

CYCLOPHOSPHAMIDE-INDUCED CYSTITIS IN CONSCIOUS FEMALE RATS: DEVELOPMENT AND PHARMACOLOGICAL VALIDATION OF AN EXPERIMENTAL MODEL OF REFERRED VISCERAL PAIN.

Hypothesis / aims of study

Cyclophosphamide (CYP)-induced bladder inflammation is a well established pre-clinical model for interstitial cystitis/painful bladder syndrome. Several studies have reported that CYP-induced cystitis results in a local inflammation, bladder overactivity and pain. Some of these reports demonstrate that CYP induces an increase in painful behaviors that peaks shortly after CYP injection and persists up to 4 hours (1,2). The aim of the present work was to develop a model of visceral pain and to evaluate clinically relevant compounds such as aspirin and ibuprofen, 2 non steroidal anti-inflammatory drugs (NSAIDs), and morphine, an agonist of opioid receptors (3).

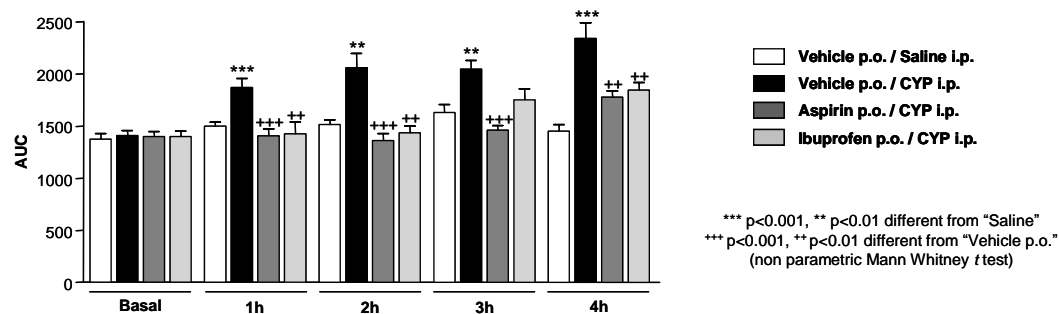
Study design, material and methods

Female Sprague Dawley rats (225-250g) were used. Visceral sensitivity in response to mechanical stimuli was determined using Von Frey monofilaments applied in the lower abdominal area, close to the urinary bladder. Each rat was placed individually in a clear plastic testing box with a metal grid floor and allowed to acclimatize for at least 30 min. Von Frey monofilaments with forces between 1 and 60 g were applied 3 times in an ascending order of strength at intervals of 5-10 sec. A score was assigned based on the animal's response: 0 = no reaction, 1 = retraction of the abdomen, 2 = change of position, 3 = licking and/or vocalization. Nociceptive parameters were expressed as nociceptive threshold (lowest filament that evoked a first score of 1), percentage of nociceptive score for each filament (% of the maximal score for 3 applications) and area under the curve (AUC, by plotting % of individual nociceptive score against Von Frey force). Chemical cystitis was induced by a single intraperitoneal (i.p.) injection of CYP at a dose of 150 mg/kg dissolved in saline (5 mL/kg). Six groups of 8-10 animals were performed. Group 1 received an i.p. injection of saline and was used as control. Groups 2 to 6 received an i.p. injection of CYP. Groups 2, 3 and 4 were treated orally (p.o.) with vehicle (5 mL/kg), aspirin (300 mg/kg) or ibuprofen (300 mg/kg) respectively. Groups 5 and 6 were administered subcutaneously (s.c.) with vehicle (5 mL/kg) or morphine (3 mg/kg) respectively. Sensitivity of the lower abdomen to Von Frey mechanical stimulation was determined before (15 min, basal response) and 1, 2, 3 and 4 hours after i.p. injection of CYP or saline. All treatments were performed 5 min prior CYP or saline injection. At the end of the experiments, rats were sacrificed and urinary bladders were removed and weighted.

Results

Compared to control, a single administration of CYP induced an increase in nociceptive score in response to noxious mechanical Von Frey forces and a decrease in mechanical threshold in response to innocuous mechanical Von Frey forces. These nociceptive responses were correlated with a significant increase in AUC (Figure 1A) and were observed 1 hour and up to 4 hours after CYP treatment. Increase in pain response caused by CYP was significantly reduced when rats received aspirin or ibuprofen p.o. compared to vehicle. This inhibition was observed between 1 and 4 hours after treatment, and was associated with a decrease in the AUC (Figure 1A) and an increase in mechanical threshold. Similarly, inhibition of nociceptive responses to mechanical stimulation was observed when rats received a single s.c. injection of morphine. This opioid agonist was able to significantly reverse the CYP effects up to 3 hours post-administration, compared to vehicle (Figure 1B). Urinary bladder weight from CYP-treated rat was significantly increased by 34% compared to control ($p < 0.001$). This increase in urinary bladder weight was reduced by 15% in the aspirin group, and 10% in the ibuprofen group, compared to vehicle ($p > 0.05$). In contrast, urinary bladder weight from the morphine group was significantly increased by 13% compared to vehicle ($p < 0.05$).

A.



B.

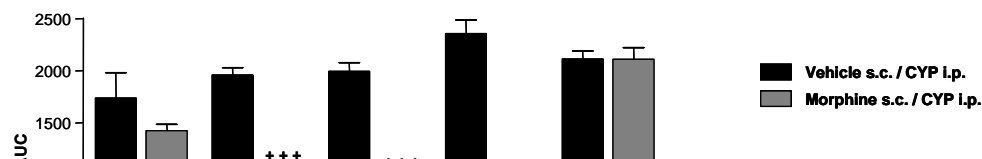


Figure 1. Effect of (A) aspirin (300 mg/kg p.o.), ibuprofen (300 mg/kg p.o.) and (B) morphine (3 mg/kg s.c.) on CYP-induced increases of nociceptive scores using Von Frey forces.

Interpretation of results

To date, measurements that have been used to assess pain associated with cystitis in rodents have been mainly behavioural. Literature reports have described that in rats, systemic CYP induces painful behaviours characterized by eye closure, abnormal posture and a decrease in respiration rate (1,2). In the present study, we describe a new approach to evaluate sensitivity of lower abdomen in response to mechanical stimuli in CYP-induced acute cystitis in rats. We clearly show that a single i.p. injection of CYP, at a dose of 150 mg/kg, increases nociceptive scores in response to noxious Von Frey forces (mechanical hyperalgesia). We also demonstrate that CYP-treated rats present a lowered mechanical threshold compared to control, known as mechanical allodynia. These pain responses were observed between 1 and 4 hours post-cystitis, and appear to be greater at the 4 hour time point. In this model, morphine reversed the referred visceral allodynia and hyperalgesia induced by CYP up to 3 hours post-administration. These results are in agreement with previous studies using morphine in rats (1). In addition, NSAIDs were able to inhibit referred visceral allodynia and hyperalgesia induced by CYP, providing the first evidence for the efficacy of aspirin and ibuprofen in CYP-induced pain in rats.

Concluding message

The present experimental model of CYP-induced cystitis is of interest as a new acute model of inflammatory visceral pain, characterized by both referred mechanical hyperalgesia and allodynia. This model can be used to characterize the physiopathology of urinary bladder pain and evaluate the effect of new treatments for interstitial cystitis/painful bladder syndrome.

References

1. Boucher M et al., 2000. J Urol, 164: 203-208.
2. Lanteri-Minet M et al., 1995. Exp Brain Res, 105: 220-232.
3. Wantuch C et al., 2007. Neurosci Lett, 421: 250-252.

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<i>Is this a clinical trial?</i>	No
<i>What were the subjects in the study?</i>	ANIMAL
<i>Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?</i>	Yes
<i>Name of ethics committee</i>	All experimental protocols were carried out in accordance with the European Community Council Directive 86/609/EEC. They were performed in accordance with French legislation concerning the protection of laboratory animals.