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Effects of nonselective and selective cyclooxygenase inhibitors on small intestinal motility in the horse

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Abstract

We investigated the effects of nonselective cyclooxygenase (COX) inhibitors (indomethacin and flunixin meglumine) and selective COX-1 (SC-560) or COX-2 (celecoxib, DUP-398 and NS-697) inhibitors on horse small bowel motility in vitro. At this purpose, samples of equine ileum were put in isolated organ baths for the motility experiments. Nonselective COX inhibitors were devoid of major effects on motility, except for an inhibition of tonic contraction shown by flunixin meglumine. SC-560, selective COX-1 inhibitor, was devoid of significant effects on ileal motility. Selective COX-2 inhibitors reduced both tonic contraction and spontaneous phasic contractions, while prostaglandin (PG) receptor antagonists were uneffective. Some of the intestinal samples were submitted to histological investigation or reverse transcription-polymerase chain reaction (RT-PCR), which revealed the presence of an inflammation reaction and the presence of both COX isoforms mRNAs. Present data support the hypothesis that the effects of COX inhibitors on horse small intestinal motility are not linked to PG depletion.

Keywords: Nonsteroidal antiinflammatory drugs; Cyclooxygenase 1; Cyclooxygenase 2; Horse; Small intestine; Motility

1. Introduction

Nonsteroidal antiinflammatory drugs (NSAIDs) are a heterogeneous class of compounds, which act by inhibiting cyclooxygenase (COX) activity and the associated synthesis of prostaglandins (PGs), as well as their effects, which include algesia, hyperpyrexia and inflammation. However, PGs are not only inflammatory mediators but exert also protective effects on several districts like the gastrointestinal tract or the kidney, and thus COX inhibition leads to well known adverse effects. The discovery of at least two COX isoforms (namely COX-1 and COX-2) has provided new insights to the understanding of both beneficial and toxic effects of NSAIDs; COX-1 was found to be constitutive in almost all tissues and mainly responsible for the synthesis of protective PGs, while COX-2 expression is induced by inflammatory stimula (Fu et al., 1990) and leads to the production of noxious PGs. These findings led to the development of selective COX-2 inhibitors as new and safer antiinflammatory agents, but further studies have challenged the safety of these drugs, revealing that COX-2 isoform could be constitutive in several tissues and contribute to the maintenance of omeosthasis (Kargman et al., 1996; Zimmermann et al., 1998; Harris and Breyer, 2001). Nonselective COX inhibitors like phenylbutazone, ketoprofen or acetylsalicilic acid, are used in equine therapy for the treatment of musculoskeletal lesions and osteoarthritis and are known to cause adverse effects which include gastrointestinal ulcers, colitis and bowel motility

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disorders (MacKay et al., 1983; MacAllister et al., 1993; Dabareiner and White, 1995). Among NSAIDs, flunixin meglumine is often used as a symptomatic medicament in the equine colic, for its ability to attenuate visceral pain. In addition to the analgesia, this compound could affect intestinal smooth muscle activity; however, an involvement of PG synthesis inhibition has not been demonstrated, despite the ability of PGs to modulate intestinal motility (Mohajer and Ma, 2000). Therefore, it would be important to establish whether the beneficial effects of NSAIDs in equine colic may also involve a reduction of intestinal muscle hyperactivity. Moreover, the occurrence of at least two COX isoforms, COX-1 and COX-2, opens the question of which is the role of each enzyme in the production of PGs in the horse bowel and it would be of great interest to evaluate the different effects of selective and nonselective COX inhibition on equine intestinal motility.

The aim of the present study was to assess the effects of nonselective (indomethacin, flunixin meglumine) and selective COX-1 (SC-560) or COX-2 (celecoxib, DUP-697, NS-398) inhibitors (Brideau et al., 2001; Smith et al, 1998; Futaki et al., 1994), on equine small intestinal preparations, in an attempt to reveal the ability of NSAIDs to modify the motility in vitro. In addition, we performed a histological evaluation of small intestine samples, in order to assess the possible presence of an inflammatory condition and reverse transcription-polymerase chain reaction (RT-PCR), aiming to demonstrate the presence of mRNA coding for COX-1 and COX-2 proteins.

2. Materials and methods

2.1. Drugs

FP receptor antagonist AL8810 (Griffin et al., 1999), atropine sulfate, flunixin meglumine, indomethacin, L-NAME and tetrodotoxin (TTX) were supplied by Sigma Chemical Co. (St Louis, MO, USA); celecoxib was from Cipla Ltd. (Mumbai, India); SC-560 was kindly provided by Dr D.W. Owens (Pfizer Inc., Groton, CT, USA). EP_1/EP_2 antagonist AH6809 (Keery and Lumley, 1988), MEN 10627 (NK_2-antagonist), DUP-697 and NS-398 were purchased from Tocris (Bristol, UK). SR 14033 (NK_1-antagonist), was supplied from Sanofi Recherche (Montpellier, FR). Compounds AH6809, AL8810, SR 14033, MEN 10627 and all NSAIDs, except flunixin meglumine, were dissolved in dimethyl sulfoxide and diluted to the final concentration with distilled water. The final maximal concentration of solvent was <0.1%. All the other drugs were dissolved in distilled water. All solutions were freshly prepared before each experiment and aliquots (10 to 100 μl) were added to the organ baths to achieve the desired molarity.

2.2. Intestinal motility in vitro

Tissues were collected from male adult horses (age range 2–4 years), slaughtered at the public abattoir in Parma. Segments of ileum (~10 cm long) were excised about 30 cm proximally to the caecum and rinsed with cooled (4 °C) modified Krebs–Heinseleit Solution (KHS) of the following composition (mM): NaCl 113.0, KCl 4.7, $MgSO_4 \cdot 7H_2O$ 1.2, $CaCl_2 \cdot 2H_2O$ 1.8, KH_2PO_4 1.2, $NaHCO_3$ 25 and dextrose 11.2. The samples were stored in cooled KHS for the 10 min-transport from the slaughterhouse to the laboratory, where the intestinal specimens were carefully rinsed with fresh KHS and cleaned from surrounding tissues and mucosa. Each segment of ileum was subsequently cut in circularly-oriented strips (25 × 3 mm), tied to both ends with silk threads and then set up into 10 ml organ baths at 37 °C, containing the solution above described, gassed with 95% O_2 and 5% CO_2 (pH 7.4). After a period of stabilization (60–120 min), the mechanical activity was measured by means of an isotonic transducer connected to the preparation, developing a passive stretch of 2–3 g throughout the entire experiment. Pilot experiments were performed to establish the optimum load to get the best spontaneous activity. The viability of preparations was assessed by checking the occurrence of measurable spontaneous contractions and by the ability of acetylcholine (0.1 μM) to evoke a contractile response (>0.1 cm shortening). Samples not showing spontaneous motility or not responding to exogenous acetylcholine were discarded.

In some experiments, electrical field stimulation (EFS) was applied with a pair of coaxial platinum electrodes, positioned 10 mm from the longitudinal axis of the preparations and used to deliver trains of square wave pulses (0.5 ms duration, 50 V amplitude) every 60 s to the tissues at a frequency of 5–10 Hz (Basile, Milano, Italy). Under these conditions, depolarization of intrinsic nerve endings and neurotransmitter release were induced (Poli et al., 1994). For each experiment, the intensity was adjusted to a level giving the 70–80% of the maximum tissue response (usually 250–300 mA).

All recordings were performed by a pen-writing polygraph (Basile, Milano, Italy). The changes of tonic contraction induced by drugs were measured as the variations of the baseline (Δ cm) of polygraph recording (showing the sustained contraction of intestinal sample) with respect to the pre-drug level assumed as "0"; the variations of spontaneous or EFS-evoked phasic contractions of the preparations (overcoming tonic contraction) were expressed as percentage of the pre-drug amplitude of contractions, assumed as 100% (Fig. 1A).

Although the effects of drugs on muscle motility are usually reversible, only one drug was tested in each preparation, in order to avoid possible drug interferences due to incomplete washing-out or to desensitization phenomena.

2.3. Histology

Intestine samples were fixed in a buffered 7% formalin solution for about 48 h. The samples were then sliced in 0.5 cm-thick sections, dehydrated with serial dilutions of