147

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INVOLVEMENT OF NITRIC OXIDE IN CYCLOPHOSPHAMIDE-INDUCED CYSTITIS IN RATS

Hypothesis / aims of study

Nitric oxide (NO) acts as a neurotransmitter in the urinary bladder. However, the involvement of NO in inducing or preventing cystitis after cyclophosphamide (CP) administration in rats remains to be clarified. Therefore, we examined the role of NO in cystitis induced by CP administration in rats.

Study design, materials and methods

10-13 weeks-old female Sprague–Dawley rats were randomly divided into the following 4 groups: Control group, CP group, CP+L-NAME (15) group, and CP+L-NAME (30) group. Rats in the CP group and both CP+L-NAME groups were intraperitoneally injected with CP (150 mg/kg), whereas rats in the Control group received an intraperitoneal injection of the same volume of saline. Furthermore, rats in CP+L-NAME (15) and CP+L-NAME (30) groups received an intraperitoneal injection of L-NAME (7.5 mg/kg and 15 mg/kg, respectively), an inhibitor of NO synthase (NOS), at 15 min before and at 3 h after the CP injection. Bladder function was examined by cystometry at 1 day after saline or CP administration. In addition, serum and urinary levels of asymmetric dimethylarginine (ADMA), an endogeneous inhibitor of NOS, were examined. Extirpated bladders of rats from all groups were used to examine the expression of inducible NOS (iNOS); protein arginine methyltransferase (PRMT)1, which is an ADMA synthase; and dimethylarginine dimethylaminohydrolase (DDAH)1 and DDAH2, which are catabolic enzymes of ADMA.

Results

Voiding intervals in rats belonging to the CP+L-NAME (30) group were significantly longer than the voiding intervals of rats in the CP group ($P < 0.05$), which were in turn significantly shorter than those of rats in the Control group ($P < 0.05$; Fig. 1A). However, the maximum voiding pressure was not different among all groups (Fig. 1B).

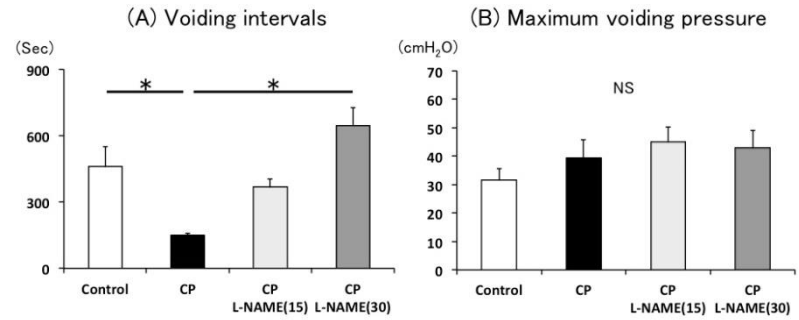

Fig.1 Results of cstometry analysis in each group. (A) Voiding intervals. (B) Maximum voiding pressure. (n = 4–6). Statistical analysis was performed by ANOVA and Bonferroni's multiple t-test. * indicates statistical significance at $P < 0.05$. NS: Not significant.

Changes (from day 0 to day 1) of urinary ADMA levels in CP-treated groups were greater than that in the Control group. Serum ADMA levels were not changed among all groups. iNOS mRNA expression was significantly increased in the CP group compared with that in the Control group ($P < 0.05$; Fig. 2), whereas the expression in CP+L-NAME (15) and CP+L-NAME (30) groups was significantly decreased compared with that in the CP group ($P < 0.05$ for both; Fig. 2). DDAH1 and DDAH2 mRNA expression levels in the CP group were decreased, but not significantly, compared with the levels in Control, CP+L-NAME (15), and CP+L-NAME (30) groups. PRMT1 mRNA expression levels were not changed among all groups.

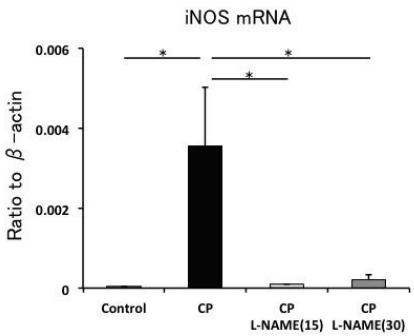

Fig. 2 iNOS mRNA expression levels in each group. (n = 4–6). Statistical analysis was performed by ANOVA and Bonferroni's multiple t-test. * indicates statistical significance at $P < 0.05$.

Interpretation of results

As expected, treatment with CP induced frequent urination with an elevation in iNOS mRNA expression levels in the bladder. Treatment with L-NAME improved voiding interval, and prevented an increase in iNOS mRNA expression in the bladder. Therefore, it is suggested that NO plays an important role in frequent urination. On the other hand, Changes of urinary ADMA levels were increased with a decrease in DDAH1 and DDAH2 mRNA expression in the bladder of rats in the CP group. This phenomenon might be a compensatory mechanism to decrease the NO production because ADMA is an endogeneous NO inhibitor.

Concluding message

In this study, we found that NO production after CP treatment might cause cystitis symptoms. Therefore, prevention of NO production, such as by L-NAME or ADMA administration, might be a useful strategy for treating lower urinary tract symptoms induced by CP administration.

Disclosures

Funding: none **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Rat **Ethics Committee:** The ethics committee of Nagoya City University