Pharmacological modulation of urinary bladder inflammation in a rat model of cyclophosphamide-induced acute bladder pain

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Objectives

Cyclophosphamide (CYP)-induced cystitis is a well characterized model of subacute, inflammatory visceral pain in rats and mice[1,2]. This model is widely used and is considered one of the closest experimental model to bladder pain syndrome/interstitial cystitis.

Recently, we showed that a single injection of CYP induces visceral pain in female rats, characterized by both referred mechanical allodynia and hyperalgesia. Aspirin, ibuprofen, 2 non steroidal anti-inflammatory drugs (NSAIDs), and morphine, an opioid agonist, were able to reverse referred visceral pain induced by CYP[3].

Our main objectives were to identify and quantify different key inflammatory mediators in urinary bladders and urines which may contribute to the antinociceptive effects of these drugs.

Methods

- **Animals:** female Sprague Dawley rats (225-250 g)
- **Chemical cystitis:** single intraperitoneal (i.p.) injection of CYP at a dose of 150 mg/kg in saline (5 mL/kg)
- **Pharmacological treatment:**

* Aspirin - 300 mg/kg p.o.; Ibuprofen - 300 mg/kg p.o.; Vehicle (5% Na_2CO_3) - 5 mL/kg p.o.
* Morphine: 3 mg/kg s.c.; Vehicle (saline): 5 mL/kg s.c.

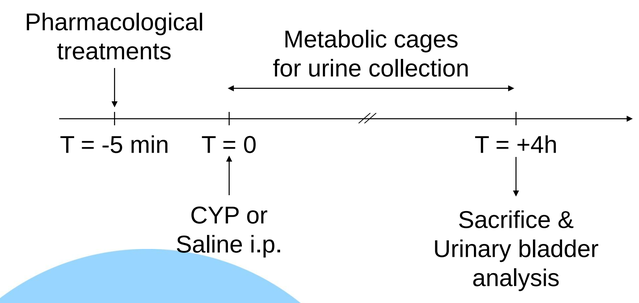
- **Macroscopic inflammatory parameters:**

- Urinary bladder wet weight and wall thickness
- Macroscopic analysis (edema & hemorrhage)[4]

- **Quantification of inflammatory mediators:**

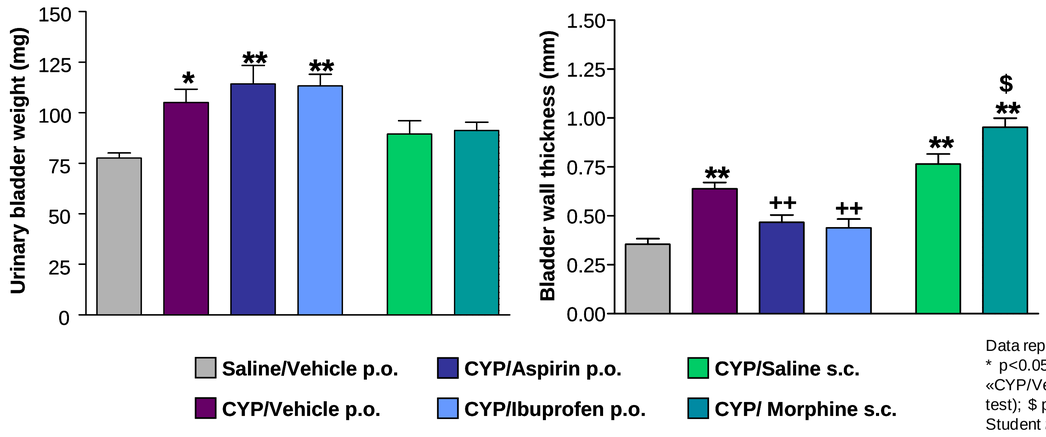
- Urinary bladders (Multiplex technology): pro-inflammatory cytokines (IL-1β and IL-6), monocyte chemotactic protein-1 (MCP-1) and vascular cell adhesion molecule (VCAM)
- Urines (enzyme immunoassays): prostaglandin E2 (PGE2), lipoxin A4 (LXA4) and 15-epi lipoxin

- **Schematic protocol:**



Results

CYP treatment induces acute urinary bladder inflammation

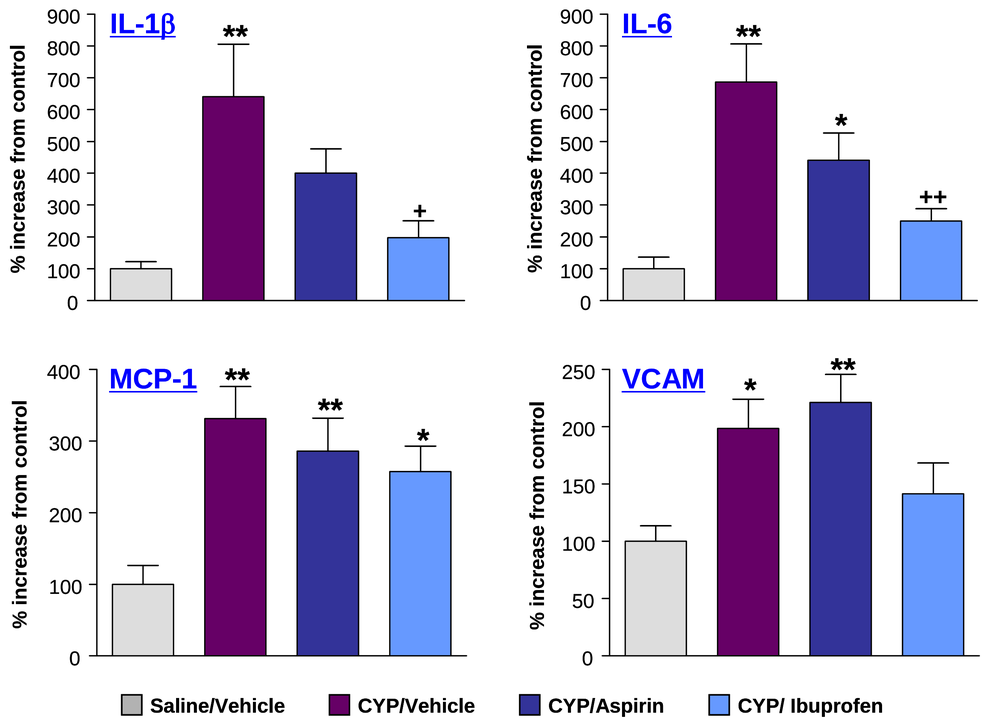


	Edema scores	Hemorrhage scores
Saline / Vehicle	0.0 ±0.0	0.0 ±0.0
CYP / Vehicle	2.4 ±0.3 **	2.0 ±0.3 **
CYP / Aspririn	1.8 ±0.4 **	1.1 ±0.2 **
CYP / Ibuprofen	1.7 ±0.5 **	1.2 ±0.4 **
CYP / Saline	1.8 ±0.3 **	1.1 ±0.1 *
CYP / Morphine	3.0 ±0.0 ** $$	2.3 ±0.3 ** $$$

Data represent mean ± s.e.m. (n=10/group)
* p<0.05, ** p<0.01 different from «Saline/Vehicle» group; ++ p<0.01 different from «CYP/Vehicle» group (one way ANOVA followed by Dunnett's multiple comparison test); $ p<0.05, $$ p<0.01, $$$ p<0.001 different from «CYP/Saline» group (unpaired Student *t* test)

- Four (4) hours after CYP injection, urinary bladder weight and wall thickness were increased by 35 and 80% respectively, associated with strong edema and erythema.
- After NSAID treatment, general inflammatory parameters were not affected except for a 30% decrease in wall thickness. In contrast, morphine treatment induced a strong increase in wall thickness and macroscopic damage.

Inflammatory mediators from the urinary bladder are increased after acute CYP

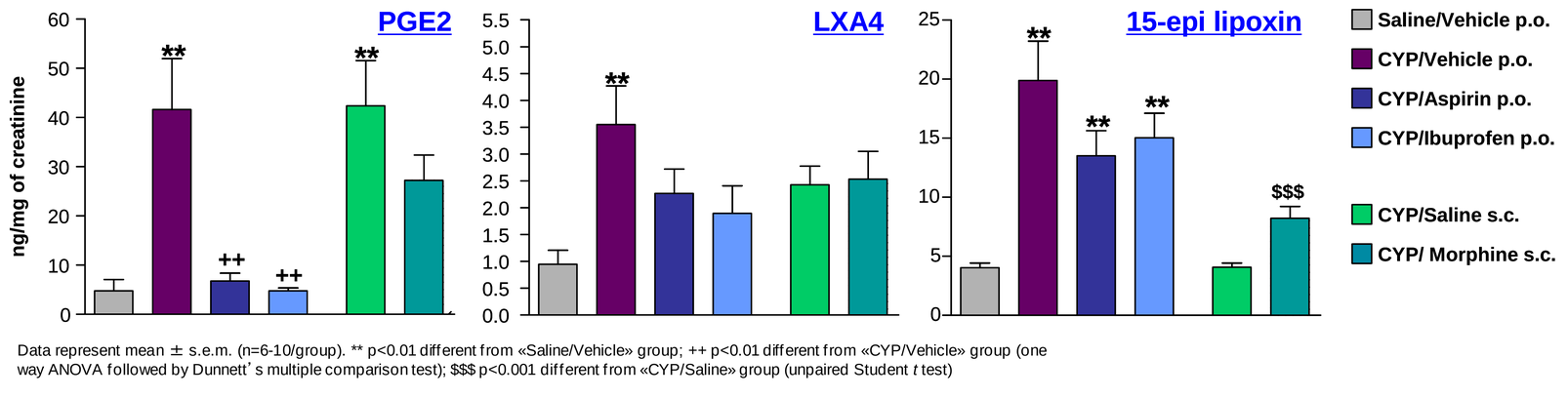


(pg/mg of proteins)	CYP / Saline	CYP / Morphine
IL-1β	15.8 ±2.7	18.8 ±2.2
IL-6	60.5 ±12.0	113.6 ±8.3 $$
MCP-1	241.6 ±46.3	601.6 ±20.7 $$$
VCAM	35.4 ±5.2	39.4 ±5.1

Data represent mean ± s.e.m. (n=6-10/group)
* p<0.05, ** p<0.01 different from «Saline/Vehicle» group; + p<0.05, ++ p<0.01 different from «CYP/Vehicle» group (one way ANOVA followed by Dunnett's multiple comparison test); $$ p<0.01, $$$ p<0.001 different from «CYP/Saline» group (unpaired Student *t* test)

- A 7 to 10-fold increase in bladder levels of IL-1β and IL-6, and a 4 to 5-fold increase in MCP-1 and VCAM were observed 4h after CYP treatment.
- Only ibuprofen reduced bladder levels of IL-1β and IL-6, while morphine increased levels of IL-6 and MCP-1.

Urinary arachidonic acid metabolites are increased after acute CYP



Data represent mean ± s.e.m. (n=6-10/group). ** p<0.01 different from «Saline/Vehicle» group; ++ p<0.01 different from «CYP/Vehicle» group (one way ANOVA followed by Dunnett's multiple comparison test); $$$ p<0.001 different from «CYP/Saline» group (unpaired Student *t* test)

- After CYP injection, significant changes were noted in the urinary levels of arachidonic acid metabolites, such as PGE2 (9-fold increase), LXA4 (4-fold increase) and 15-epi lipoxin (5-fold increase).
- A strong decrease in urinary PGE2 was noticed after both aspirin and ibuprofen treatments, while morphine-treated rats presented an increase in urinary 15-epi lipoxin.

Conclusions

A single i.p. injection of CYP induces an acute inflammation in the urinary bladder 4h post-administration. This reaction is confirmed by high levels of pro-inflammatory cytokines, IL-1β and IL-6, and an increase in chemokine and adhesion molecules, suggesting a strong recruitment of monocytes/macrophages. High levels of lipid-derived mediators are also present in urines. These metabolites play a key role in the regulation and resolution of acute cystitis. In summary, altered visceral sensation following CYP seems to be mediated by inflammatory reactions in the urinary bladder. Antinociceptive effects of NSAIDs are associated with a decrease in key inflammatory mediators such as IL-1β, IL-6 and PGE2, known to promote the sensitization of bladder nociceptors. In contrast, reversal of bladder pain by morphine may not be linked to a direct modulation of bladder inflammation.

References

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