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A novel model for NSAID induced gastroenteropathy in rats☆

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ABSTRACT

Introduction: Progress in management of Nonsteroidal anti-inflammatory drug (NSAID) induced gastrointestinal toxicity requires the availability of appropriate experimental animal models that are as close to humans as feasible. Our objective was to develop a rat model for NSAID-induced gastroenteropathy and also to simulate the common clinical scenario of co-administration of NSAID and proton pump inhibitor (PPI) to explore if PPI contribute to exacerbation of NSAID-enteropathy.

Methods: Rats were treated twice daily with pantoprazole sodium (PTZ; 10 mg/kg peroral) or vehicle for a total of 10 days. In some experiments, Diclofenac sodium (DCF; 9 mg/kg) or vehicle was administered orally twice daily for the final 5 days of PTZ/vehicle administration. After the last dose on 9th day, rats in all the groups were fasted but water was provided ad libitum. 12 h after the last dose on 10th day, rats in all the groups were euthanized and their gastrointestinal tracts were assessed for haemorrhagic lesions, lipid peroxidation, intestinal permeability and gastrointestinal luminal pH alterations. Changes in haemoglobin, haematocrit and serum levels of albumin, total protein, ALT and bilirubin were calculated.

Results: The macroscopic and histological evidence suggested that administration of DCF resulted in significant gastroenteropathic damage and co-administration of PTZ resulted in significant exacerbation of NSAID enteropathy, while attenuation of NSAID induced gastropathy was observed. Our results were further supported by the significant decrease in haemoglobin and haematocrit levels and serum levels of albumin and total proteins, an increase in oxidative stress and intestinal permeability with the use of DCF either alone or in combination with PTZ.

Conclusions: This model was developed to simulate the human clinical situation during NSAID therapy and indeed the present DCF regimen caused both gastric and small bowel alterations, such as multiple erosive lesions, together with a decrease in haemoglobin, haematocrit, serum albumin, serum total protein levels and IP alteration, known to occur in patients receiving NSAIDs. Additionally, this paper provides yet another evidence for PPI induced exacerbation of NSAID enteropathy.

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) which are among the most prescribed drugs worldwide (Brune & Patrignani, 2015; Conaghan, 2012) are often preferred because of their low abuse potential, long history of clinical use and robust efficacy in alleviating pain and inflammation (Atchison, Herndon, & Rusie, 2013). However, their use is associated with adverse events in the upper gastrointestinal (GI) tract of experimental animals and humans, and now it is being increasingly appreciated that these drugs can also exert detrimental effects on the lower GI tract, with potential serious outcomes like; ulceration, perforation, overt bleeding and diaphragm-like strictures (Fornai et al., 2014; Scarpignato & Hunt, 2010; Zeino, Sisson, & Bjarnason, 2010). The frequency and severity of lower GI toxicity of NSAIDs such as Diclofenac sodium (DCF) is frequently underestimated (Scarpignato & Hunt, 2010; Zeino et al., 2010). The fact that there are no proven-effective treatments for NSAID-enteropathy likely contributes to the lack of recognition of this serious condition (Wallace, 2012). This is a major clinical concern as NSAID induced enteropathic damage is more difficult to detect, more expensive to treat, and is associated with longer hospital stays and higher mortality rates, compared to gastropathic damage (Lanas et al., 2009; Wallace, 2013).

The most common approach used clinically to minimize NSAID induced gastropathic injury has been the co-administration of a proton pump inhibitor (PPI) with the NSAID (Wallace, 2012). This has been shown to significantly attenuate the incidence and severity of gastro-duodenal damage (Scarpignato & Hunt, 2010; Scheiman et al., 2006), but recent evidence from animal studies suggest that these

Abbreviations: DCF, Diclofenac sodium; EB, Evans Blue; Hb, Haemoglobin; HCT, Haematocrit; LPO, Lipid peroxidation; MDA, Malondialdehyde; NSAIDs, Nonsteroidal anti-inflammatory drugs; PPIs, Proton pump inhibitors; PTZ, Pantoprazole sodium; ROS, Reactive oxygen species..

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gastroprotective drugs rather synergistically exacerbate NSAID-induced small intestinal injury and bleeding (Satoh, Amagase, & Takeuchi, 2014; Wallace et al., 2011). There are several clinical studies too that report high levels of intestinal damage in healthy volunteers taking NSAIDs plus a PPI (Fujimori et al., 2010; Goldstein et al., 2005; Maiden, Thjodleifsson, Theodors, Gonzalez, & Bjarnason, 2005).

Thus, it is imperative to find novel therapeutic agents to prevent the NSAID-induced gastroenteropathy as well as the PPI induced exacerbation of NSAID enteropathy. In this purview, future studies need to include a more rigorous evaluation of the therapeutic interventions or novel NSAIDs in the entire GI tract, rather than focusing almost entirely on gastro-duodenal region. In addition, we also need to examine the GI safety of these compounds when administered together with PPIs, as PPIs have become the mainstay therapy for preventing NSAID-induced gastro-duodenal damage.

Many studies have been done for investigating the pathogenesis of GI lesions and for screening drugs for the treatment of GI ulcers in humans, however, in most of those studies or the models thereof, the focus has been on either NSAID-induced gastric (gastropathy) or small intestinal (enteropathy) injury (Tajima, 2014; Wallace, 2012). Likewise, to model NSAID induced gastropathy and/or enteropathy different dose of NSAIDs, dosing regimen and rats with different nutritional status (fed and non-fed rats) have been used (Atchison, Balakumaran, et al., 2000; Cheung, Kim, Park, & Kim, 2014; Fornai et al., 2014; Kim et al., 2005; Satoh et al., 2014; Saud, Nandi, Ong, Finocchiaro, & Levine, 2005). However, these approaches have their own limitations and do not mimic the clinical scenario of NSAID use in humans and may not give the complete idea about the entire GI safety/efficacy/toxicity of either novel and current NSAIDs or any other therapeutic agent aimed at preventing GI toxicity of NSAIDs (Blackler, Syer, Bolla, Ongini, & Wallace, 2012), thus increasing the chances of failure or safety concerns at a later stage. For example, the drugs which may prove to be gastroprotective in model of NSAID induced gastropathy may not be effective in preventing enteropathic damage or rather prove to be detrimental in nature; like the case with PPIs (Wallace et al., 2011).

This likely necessitates the development of animal models with both gastric (gastropathy) as well as small intestinal (enteropathy) injury simulating the clinical scenario of NSAID use, that is; a model for NSAID-induced gastroenteropathy. Indeed, this approach will make the data more predictive of the human response, therefore, providing more insight on the potential GI toxicity of current and novel NSAIDs or efficacy/safety of drugs intended for use as treatments of the GI toxicity of NSAIDs. In the present study, we have developed a rat model for NSAID-induced gastroenteropathy. In addition, we simulated the common clinical scenario of co-administration of NSAID and PPI to explore if PPI contribute to exacerbation of NSAID-enteropathy.

2. Materials and methods

2.1. Chemicals and assay kits

Diclofenac sodium (DCF) and Pantoprazole sodium (PTZ) were purchased from Yarrow Chem Products, Mumbai, India. Sodium bicarbonate (NaHCO3) and carboxymethyl cellulose (CMC) were products of Himedia, Mumbai, India. The assay kits for total protein, albumin, alanine transaminase (ALT) and bilirubin (both total and direct) were products of ERBA Diagnostics Mannheim GmbH, Mannheim, Germany. Heparin was purchased from Biological E. Ltd., Hyderabad, India. Heparinised capillaries were purchased from Himedia, Mumbai, India. Rodent diet was obtained from VRK nutrition, Pune, India. All other reagents used in this study were of analytical grade and procured from a leading chemical house in India.

2.2. Animals

Male, Wistar rats (n = 24), 4–6 months old, weighing 200–250 g were obtained from the animal house of B. V. Patel PERD Centre, Ahmedabad, India. All animals were housed in clean, polypropylene cages (three animals per cage) and were allowed to acclimatize for a week before any experiment. A 10% air exhaust conditioning, relative humidity (60 ± 5%), temperature (25 ± 3 °C) and 12:12 h light:dark cycle was maintained in the animal house facility (Reg. No. 1661/PO/a/12/CPCSEA) in accordance with Good Laboratory Practise (GLP) mentioned in Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines. Balanced rodent food pellet and water was provided ad libitum. All experimental protocols were reviewed and accepted by the Institutional Animal Ethics Committee prior to initiation of the experiment.

2.3. Experimental design

2.3.1. Preparation of drugs for administration

Doses required for the study were calculated according to the body weight of the experimental animals. Required quantity of DCF was suspended in 1% CMC and PTZ was suspended in 1% CMC containing 1% NaHCO3 for oral administration (Satoh et al., 2014). Drugs were prepared just before the experiments and administered in a volume of 0.2 ml/100 g body weight. The same volume of a solution containing 1% CMC was used as a vehicle. A small volume of drug vehicle was employed in order to minimize any possible interference with the effects of test drugs, as a consequence of repeated daily administrations.

2.3.2. Induction of gastrointestinal damage/dosage regimen

Animals were divided into 4 groups: Group 1: Treated with vehicle for 10 days, referred as NC group henceforth, Group 2: Treated with PTZ (10 mg/kg body weight peroral, *p.o.*) twice daily for 10 days, referred as PTZ group henceforth, Group 3: Treated with vehicle for 10 days and DCF (9 mg/kg body weight, *p.o*) twice daily on the final 5 days, referred as DCF group henceforth, Group 4: Treated with PTZ (10 mg/kg body weight *p.o.*) twice daily for 10 days and DCF (9 mg/kg body weight, *p.o*) twice daily on the final 5 days, referred as DCF + PTZ group henceforth. The dose and duration of DCF treatment was selected on the basis of previous literature (Reuter, Davies, & Wallace, 1997; Satoh, Amagase, & Takeuchi, 2012; Satoh et al., 2014; Wallace et al., 2011) and preliminary studies. In preliminary studies, DCF at the dose of 9 mg/kg body weight twice daily when administered for 5 days (6th to 10th day) was effective in producing gastric as well as intestinal ulcers without any mortality, while, slightly higher dose of DCF (10 mg/kg twice daily for 5 days) resulted in death of all the rats on fifth day and lower dose did not produce lesions in all rats (data not shown). Dose of PPI was taken as per earlier studies where, PPIs at the dose of 10 mg/kg have been shown to sufficiently inhibit gastric acid secretion (>99%) in rats (Wallace et al., 2011). In previous clinical studies, therapeutic dose of NSAIDs has been reported to cause gastroenteropathic damage in humans and PPIs have been reported to exacerbate the enteropathic damage (Fujimori et al., 2010; Kuramoto et al., 2013; Maiden, 2009; Watanabe et al., 2008). Here, our prime objective was the development of a novel model for gastroenteropathy in rats which may be used in assessing the entire GI safety/efficacy/toxicity of therapeutic agent aimed at preventing the GI toxicity of NSAIDs, thus reducing the chances of failure or safety concerns at a later stage. PTZ was administered 30 min (min) prior to DCF administration. After the last dose on 9th day, rats in all the groups were fasted but water was provided ad libitum. Twelve hours after the last dose of the test drugs on 10th day, rats were anaesthetised with isoflurane, blood samples were collected from each animal and later euthanized by CO2 asphyxiation.