学 位 論 文 の 要 旨

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学 位 論 文 名　**HEAVY WATER INHIBITS THE EXPRESSION OF TGF-β1 AND THE DEVELOPMENT OF KAOLIN-INDUCED HYDROCEPHALUS IN MICE.**

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論 文 内 容 の 要 旨

Introduction

Communicating hydrocephalus (CH) is caused by various insults. The pathogenesis of CH following subarachnoid hemorrhage (SAH) is assumed to be an impairment of cerebrospinal fluid flow and/or absorption due to leptomeningeal fibrosis (LM) resulting from an inflammatory response. Therefore, antifibrotic therapy is expected to prevent the development of CH. Deuterium is a stable isotope of hydrogen and heavy water (D_2O) has many isotope effects on biological systems. Previous studies demonstrated that D_2O inhibits the proliferation of fibroblasts by overstabilization of microtubules, resulting in the inhibition of collagen synthesis. D_2O has inhibitory effects on the secretion of several hormones, suggesting that D_2O interferes with fibrosis by inhibiting the production of cytokines. The purpose of the present study is to investigate the preventive effects of D_2O on the development of leptomeningeal fibrosis and hydrocephalus induced by kaolin in mice, a model of CH following inflammation, with reference to the effects of D_2O on cytokine production.

Materials and Methods

Male Jcl:ICR mice aged 10-12 weeks were used. To induce hydrocephalus, mice were injected with 5 μl of 2% aluminum silicate (kaolin) in distilled water (DW) into the cisterna magna. The kaolin-injected mice were divided into two groups: (1) a H group received continuous H_2O administration as tap water, and (2) a D group received continuous 30% D_2O administration as tap water from 7 days before injection to the date when sacrificed. H_2O-administered mice injected with DW were designated as a sham-operated (sham) group. Water intake and body weight were measured every 7 days. Locomotive

activities were analyzed on day 0 before injection and on days 7, 14, and 28. Histological examinations in the cisterna magna were performed on days 7, 14, and 28. Transforming growth factor-β1 (TGF-β1) and Mac-3 immunostaining was performed on days 7 and 14. Serum levels of TGF-β1, fibroblast growth factor-2 (FGF-2), platelet derived growth factor (PDGF)-BB and interleukin (IL)-6 were measured by ELISA on days 7 and 14. Proliferative activity in the fibrosis was assessed by the cell density analysis and 5-bromo-2'-deoxyuridine (BrdU) labeling index on day 14. Collagen amount in the cisterna magna was measured with a collagen stain kit on day 28. The cerebral ventricular volume was measured using Scion Image software on day 28. Side effects of D_2O were assessed by biochemical analyses and histological examinations in the brain, intestine, kidney and testis on day 28. Except for the behavioral analysis, we used a factorial ANOVA followed by a *post-hoc* pairwise comparison (Scheffe's F). For the behavioral analysis, we used repeated measure ANOVA. Statistical significance was inferred at $p < 0.05$.

Results

Body weight, water intake, and locomotive activity

Body weights in the H group on days 14 and 28 were significantly lower than those in the D group, while there were no significant differences in body weight between H and sham, or D and sham group. There were no significant differences in water intake or locomotive activity among the groups.

Change in the ventricular size, quantitative analyses of the collagen, cell density, and BrdU labeling index

The quantitative analyses revealed that the ventricular volume in the H group (15.3 ± 3.98 mm^3) was significantly higher than that in the sham group (1.3 ± 0.35 mm^3), while that in the D group (3.3 ± 0.70 mm^3) was significantly smaller than that in the H group. There was no significant difference in ventricular volume between the D and sham groups. The H group demonstrated extensive fibrosis on day 28, whereas less fibrosis was seen in the D group. The collagen deposits in the D group were significantly smaller than those in the H group (H: 145.3 ± 15.80 μg, D: 33.1 ± 9.62 μg). Cell density and BrdU labeling index in the fibrosis were significantly lower in the D group than in the H group (H: 6986.6 ± 869.16 cells/mm^2 and 7.5 ± 1.77%, D: 4970.6 ± 1173.92 cells/mm^2 and 3.0 ± 1.43%) on day 14.

TGF-β1 immunostaining and serum levels of cytokines

TGF-β1-positive cells in the D group were fewer than those in the H group on day 7, and TGF-β1 immunoreactivity was negative in the D group on day 14. TGF-β1-positive cells in the fibrosis were Mac-3-positive macrophages. The choroid plexus epithelium and meninges in the H group were TGF-β1-positive on days 7 and 14, however, in the D group, the choroid plexus and meninges were negative for TGF-β1 on both days. The serum level of total TGF-β1 was not significantly different among the groups on day 7, whereas the serum level of total TGF-β1 in the D group on day 14 (148.5 ± 59.32 pg/ml, n=4) was significantly

lower than that in the H group (307.8 ± 44.82 pg/ml, n=4). The serum levels of FGF-2, PDGF-BB, and IL-6 on day 14 showed no significant differences among the groups.

Side effects

Histopathological examinations in the brain, intestine, kidney, and testis showed no apparent abnormal findings related to D_2O toxicity. Biochemical analyses revealed that there was no significant difference between the D and sham groups except for blood urea nitrogen (BUN), whereas there were no significant differences in any items between the H and D or between the H and sham groups.

Discussion

D_2O effects on fibrosis in the development of kaolin-induced hydrocephalus

The present histopathological study showed that D_2O inhibited progression of hydrocephalus and severe fibrosis induced by kaolin in the cisterna magna. Cell density and collagen assays in conjunction with the BrdU labeling index suggested that D_2O inhibited the fibroblast proliferation and collagen synthesis. These results indicated D_2O may directly interfere with fibroblast proliferation by overstabilizing microtubules, thereby decreasing collagen production.

D_2O effects on cytokine expression

In the present study, the serum level of total TGF-β1 was significantly higher in the H group than in the D group on day 14. In the H group, TGF-β1 immunoreactivity apparently increased in the macrophages, choroid plexus, and meninges, which are known as sources of TGF-β1 secretion. On the other hand, in the D group, these cells and tissue were TGF-β1-negative on day 14, which corresponded well to the serum TGF-β1 level. These results indicated that D_2O inhibited TGF-β1 expression in the macrophages, choroid plexus, and meninges, which may also contribute to inhibiting fibroblast proliferation and collagen synthesis.

D_2O toxicity

Oral administration of 30% D_2O did not show apparent toxicity in the histopathological study. Biochemical analyses revealed that BUN in the D group was significantly, albeit mildly, higher than in the sham group, whereas there was no significant difference between the H and D groups. BUN in the D group increased to a lesser extent than in the past report, and creatinine was not significantly different among the groups, while the kidney was histologically intact. Administration of 30% D_2O in the present study thus seems almost harmless.

Conclusions

D_2O prevented the development of kaolin-induced hydrocephalus in mice, and inhibited intrameningeal fibrosis and upregulation of TGF-β1.