学 位 論 文 の 要 旨

氏 名 山 口 恵 実

学 位 論 文 名	Experimental Investigation of the Role of Endothelin-1 in Idiopathic Portal Hypertension
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著 者 名	Emi Yamaguchi, Akira Yamanoi, Takashi Ono and Naofumi Nagasue

論 文 内 容 の 要 旨

INTRODUCTION

Idiopathic portal hypertension (IPH) is defined as an elevation of portal pressure in the absence of cirrhosis or any extrahepatic portal venous obstruction and is clinically characterized by portal hypertension, splenomegaly and pancytopenia. Several clinicopathological studies and relevant animal experiments have suggested that IPH might be caused by an immunological mechanism or chronic ingestion of arsenicals, but the precise mechanisms of IPH have not been elucidated in detail.

Endothelin-1 (ET-1) is one of the major humoral factors modulating portal pressure. Previous reports including our previous study showed that ET-1 was elevated in the peripheral blood of chronic liver disease or IPH patients, and that ET-1 was released from B lymphocytes in the germinal center of lymphoid follicles of the spleen.

The aim of the current study is to investigate how ET-1 is involved in the development of portal hypertension induced by chronic arsenical ingestion, and which kind of hematocytes induces ET-1 under various kinds of stimuli.

MATERIALS AND METHODS

Portal pressure and venous ET-1 concentrations were measured in male Sprague-Dawley rats which were housed for 8 to 20 weeks with free access to drinking water containing 5, 10, 50 or 100 p.p.m. of sodium arsenate. ET-1 expression in the spleen was assessed by immunohistochemical staining. B and T lymphocytes and monocyte-derived macrophages (MDMs) cultured from healthy human peripheral blood were stimulated with sodium arsenite, sodium arsenate, lipopolysaccharide (LPS) and interferon-γ (IFN-γ). ET-1 production of these cells was assessed by measuring ET-1 concentrations in the cell culture supernatants by ELISA.

RESULTS AND DISCUSSION

The low dose arsenic (5 or 10 p.p.m.) group tended to have an elevated portal pressure (p = 0.173, r^2 = 0.120), and the portal venous ET-1 concentration increased over the duration (p = 0.047, r^2 = 0.236). Such change in portal pressure and ET-1 concentration were not seen in the high dose arsenic group (50 or 100 p.p.m.). Immunohistochemical staining for ET-1 of the spleen failed to show the increased ET-1 expression in any splenic cells. We speculate that exposing to arsenical over a period of years may be necessary for the remarkable increase in portal ET-1 concentration and expression of ET-1 in the spleen. Furthermore, arsenic poisoning is classified as acute intoxication or as chronic poisoning. Low dose arsenic exposure may lead to chronic poisoning such as IPH, whereas high dose exposure results in acute intoxication.

As no direct evidence that ET-1 was released from the spleen especially from B lymphocytes have been shown, we accordingly tried in the present *in vitro* study to clarify which cells, such as lymphocytes, macrophages and splenic cells, could produce ET-1. No spontaneous release of ET-1 by arsenic stimulation was however observed in any

hematocytes obtained from human peripheral blood.

There are some clinical and experimental reports that IPH is associated with infectious disease, and an important role of ET-1 in the pathogenesis of LPS-induced inflammatory reactions was speculated. Thus, to clarify whether ET-1 is exactly produced by B lymphocytes, LPS and IFN-γ, which are infection-related factors, are used for the stimulation of hematocytes. Stimulation with either LPS (10 ug/ml) or IFN-γ (100-1000 U/ml) significantly increased ET-1 release from B lymphocytes. ET-1 release from MDMs was significantly increased by LPS treatment (1-10 U/ml) in a dose-dependent manner. T lymphocytes did not release ET-1 in response to either LPS or IFN-γ stimulation. These results may suggest that immunological factors might be necessary for the development of IPH in combination with arsenicals.

CONCLUSION

The present study indicates that long-term ingestion of arsenicals might elevate portal pressure through the accelerated ET-1 production. LPS and IFN-γ clearly induced ET-1 synthesis not only in B lymphocytes but also in MDMs, although exposure to arsenic only did not affect these cells. This study partially supports our hypothesis that IPH might be promoted by ET-1 production from B lymphocytes in response to certain substances.