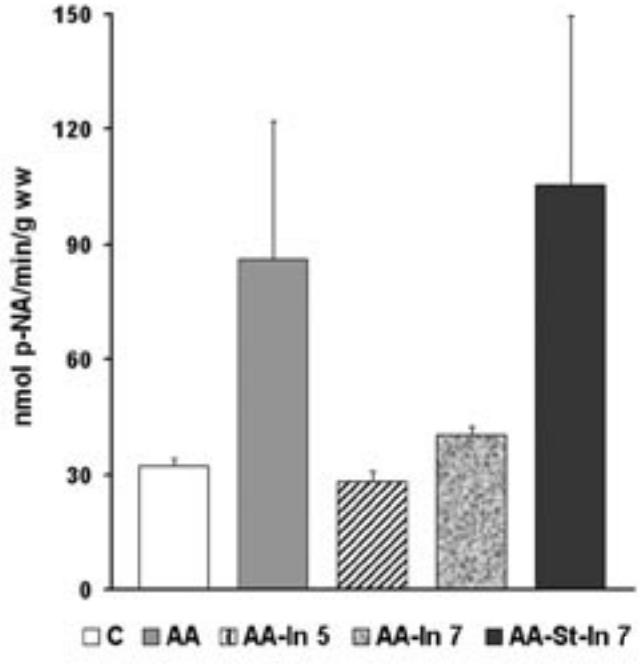

Fig. 1. *GGTP activity in the gastric mucosa of control and arthritic animals with and without indomethacin and stobadine administration (C – control group; AA – arthritis group ; AA-In 5 – arthritis animals administered indomethacin in the dose of 5 mg/kg b.w.; AA-In 7 – arthritis animals administered indomethacin in the dose of 7 mg/kg b.w.; AA-St-In 7 – arthritis animals administered indomethacin in the dose of 7 mg/kg b.w. and stobadine dipalmitate in the dose of 15 mg/kg b.w.)*

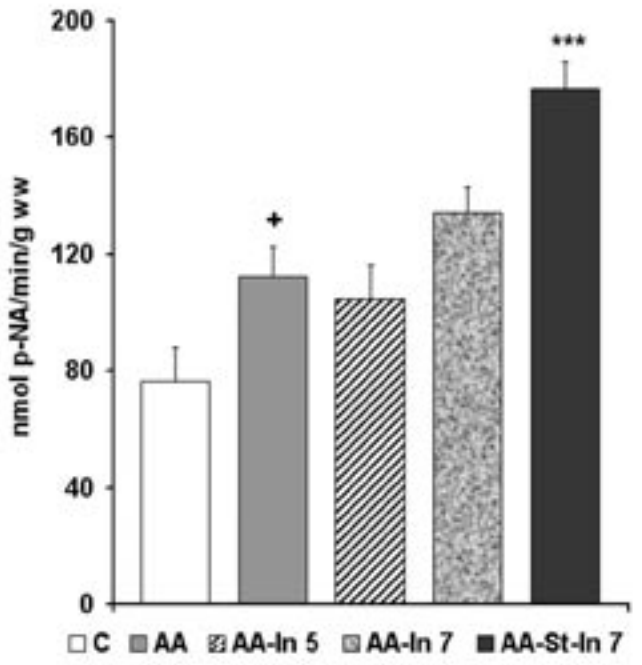

Fig. 2. *GGTP activity in the ileum of control and arthritic animals with and without indomethacin and stobadine administration. (For further explanation see Fig. 1.)*

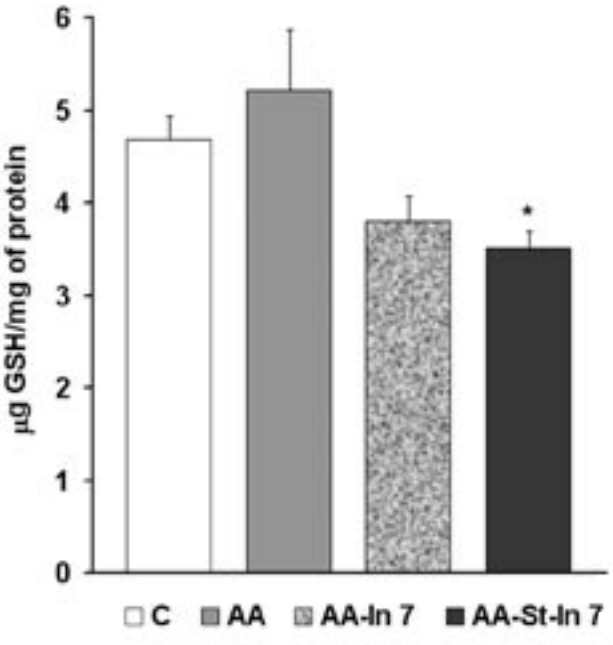

Fig. 3. *GSH level in the ileum of control and arthritic animals with and without indomethacin and stobadine administration. (For further explanation see Fig. 1.)*

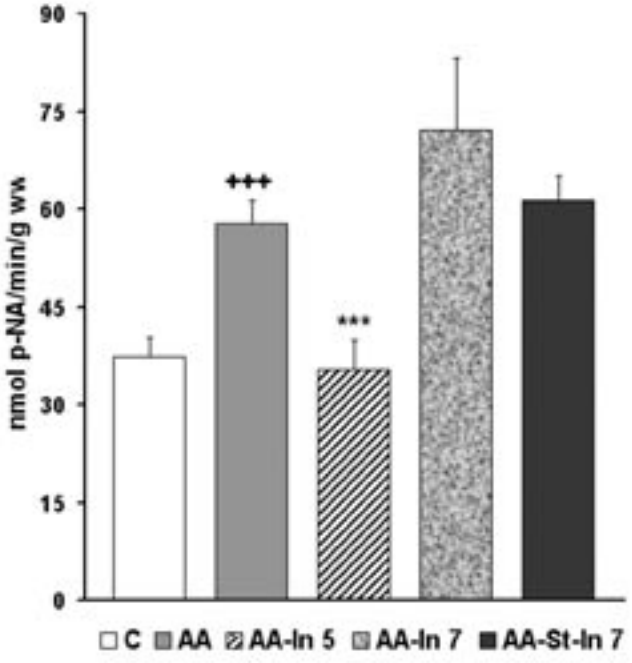

Fig. 4. *GGTP activity in the spleen of control and arthritic animals with and without indomethacin and stobadine administration. (For further explanation see Fig. 1.)*

CONCLUSIONS

The additive adverse effect of arthritis induction and indomethacin administration was demonstrated on the basis of gastric mucosa damage observations. A gastroentero-protective effect of stobadine was not confirmed. Further studies should be performed (e.g. with different stobadine doses) to confirm or to disprove this finding. The positive effect of stobadine was clearly demonstrated in morphological and biochemical parameters of the spleen and in the survival of animals.

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