EXPERIMENTAL STUDY ON THE DEVELOPMENT
OF LIVER CELL CARCINOMA
— ROLE OF HISTAMINE IN CHRONIC LIVER INJURY —

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ABSTRACT

Role of autotallergic mechanism on the chronicity of liver diseases with special reference to histamine, which is said to be one of the chemical mediators and plays a large part in antigen antibody reaction, was investigated experimentally.

Under the administration of homologous liver antigen with histamine to rabbits, liver injury closely resembling chronic active hepatitis could be developed, which progressed into liver cirrhosis with pseudolobulus formation. This result may indicate that the progression of chronic hepatitis into liver cirrhosis can be regarded as one process. Estrogen promotes antigen antibody reaction and plays a large part in prolongation and aggregation of liver diseases. Furthermore, liver cell carcinoma was demonstrated in one animal and epithelial hyperplasia of bile ductules in two animals. This fact may suggest that chronic hepatitis changes into malignant tumor and perpetual antigenic stimulation has a large role in the development and progression of malignant tumor and role of histamine should never be ignored.

Although many clinical and experimental investigations are required to clarify the mechanism of chronicity of liver diseases, the immunological disturbances including autoallergic mechanism cannot be ignored and the role of histamine, which has been said playing a large role in such process, must also be taken into consideration. The present study was designed to investigate the relation of chronicity of liver diseases and histamine experimentally with further probability of participating in malignant changes.
MATERIAL AND METHODS

Material: male albino rabbits, weighing about 3 kg of body weight.
Antigen: homologous liver solution obtained from normal rabbits and diluted in physiological saline to 1 to 6.
Adjuvant: Freund's complete adjuvant.
Histamine: free base histamine diluted by distilled water to 1 mg/ml.
Follicular hormone: estradiol-17-β-valerate in oil solution.

Methods: five ml of homologous antigen in 5 ml of complete Freund's adjuvant was administered at 15 to 20 sites of each rabbits hypodermically. After two weeks only antigen was injected via ear vein. Histamine was injected at the dosage level of 0.5 mg/kg of body weight every day through ear vein. Follicular hormone was administered intramuscularly in an amount of 3.0 mg/kg every four weeks. Prior to sensitization, the serum protein, especially gammaglobulin fraction, was examined.

RESULTS

a) Antigen in adjuvant (15 animals)
Histological findings in this group revealed increase in number and size of Kupffer's cells and spotty necroses in parenchyma as well as an increase in fiber formation with cell infiltration in mesenchyma. The serum gammablobulin level increased gradually (Fig. 1).

b) Histamine administration (9 animals)
Conspicuous histological findings of this group was fiber formation in the mesenchyma. The serum gammaglobulin level increased gradually (Fig. 1).

c) Histamine with homologous liver antigen (31 animals)
The gammaglobulin level in this group increased comparatively rapidly (Fig. 1). Histologically, granulomatous changes, proliferation of plasma cells and enlargement of Kupffer's cells in parenchyma were evident. In mesenchyma, fiber formation with lymphocytic proliferation was seen in almost all animals. However, destruction of limiting plates closely resembling active chronic hepatitis was most outstanding in this group and finally they progressed into liver cirrhosis with pseudolobulus formation (Fig. 2, 3). It is noteworthy, however, that there could be noticed also liver cell carcinoma (Fig. 4), which metastasized to the lungs (Fig. 5), spleen and kidneys (Fig. 6) and epithelial hyperplasia of bile ductules (Fig. 7) in this group.
d) Follicular hormone and antigen with adjuvant (11 animals)

Seven weeks after sensitization, the serum gammaglobulin was markedly

![Graph showing change of serum γ-globulin in liver injury induced substances (Rabbits).]
Fig. 2 Histamine and homologous liver extract in complete Freund's adjuvant (Rabbit, Hematoxylin and eosin, × 92). Border between parenchyma and portal tract is not sharp because of destruction of limiting plates of parenchyma. Closely resembling active chronic hepatitis.

Fig. 3 Histamine and homologous liver extract in complete Freund's
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Fig. 4 Histamine and homologous liver extract in complete Freund's adjuvant (Rabbit, after 34 weeks' sensitization, Hematoxylin and eosin, x 170). —Development of malignant tumor—. The cancer cells are enclosed by connective tissue and occupy the mid portion of the figure and almost normal liver cells can be seen in the lower portion.

Fig. 5 Histamine and homologous liver extract in complete Freund's adjuvant (Rabbit, same animal as Fig. 4. Hematoxylin and eosin, × 96) Metastasis to the lung.
Fig. 6  Same animal as Fig. 4. Metastasis to the kidney. (Hematoxylin and eosin, × 96).

Fig. 7  Histamine and homologous liver extract in complete Freund's adjuvant (Rabbit, after 40 weeks' sensitization Hematoxylin and eosin, × 170) Marked proliferation of bile ductules and epithelial hyperplasia of bile ductules can be seen enclosed by connective
elevated and hypergammaglobulinemia was evident (Fig. 1). Histology showed enlargement and proliferation of Kupffer's cells in parenchyma. Furthermore, destruction of limiting plates was seen which progressed into pseudolobulus formation (Fig. 8).

**DISCUSSION**

On the mechanism of the development of chronic hepatitis is still in controversy and many attempts have been made to induce it in experimental animals with sensitization. The authors have been studying the role of histamine in liver diseases because it plays a large part in antigen/antibody reaction. Moreover, it is well known that histamine promotes fibroplastic activity and the transition into cirrhosis depends more on the fibroplastic than the immunologic component, the role of histamine cannot be ignored.

The histological findings of active form of chronic hepatitis manifest that of mesenchymal hypersensitivity, which may progress into cirrhosis and more importantly malignant changes. This mechanism still remains obscure, however,
more recently clinical cases dealing with chronic hepatitis which progressed into liver cell carcinoma have published. It is also well known that malignant diseases complicate with autoallergic diseases in high frequency and malignant changes are extremely high in autoallergic diseases. Although it cannot be easily determined whether his process is resulted from primary disease itself or not, perpetual stimulation by antigen with resultant degenerative and regenerative process of cells must be taken into account. If autoallergic mechanism is involved in the development of chronic hepatitis, it may be said that there exists a close relationship between chronic hepatitis, liver cirrhosis and liver cell carcinoma. In fact, as mentioned above, this process is well documented clinically.

On the other hand, in considering the patients with “lupoid” hepatitis from immunological aspect including autoallergic process, the effect of follicular hormone must also be taken into consideration. On the role of estrogen in liver diseases, it has already reported that estrogen is one of the prolongative and aggravating factors. In the present study estrogen was given during the sensitization period and resulted in the development of these results that estrogen aggravates liver injuries with resultant hypergammaglobulinemia. Furthermore, it is said that follicular hormone and male sex hormone react antagonistically upon the methylation of histamine, the former inhibitory and the latter promotive. Thus hyperestrogenemia may also participate in the development of hyperhistaminemia in chronic liver diseases.

CONCLUSION

1) The role of immunological process with special reference to histamine in the chronicity of liver diseases was investigated experimentally.

2) The progression of chronic hepatitis into liver cirrhosis was demonstrated experimentally and can be regarded as one process.

3. Estrogen promotes antigen/antibody reaction and plays a large part in prolongation and aggravation of liver diseases.

4) With prolonged sensitization of rabbits with homologous liver antigen, liver cell carcinoma and epithelial hyperplasia of bile ductules were developed. This results may suggest that chronic hepatitis changes into malignant tumor and perpetual antigenic stimulation has a large role in the development and progression of malignant tumor.

REFERENCES


