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THE RELATIONSHIP BETWEEN THE KYNURENINE PATHWAY IN THE BLADDER AND HEMORRHAGIC CYSTITIS INDUCED BY CYCLOPHOSPHAMIDE

Hypothesis / aims of study

Cyclophosphamide (CYP), an anticancer drug, causes hemorrhagic cystitis as a serious adverse effect. Mesna is used for prevention of cystitis induced by CYP but can not prevent all cases. Moreover, the pathological mechanisms of cystitis have not been revealed completely. Tryptophan is metabolized to kynurenine with indoleamine-2,3-dioxygenase (IDO), and this pathway is called the kynurenine pathway. The two important metabolites in the kynurenine pathway are kynurenic acid (KYNA) and quinolinic acid (QUIN). KYNA is made from kynurenine by kynurenine aminotransferase (KAT), which has neuroprotective activity. On the other hand, QUIN has a neurotoxic property and is made with 3-hydroxyanthranilic acid oxygenase (HAAO). The amount of IDO in the cerebrospinal fluid is increased by inflammation [1]. However, the relationship between the kynurenine pathway and cystitis has not been reported. Thus, we examined the role of the kynurenine pathway in bladder function by using a rat model of cystitis induced by CYP.

Study design, materials and methods

We used 10- to 11-week-old female Wistar/ST rats. Cystitis was induced by intraperitoneal injection of CYP (150 mg/kg body weight). Control rats were administered the same volume of saline. At 24 hours after injection, we performed conscious cystometry (80 μ L/min). Then, we used other rats for tissue evaluation. We injected CYP or saline at the aforementioned dose and removed the bladders 24 hours after injection. We evaluated bladder hypertrophy based on bladder weight-to-body weight ratio. We measured the mRNA expression levels of IDO1, KAT1, and HAAO in the bladders by using real-time polymerase chain reaction analysis and the tryptophan and kynurenine contents of the bladders by using ultraperformance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). Statistical analysis was performed by using the Student *t* test.

Results

Intercontraction intervals were significantly decreased in the cystitis rat model in comparison with those of the control rats ($p < 0.05$). The bladder weight-to-body weight ratio of the cystitis models was significantly higher than that of the control rats ($p < 0.01$). In the cystitis models, the mRNA expression level of IDO1 in the bladder was approximately 23 times as much as the levels in the control rats ($p < 0.01$, Figure 1 left), and that of HAAO was increased to twofold that of the control rats ($p < 0.01$, Figure 1 middle). On the other hand, the expression level of KAT1 did not significantly change between the two groups (Figure 1 right). The kynurenine and tryptophan levels in the urinary tissues from the cystitis rats were upregulated about 18 times (Figure 2) and twice more, respectively, than those in the urinary tissues from the control rats.

Interpretation of results

Shortening of intercontraction intervals and hypertrophy of the bladder are known symptoms of cystitis. In this study, these symptoms were observed as in existing reports. In the bladder of the cystitis models, the mRNA expression level of IDO significantly increased and the kynurenine content also increased in comparison with those in the control rats. These data suggest that the tryptophan metabolism in the kynurenine pathway in the bladder was promoted by cystitis induced by CYP. Moreover, our results suggest that the production of QUIN increased on the basis that the mRNA expression level of HAAO, the producing enzyme of QUIN, increased. QUIN is known to act as a neurotoxin, so we speculated that QUIN acted on the sensory nerves of the bladder and that the increase in QUIN was a factor that shortened the micturition intervals in the cystitis rats.

Concluding message

To our knowledge, this study is the first that focused on the relationship between the kynurenine pathway in the bladder and hemorrhagic cystitis. In this study, we found that CYP induced hemorrhagic cystitis with enhancement of the kynurenine pathway in the bladder, suggesting that the kynurenine pathway might be a new target for treatment and prevention of cystitis. Therefore, we are currently performing further study that involves, for example, measurement of KYNA and QUIN levels in the bladder by using UPLC-MS/MS to determine the detailed mechanisms.

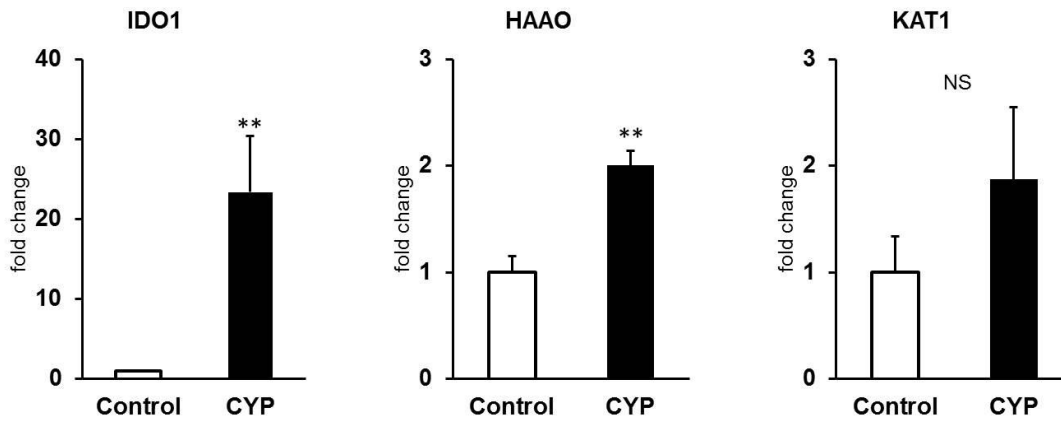


Figure 1. The mRNA expression levels of IDO1, HAAO, and KAT1 in the bladder. n = 3–6. *p < 0.01, Student *t* test. NS: no significance, IDO1: indoleamine-2,3-dioxygenase, HAAO: 3-hydroxyanthranilic acid oxygenase, KAT1: kynurenine aminotransferase 1

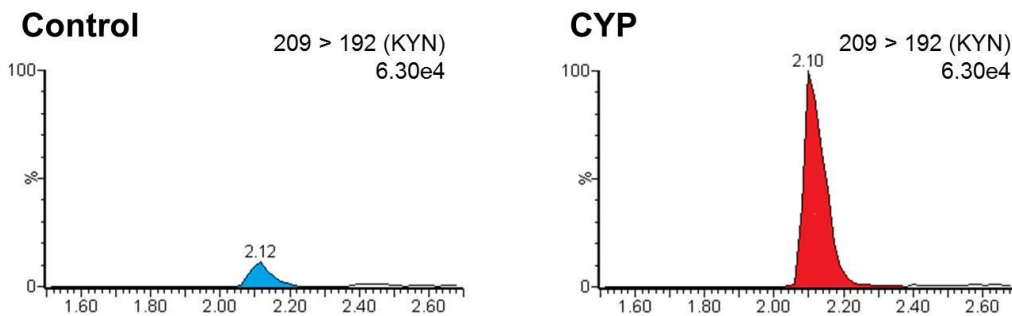


Figure 2. The chromatogram of kynurenine (KYN) in each group. The peak area indicates the abundance of KYN.

References

1. Yan EB, et al. J Neuroinflammation. 12:110. 2015.

Disclosures

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