SERUM PROTEASE INHIBITORS IN OPISTHORCHIASIS, HEPATOMA, CHOLANGIOCARCINOMA, AND OTHER LIVER DISEASES

SUPRANEE CHANGBUMRUNG, PANATA MIGASENA, VENUS SUPAWAN, PIMATHORN JUTTIJUDATA and TALABPORN BUAVATANA

Department of Tropical Nutrition and Food Science, Faculty of Tropical Medicine, Mahidol University, Bangkok 10400, Thailand.

INTRODUCTION

Human liver fluke infection *(Opisthorchis viverrini)* is endemic in the northeast of Thailand (Harinasuta and Vajrasthira, 1960; Sornmani *et al.*, 1981), where the consumption of raw fish or half-cooked fish is popular (Migasena, 1982). The prolonged and frequent exposure to *Opisthorchis* infection may produce liver cancer (Harinasuta and Vajrasthira, 1960; Bhamarapravati and Viranuvatti, 1966; Sonakul *et al.*, 1978; Thamavit *et al.*, 1978).

Changbumrung *et al.*, (1982) reported an increase of surum protease inhibitors (PI), i.e α_1-antitrypsin (A1PI), α_2-macroglobulin (α_2M) and α_1-antichymotrypsin (Ach), in human opisthorchiasis. Serum A1PI is also reported to be increased in malignancies and liver diseases (Snyder and Ashwell, 1971; Carlson and Eriksson, 1980). The purpose of this study is to examine the possibility that PI may play a role in liver diseases, whether the increase of PI in opisthorchiasis is either synergistic or antagonistic to either infection or malignancy or both. The serum PIs were investigated in opisthorchiasis, hepatoma, cholangiocarcinoma and other liver diseases, i.e. amoebic liver abscess, hepatic cirrhosis, hepatitis, carcinoma of the head of the pancreas including healthy controls.

MATERIALS AND METHODS

Fasting venous blood was obtained from patients suffering from various hepatic diseases and liver fluke infection. Those patients with opisthorchiasis were admitted to the Hospital for Tropical Diseases, and all came from the northeast of Thailand. In the case of all the other conditions the patients were from Pramongkutklao Hospital and unfortunately there was no record of the domicile of the patients. It was known that those with cholangiocarcinoma were all infected with *O. viverrini* (Table 1).

The control sera were obtained from laboratory personnel and healthy volunteers from the Armed Forces. Samples from both men and women were used as serum concentration of α_2M is reported to be slightly higher in women than men (Schultze and Heremans, 1966).

The hepatic diseases, i.e. hepatoma or primary liver cells carcinoma was diagnosed histopathologically. The liver aspiration was carried out in the cases of amoebic liver ab-

Table 1
Demographic data of cases studied.

Condition	No. Sex (M + F)	Age range (Years)	Average age (Years)
Healthy controls	27M + 13F	20 – 51	37
Opisthorchiasis	43M + 20F	13 – 68	35
Hepatoma	30M	20 – 69	47
Amoebic liver abscess	11M + 1F	15 – 65	35
Cholangiocarcinoma	6M	30 – 61	42
Hepatic cirrhosis	5M	40 – 67	50
Hepatitis	2M + 3F	14 – 67	36
Carcinoma of the head of pancreas	4M + 1F	33 – 63	50

scess. The hepatic cirrhosis and hepatitis were based on clinical and biochemical background. The cholangiocarcinoma and carcinoma of the head of pancreas were confirmed by surgery. In opisthorchiasis, the number of *O. viverrini* ova in the faeces determined by Stoll's technique (1926) was in the range of 400–54,640 ova per gram faeces.

Serum was separated, then stored at −20 °C and thawed before electrophoresis. Rocket immuno-electrophoresis was carried out according to the Laurell technique (1972) for serum A1PI, Ach and α_2M. The antisera produced in rabbits for the different proteins were purchased from Behring Institute, Marburg, Germany. Quantitation was made using control sera obtained from this manufacturer. Bilirubin measurements were made by the method of Malloy and Evelyn (1937) modified by Ducci and Watson (1945).

RESULTS

Serum concentrations of A1PI, Ach and α_2M for all the groups studied are shown in Table 2. In Fig. 1 the results are presented as a bar chart to show the percent deviation of the median from the controls for each disease. Patients with cancers tend to have high concentration of PIs while the groups of patients with opisthorchiasis and hepatitis tend to show lower concentrations. Only those patients with hepatic cirrhosis have highly elevated α_2M concentrations, whereas all other groups tend to have low concentrations.

Analysis of the data was done using non-parametric tests, as distribution of results tended to be asymmetric. In the case of opisthorchiasis the concentration of PIs was only slightly elevated but significant for both A1PI ($p < 0.0005$, Mann-Whitney U test) and α_2M ($p < 0.0143$, Mann-Whitney U test). When control and other groups were tested by the Mann-Whitney U test, the significance level of A1PI was at $p < 0.001$ for all groups, for Ach was $p < 0.005$ or less, except for opisthorchiasis ($p < 0.0643$), and for α_2M significance level ranged from $p < 0.03$ or less.

The significant difference between opis-

thorchiasis and other liver diseases, the significance level of Ach ranged from $\bar{p} < 0.0465 - \bar{p} < 0.001$; for A1PI it ranged from $p < 0.0162 - p < 0.001$ except for hepatic cirrhosis ($\bar{p} < 0.0630$), and for α_2M it ranged from $p < 0.0526 - p < 0.001$ except for hepatitis ($\bar{p} < 0.0869$) and amoebic liver abscess (NS).

For the difference between hepatoma and hepatitis, the significance level for Ach was $p < 0.001$. Correlation between age and PIs were examined using Spearman rank correlation coefficient which indicated no correlation between age and each PI.

Serum bilirubin concentrations in patients infected with *O. viverrini* were in normal range (mean and standard deviation of total bilirubin = 0.6 ± 0.4, direct-reacting bilirubin = 0.3 ± 0.2 mg/dl).

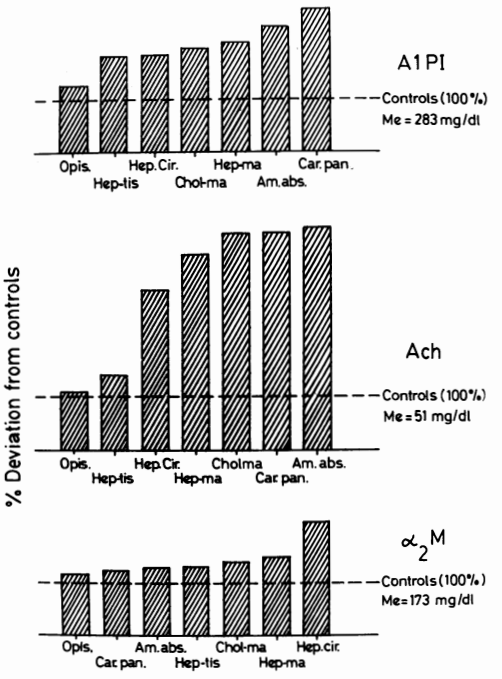

Fig. 1–Increase in median (Me) of serum PI in human liver fluke infection (Opisthorchiasis) and liver diseases. Opis. = opisthorchiasis, Hep-tis = hepatitis, Hep. cir. = hepatic cirrhosis, Chol-ma = cholangiocarcinoma, Am. abs. = amoebic liver abscess, Car. pan. = carcinoma of the head of pancreas.

DISCUSSION

The function of the serum PIs may be to restrict the activity of proteases released during physiological and pathological processes both in the blood stream and in extravascular tissues. During the inflammatory response in infection when granulocytes migrate into the tissue, eventually releasing their enzymes which might result in vascular injury and other tissue damage at the site of inflammation, A1PI may be important as an inhibitor of the proteases released from the granulocytes for limiting this adverse effects in the tissue (Koj, 1974). Evidence has been shown that lysosomal proteases can be inhibited by A1PI and α_2M (Kuepper and Bearn 1966; Koj *et al.*, 1972; Barret and Starkey, 1973). Ach is rather specific in reacting only with chymotrypsin (Hensen and Loeliger, 1963).

Histological studies on 154 cases of liver fluke infection indicated a prevalence of liver carcinoma of 56.6% and that the tumours were cholangiocarcinoma in 67 cases (77%), hepatocellular carcinoma in 9 cases (10.3%) and the others (Sonakul *et al.*, 1978). In addition in 14 autopsy cases of cholangiocarcinoma, 11 cases were associated with opisthorchiasis (Bhamarapravati and Viranuvatti, 1966). In an animal experiment, *O. viverrini*-infected hamsters fed with dimethyl nitrosamine, which is a potent carcinogen, all developed cholangiocarcinoma (Thamavit *et al.*, 1978). In patients suffering from opisthorchiasis, serum bile acids i.e. taurocholic acid, taurochenodeoxycholic acid, glycochenodeoxycholic acid, were increased when compared with the healthy controls (Miga-

Table 2

Serum protease inhibitors, A1PI, Ach and α_2M (mg/dl) in liver fluke infection, various hepatic diseases and healthy controls.

Groups studied	No.	A1PI	Ach	α_2M
Controls	40	283* (146-415)	51 (29-101)	173 (126-319)
Opisthorchiasis	63	360 (214-729)	55 (25-160)	204 (121-300)
Hepatoma	30	587 (163-796)	185 (80-248)	263 (164-431)
Amoebic liver abscess	12	673 (146-954)	211 (120-266)	227 (123-428)
Cholangiocarcinoma	6	555 (434-791)	205 (168-323)	247 (213-287)
Carcinoma of the head of pancreas	5	766 (556-826)	206 (166-238)	217 (182-334)
Hepatic cirrhosis	5	517 (454-974)	152 (103-326)	376 (211-488)
Hepatitis	5	512 (419-551)	72 (54-118)	231 (217-286)

* Median, and the range is shown in parenthesis.
By the Kruskal Wallis one-way analysis of variance for all groups, the significance level of A1PI, Ach and α_2M was $P < 0.001$.

sena *et al.*, 1983). Nitroso bile acid conjugates such as N-nitrosotaurocholic acid and N-nitrosoglycocholic acid showed the mutagenic effect using Ame's test (Puju *et al.*, 1982). This evidence indicated that *O. viverrini* acts therefore as promoter of chemically induced cholangiocarcinoma.

Carlson *et al.*, (1981) suggested that A1PI was PI which may have an important protective role in liver disease. Tissue A1PI is present in neoplastic tissue macrophages, peripheral blood monocytes and is identical with serum A1PI (Isaacson *et al.*, 1981). They stated with confidence that A1PI is synthesised by human macrophages. A1PI is also present in granulocytes (Benitez-Bibiesca and Frere-Horta, 1978; Isaacson *et al.*, 1981). In this study, the high serum A1PI concentration found in cholangiocarcinoma, hepatoma etc. is probably derived from the white cell infiltration associated with the disease. Ach concentration increases markedly in liver cancer. It is therefore possible that the elevated concentrations of serum PIs seen is a nonspecific response to inflammation as seen in acute arthritis (Steinbuch and Audran, 1974). However the increase in the latter is moderate.

The high concentration of PI seen in the hepatic cancers may be a host response mechanism to an accumulation of proteases

which occurs due to a defect in the control of the mechanisms involved in cell synthesis as Stubblefield and Brown (1977) postulated protease theory in cancer. This switching mechanism would have to be regulated by other control mechanisms, otherwise an excess of PI might itself harm the cell since PIs are toxic.

The serum concentration of PIs is only slightly elevated in those patients with opisthorchiasis, whereas in those with cholangiocarcinoma the increase was very marked. Serum bilirubin concentrations in those patients with *Opisthorchis* infection was normal indicating that infection was mild and that the bile duct was problably still free of obstruction. A correlation of the PIs against age in those infected with *O. viverrini* was not found to be significant. This does not necessarily mean that increased exposure to opisthorchiasis is not accompanied by an increase in the serum PIs. It is possible therefore that the serum concentration of PIs may be useful as a screening tool for surveying the community for risks of cholangiocarcinoma in areas where opisthorchiasis is known to exist. It has been shown that severe malnutrition depress the concentration of PI, and slight increases have been found to occur in patients with nephritis (Schelp *et al.*, 1979; Werner, 1969). Such conditions do not necessarily preclude the usefulness however in the manner suggested above.

SUMMARY

Serum α_1-antitrypsin, α_1-antichymotrypsin and α_2-macroglobulin increased significantly in patients suffering from liver diseases : hepatoma, amoebic liver abscess, hepatitis, hepatic cirrhosis, cholangiocarcinoma, carcinoma of the head of pancreas including liver fluke infection (opisthorchiasis).

Marked increase of α_1-antitrypsin and α_1-antichymotrypsin were found in cholangiocarcinoma, carcinoma of the head of pancreas, amoebic liver abscess, hepatic cirrhosis and hepatoma. α_2-macroglobulin increased markedly in hepatic cirrhosis. The concentrations of protease inhibitors found in opisthorchiasis were only moderately elevated.

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