463

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INCREASE IN ARACHIDONIC ACID METABOLITES IN THE URINE OF FEMALE RATS WITH CYCLOPHOSPHAMIDE-INDUCED CYSTITIS.

Hypothesis / aims of study

Cyclophosphamide (CYP)-induced bladder inflammation is a well established pre-clinical model for interstitial cystitis. CYP treatment results in a local inflammation, bladder overactivity and painful sensation. Bladder inflammation could excite the detrusor muscles with chemical mediators and activate the afferent nerve terminals. Mediators of the arachidonic acid cascade have been suggested to play a role in contributing to tone changes of the detrusor and modulating activity of bladder afferent nerves (1). The present study aimed to investigate the changes in urinary prostanoid mediators, particularly prostaglandin E2 (PGE2), leukotriene B4 (LTB4), thromboxane B2 (TXB2) and isoprostane, in the experimental model of CYP-induced cystitis in the female rat, and to correlate the levels of these mediators with progression of urinary bladder inflammation.

Study design, materials and methods

Twelve female Sprague Dawley rats (225-250 g) were used in the present study. Chemical cystitis was induced by a single intraperitoneal injection of CYP (150 mg/kg in saline, 10 mL/kg n=8). Four rats were used as control. Urines were collected using metabolic cages and measured over an hour at different time points before and after CYP administration: -24 (control), +2, +6, +24, +48 and +72 hours. One hour before urine collection, rats received 2 ml of water by gavage to ensure enough urine volume for assays. Some rats were then sacrified at -24 (control, n=4), +24 (n=4) and +72 hours (n=4), and bladders removed. Different inflammatory parameters were assessed: whole urinary bladder wet weight, urinary bladder wall thickness, gross macroscopic analysis (oedema & haemorrhage using Gray's criteria) and myeloperoxidase (MPO, index of granulocyte infiltration) activity. PGE2, LTB4, TXB2 and isoprostane were quantified using enzyme immunoassays (EIA) and normalised to total urine volume collected over an hour.

Results

Compared to control, a single administration of CYP (150 mg/kg) increased urinary bladder wall thickness, urinary bladder wet weight, macroscopic scores and MPO activity (Table 1). Some of these parameters were greater 72 hours post-CYP injection (Table 1). Gross macroscopic analysis showed the presence of an oedema, a dilatation of blood vessels and some erythema.

		Control	CYP +24 hours	CYP +72 hours
Whole bladder wet weight (mg)	Mean ± s.e.m.	56.7 2.2	81.9* 9.2	118.4***++ 5.0
Bladder wall thickness (mm)	Mean ± s.e.m.	0.96 0.09	1.31 0.12	1.24 0.12
Score oedema	Mean ± s.e.m.	0.0 0.0	1.8*** 0.3	1.0***++ 0.0
Score haemorrhage	Mean ± s.e.m.	0.0 0.0	1.8*** 0.3	1.5*** 0.3
MPO activity (mU/mg bladder)	Mean ± s.e.m.	0.4 0.1	26.6*** 4.5	36.3*** 3.0

Table 1. Alterations in inflammatory parameters from female rats treated with CYP (*p<0.05, ***p<0.001 different from "control" value; ++p<0.01 different from "CYP +24hours" value; n=4; one way ANOVA followed by Newman-Keuls multiple comparison test).

CYP-induced bladder inflammation also resulted in a significant increase in urinary levels of PGE2, LTB4 and TXB2 compared to basal (-24 hours). This increase is observed between 2 and 24 hours post-CYP administration, with a peak level at 6 hours (Table 2). Isoprostane level was also significantly increased 6 and 72 hours post-CYP injection, compared to basal (Table 2).

		PGE2	LTB4	TXB2	Isoprostane
-24 hours (basal)	Mean ± s.e.m.	2.076 0.465	1.217 0.196	1.252 0.074	1.449 0.195
+2 hours	Mean ± s.e.m.	6.535*** 1.740	1.166 0.329	2.649* 0.045	1.124 0.286
+6 hours	Mean ± s.e.m.	9.654*** 2.000	4.001*** 0.842	3.327*** 0.526	3.872*** 0.734
+24 hours	Mean ± s.e.m.	2.573 0.335	0.472 0.110	3.153*** 0.681	0.713 0.160
+48 hours	Mean ± s.e.m.	3.037 0.616	0.985 0.365	1.393 0.149	1.120 0.170
+72 hours	Mean ± s.e.m.	2.773 0.617	1.997 0.324	0.840 0.160	2.867* 0.678

Table 2. PGE2, LTB4, TXB2 and isoprostane levels in urine from female rats treated with CYP (values represent ng of peptide in whole urine volume. *p<0.05, ***p<0.001 different from "basal" value, n=4-8; one-way ANOVA followed by Bonferroni/Dunnett test).

Interpretation of results

As previously described (2), a single intraperitoneal dose of CYP produced urinary bladder inflammation, mainly characterized by an increase in urinary bladder weight, sign of a moderate oedema, and an increase in MPO activity, index of granulocyte infiltration. These changes were observed at 24 hours and more marked 72 hours after CYP administration. We also observed an increase in urinary PGE2, LTB4, TXB2 and isoprostane, at early time point (6 hours) post-CYP injection. Previous studies have reported an increase in urinary bladder COX-2 and PGE2 expressions, and urinary PGE2 in the same experimental model (2). Other reports described an increase in urinary prostaglandin PGE2 in patients with overactive bladder (3). The present study confirms some of these data, and also shows that other arachidonic acid metabolites, LTB4, TXB2 and isoprostane, are altered in CYP-induced acute bladder inflammation. Their kinetic of release during inflammation suggests that they seem to be involved in the acute phase of the inflammatory process.

Concluding message

Arachidonic acid metabolites, like PGE2, LTB4, TXB2 and isoprostane, are differentially regulated in the course of CYP-induced cystitis, and may play an important role in the genesis of inflammatory parameters and eventually in bladder overactivity observed in rats pre-treated with CYP. These mediators may be used as urinary biomarkers to assess progression of CYP-induced acute cystitis.

References

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