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Effect of colchicine on hepatic glycosaminoglycan metabolism in mice infected with *Schistosoma japonicum*[1]

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AIM: To study the effect of colchicine on hepatic glycosaminoglycan (GAG) metabolism in mice infected with *Schistosoma japonicum*. **METHODS:** The amount of GAG was measured in the liver of mice infected with *Schistosoma japonicum* for 6—16 wk and treated with colchicine. **RESULTS:** Six weeks after infection, the GAG content of infected mice's livers and uninfected control was 14 ± 2 μg/g liver and 4 ± 1 μg/g liver, respectively. The GAG cintent reached a peak about 6 times of the normal level in the 10th wk after infection, being 56 ± 9 μg/g and 10 ± 1 μg/g for infected and uninfected mice respectively, and then declined. Treatmetn of infected mice with colchicine reduced the total GAG content to 22 ± 3 μg/g liver, but not suppress the GAG content below the normal level in the 10th wk. Microscopic hepatic egg granuloma. **CONCLUSION:** Colchicine may exerts its GAG reduction effect through the suppression of granuloma formation.

KEY WORDS *Schistosoma japonicum*; colchicine; glycosaminoglycans; liver

Hepatic fibrosis in schistosomiasis is associated with accumulation of collagen and extracellular glycosaminoglycans (GAG)[1]. The liver GAG content of *S mansoni*-infected mice was 5 times higher in acute infection[2]. In advanced liver cirrhrosis in man, the heparan sulfate content increased to 4.7 times the normal value and the dermatan sulfate content increased by a factor of 15[3]. Since GAG content and composition changed greatly in live cirrhosis, agents that mediate the metabolism of GAG and collagen might be used as antifibrotic drugs. Colchicine was used in the treatment of patients with schistosomal hepatic fibrosis and cirrhosis of other etiology with good results[4]. In the present study we studied the effect of colchicine on hepatic GAG content in the first 16 wk after the mice were infected with *S japonicum* in order to define the mechanism of action of colchicine on the heptic fibrosis in schistosomiasis.

MATERIALS AND METHODS

Mice and parasites Mice of Kunming strain were from a breeding colony in Shanghai Medical University. Cercariae were obtained from a life cycle maintained by the Department of Epidemiology of the same university. The parasites were originally isolated in Anhui Province in 1975 and have been maintained in the snail *Oncomelania hupensis*.

Infection and treatment Each mouse was infected with 20 cercariae penetrating the abdominal skin in supine position after 6 wk. The mice were divided into 2 groups. One group of mice received colchicine (0.2 mg·kg^{-1} ig, 6 times a wk, and the other group did not receive any. All the mice were kept under the same conditions. After 6, 10, 13, and 16 wk of infection, 1/4 of mice in each group were killed by cervical dislocation and the liver GAG were extracted and assayed.

GAG extraction The liver was homogenized and boiled at 100 ℃ for 5 min. Lipids were removed with 10 vol of acetone and 5 vol of methanol : chloroform (1 : 3, vol/vol), and the sample was dried at 25 ℃.

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After treatment with NaOH 0.5 mol·L^{-1} at 4 ℃ overnight, the sample was dialyzed against phosphate buffer 0.06 mol·L^{-1} pH 7.6, and then digested with pronase E (25 μg/g dry weight of delipidated sample). Protein and peptides were precipitated from the digest with cold TCA and removed by centrifugation. The supernatant was dialyzed against distilled water and the GAG was concentrated by precipitation with 5 % cetylpyridium chloride[15] for microanalysis of GAG.

Crude GAG sample (0.1 ml) was stained with 0.05 % Alcian blue 0.5 ml[16] at room temperature for 1 h. The resultant complex was washed with cold (4 ℃) NaAc 50 mmol·L^{-1}, then dissolved in NaAc 50 mmol·L^{-1} (heated at 60 ℃ for 10 min), pH 5.8, containing 2 % sodium dodecyl sulfate. The absorption at 620 nm was measured against hyaluronic acid and was linear in the range of 2—10 mg·L^{-1}.

Microscopic examination Livers of mice were fixed in 10 % neutral formalin. Paraffin sections were observed under light microscope.

RESULTS

The formation of granulomas in mouse livers infected with *S japonicum* was observed microscopically. The liver from the uninfected mice were grossly normal (Fig 1A, Plate 2). The sections from mice infected for 6—10 wk showed a great number of granulomas in the portal regions. Fig 1B (Plate 2) showed an eosinophilic abscess in a mouse liver 6 wk after infection. The eosinophilic abscesses gradually turned into fibrotic granulomas at 10th wk of infection (Fig 1C, Plate 2). Much less granulomas were found at 13 wk, the granulomas in 6—13 wk were at about the same developmental age, ie, all the mice appeared to have a single wave of egg laying and concomitant granuloma formation.

The inhibitory effect of colchicine on the formation of liver granulomas in mice infected with *S japonicum* was obvious. the size of granulomas in the portal region was much smaller than that in the untreated mice. Most of the granulomas regressed, only a few small abscesses remained in livers of mice treated with colchicine (Fig 1D, Plate 2).

Six weeks after infection, the GAG content of liver was higher in the infected mice than that in the uninfected mice ($P<0.01$). Ten weeks after infection, the GAG content of the liver of infected mice was 6 times that of the uninfected mice. After 13 wk of infection the GAG content decreased but still remained to be higher *vs* the control ($P<0.05$). The GAG content of mice infected for 16 wk was not significantly different from that in the controls. It appeared that the GAG content rose and waned with the formation and regression of granulomas (Tab 1).

Tab 1. Effect of colchicine on liver glycosaminoglycan content (μg/g wet weight liver) of schistosome-infected mice. $\bar{x}\pm s$.

	Normal	Infected	Treated
6 wk	4±1 (5)	14±2 (5)	—
10 wk	10±1 (8)	56±9 (8)	22±3 (9)
13 wk	11±1 (7)	20±2 (7)	16±2 (8)
16 wk	8±1 (7)	12±2 (9)	9±1 (9)

Number of ♀ mice (28±2 g) in parenthesis.

Mice treated with colchicine for 4 wk beginning from the 6th wk of infection had a liver GAG content of about 50 % of that in untreated infected mice ($P<0.05$). After 13 wk of infection, ie, having been treated with colchicine for 7 wk, the GAG content was still slightly less than that in untreated infected ($P>0.05$). At 16 wk, the GAG contents in the control, the infected, and the treated mice showed no difference.

It was obvious that colchicine partially blocked the GAG accumulation in infected mice especially at 10th wk after infection.

DISCUSSION

The liver GAG content of *S japonicum* in-

fected mice and colchicine treated animals was assayed at 6th, 10th, 13th, and 16th wk, with the highest level at the 10th wk. On the other hand, the microscopic examination of liver sections showed that most eggs in the granuloma matured at 10th wk. At 13th and 16th wk, the GAG content decreased gradually parallel with the regression of granulomas. Thus the increasing GAG synthesis must be the result of granuloma formation. Our results also showed that colchicine inhibited the formation of hepatic egg granuloma and concomitantly inhibited the increase of GAG content in infected animals. Thus colchicine may exert its effect through the suppression of granuloma formation.

The regression of granulomas in infected mice at 13th, 16th wk render the observation and explanation of colchicine efficiency more difficult. Although this phenomenon had been seen in other animal models[5] for reasons yet unknown, it is likely that the explanation of the action of colchicine on the bases of 10th wk experimental results is reasonable, because the difference between the liver GAG content and microscopic observation of infected and treated mice during the increasing phase is remarkable.

Although it has been known that schistosome egg granulomas played an important role in GAG and collagen synthesis and secretion[8], the cells that synthesize GAG and substrates that induce the GAG synthesis and secretion are to be further delineated. Colchicine could also possibly decrease the liver GAG content through accelerating the GAG degradation. Therefore, to fully explore the mechanism by which colchicine decreased the liver GAG content in schistosome-infected mice, further experiments that measure the effect of drug on GAG synthesis and degradation rate *in vivo* and *in vitro* are necessary.

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秋水仙碱对日本血吸虫感染的小鼠肝葡糖胺基聚糖代谢的影响

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目的：研究秋水仙碱对日本血吸虫感染的小鼠肝葡糖胺基聚糖（GAG）代谢的影响。**方法**：被日本血吸虫感染 6—16 wk 的小鼠用秋水仙

碱治疗，测量小鼠肝 GAG 的含量，显微镜观察感染后第6，10，13 wk 小鼠肝脏组织切片。**结果**：感染6 wk 以后，小鼠肝 GAG 含量显著高于未感染的对照组。GAG 含量在第10 wk 达到最高峰，约为正常水平的6倍；感染组为56±9 μg/g，无感染组为10±1 μg/g。10 wk 后，无感染组 GAG 水平开始下降。秋水仙碱治疗使感染10 wk 小鼠的 GAG 含量比感染对照组降低了约50 %，即22±3 μg/g，说明在第10 wk 秋水仙碱不能使 GAG 含量下降到正常水平以下。 **结论**：秋水仙碱可能通过抑制肉芽组织的形成从而降低被日本血吸虫感染的小鼠肝 GAG 的含量。

关键词 日本血吸虫；秋水仙碱；葡糖胺基聚糖类；肝

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Endogenous adenosine and ATP-sensitive potassium channel modulate anoxia-induced electrophysiological changes of pacemaker cells in sinoatrial node of guinea pigs[1]

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AIM: To investigate the electrophysiological effects of adenosine deaminase (ADase, an enzyme converting adenosine to inosine and ammonia), 8-phenyltheophylline (8-PT, a non-selective antagonist of adenosine receptors) and glibenclamide (Gli, a potent blocker of ATP-sensitive K^+ channels) on anoxic pacemaker cells of SA node. **METHODS**: Anoxia of pacemaker cells in SA node of guinea-pig was induced by perfused for 20 min with a modified K-H solution gassed with 100 % N_2 deprived of glucose. Parameters of action potentials including maximal diastolic potential (MDP), amplitude of action potential (APA), duration of 90 % repolarization (APD_{90}), maximal rate of depolarization (V_{max}), rate of pacemaker firing (RPF), and velocity of diastolic (phase 4) depolarization (VDD) were recorded using intracellular microelectrodes. **RESULTS**: Anoxia increased MDP, APA, and V_{max} and decreased VDD, RPF in a time-dependent manner. ADase 10 $U \cdot L^{-1}$, 8-PT 0.1 $\mu mol \cdot L^{-1}$ and Gli 10 $\mu mol \cdot L^{-1}$ significantly attenuated the electrophysiological changes of pacemaker cells in sincatrial node induced by anoxia. **CONCLUSION**: Endogenous adenosine and ATP-sensitive K^+ channels may play an important role in the generation of anoxic bradycardia in guinea pigs.

KEY WORDS anoxia; adenosine; adenosine deaminase; glyburide; potassium channels; sinoatrial node; electrophysiology

Endogenous adenosine is primarily formed from dephosphorylation of AMP that may occur intracellularly or extracellularly[1] and may be derived from hydrolysis of s-adenosylhomocysteine (SAH)[2]. In hypoxic myocardium, adenosine release from SAH hy-

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