Plasma lysozyme level and reticuloendothelial system function in human liver disease

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Summary

Plasma lysozyme levels have been reported to reflect the functional status of the reticuloendothelial system (RES). We measured plasma lysozyme levels in 22 patients with acute hepatitis and 21 patients with cirrhosis and a mesocaval shunt. In 17 of these patients RES function was assessed by measuring the disappearance rate (t/2) of radio-labelled sulphur colloid. In acute hepatitis plasma lysozyme levels and colloid t/2 were significantly lower than in healthy controls and cirrhotics. In the acute hepatitis patients, the plasma lysozyme levels rose significantly two weeks after admission as the hepatitis improved. The colloid t/2 of the 17 patients with liver disease was significantly correlated with the plasma lysozyme level (r = +0.66, p = 0.005).

These results suggest that in human liver disease, in comparison with animal experiments, plasma lysozyme is dependent on RES functional status in the sense that a more active RES will result in a lower lysozyme level.

Introduction

About 15% of liver cells are reticuloendothelial cells [1]. In healthy people and patients with liver disease the reticuloendothelial system (RES) has an important function in preventing the potential harmful complications of gut-derived endotoxemia [2–4]. In clinical medicine RES function has received little attention [2]. It is possible to measure phagocytic RES function by determining the rate of disappearance of injected radio-labelled colloids which are taken up mainly by the liver RES [5], but this has not become a routine technique for measuring RES function in man.

Recently Kokoshis and Di Luzio [6] proposed that serum lysozyme is a good index for RES functional status. Stimulation of macrophages results in increased serum lysozyme levels. The present study was undertaken to determine whether

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lysozyme is helpful in evaluating RES function in human liver disease. In two groups of patients with respectively acute hepatitis and cirrhosis of the liver, plasma lysozyme levels were determined and related to colloid clearance.

**Patients and methods**

**Acute hepatitis**

Twenty-two in-patients (12 males and 10 females) with acute hepatitis were studied. The mean age was 36.3 years (range 19–70). The etiology was hepatitis B in 14, drug hepatitis in 6, hepatitis A in 1 and in 1 acute cardiac failure. The day after admission and 14 days later heparinized blood was drawn and plasma prepared for lysozyme estimation. During the first week of admission colloid clearance was measured. The diagnosis acute hepatitis was based on serum values of bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and liver biopsy.

**Cirrhosis of the liver**

Twenty-one out-patients (11 males and 10 females, mean age 55.0 years, range 32–74) with cirrhosis of the liver, in whom a mesocaval shunt had been performed six months to five years previously, were examined. The cause of cirrhosis was alcohol in 11, chronic active hepatitis in 4, unknown in 4 and primary biliary cirrhosis in 2. Three patients had a history of recent alcohol consumption. Patients who had undergone a splenectomy were not included. Blood was drawn for lysozyme estimation and liver tests (bilirubin, AST). Thereafter colloid clearance was measured.

**Lysozyme**

The plasma lysozyme activity was measured using *Micrococcus lysodeikticus* as a substrate (Lysozyme reagent kit, Worthington Biochemical Co, Freehold, N.J., U.S.A.). Plasma samples were added to the cell suspensions and the decrease in light absorption as a result of cell lysis was recorded from 30 to 180 s after addition of the plasma sample. The values obtained were compared to a standard curve simultaneously prepared using known concentrations of egg white lysozyme. Normal values were obtained from 20 healthy laboratory and hospital workers.

**RES function**

The function of the RES was assessed with $^{99m}$Tc technetium-sulphur-colloid [5,7]. In the right antecubital vein a dose of 2.96 MBq (80 µCi) in 1 ml (0.3 mg S colloid) was injected while the left arm was held in a large volume Liquid Scintillation Counter (Armac, Packard). The left forearm activity was counted for 15 min. The disappearance curve is derived by computer and the half-time ($t/2$) in minutes is calculated during a 3-min period starting 1 min after the maximal number of counts. This technique was only available during the second part of the study and was performed in eight patients with acute hepatitis and nine with cirrhosis and a mesocaval shunt. Normal values were obtained from 15 healthy hospital workers. All patients and control subjects gave informed consent. The radiation dose to a normal liver in the RES function measurement is calculated to be $2.7 \times 10^{-4}$ Gray ($27 \times 10^{-3}$ rad) [8].

For comparison of two means statistical analysis was done by two-tailed paired or unpaired Student’s *t* test.
**Results**

**Clinical data**

Most hepatitis patients were jaundiced, mean peak bilirubin 209 μmol/l (range 17–570, normal reference value up to 12 μmol/l). The mean ALT level (normal up to 30 U/l) in acute hepatitis was 1811 U/l (range 616–6830). The patients with a cirrhosis had a mean AST level (normal up to 30 U/l) of 78 U/l, with a range of 14–205. The mean bilirubin level was 58 μmol/l (range 12–395).

**Plasma lysozyme**

In acute hepatitis the mean plasma lysozyme on the day after admission (day 2) was 3.45 ± 2.96 mg/l (mean ± S.D., Fig. 1). This value was significantly lower than

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Fig. 1. Plasma lysozyme levels in patients with acute hepatitis (day after admission) and ambulant patients with cirrhosis of the liver and a mesocaval shunt.
that of patients with cirrhosis and a mesocaval shunt (6.94 ± 2.45 mg/l, p < 0.001). Lysozyme in acute hepatitis differed significantly from healthy controls (6.05 ± 1.63 mg/l, p < 0.005). In contrast in cirrhosis plasma lysozyme was not significantly different from normals (0.10 < p < 0.20). Sixteen of the 22 hepatitis patients were still in hospital on day 16 and their mean lysozyme at that moment was 3.86 ± 2.75 mg/l. In 10 of these 16 patients plasma lysozyme was higher than on admission. There was no significant correlation between ALT level and plasma lysozyme on admission (r = +0.13).

**RES function**

The t/2 of $^{99m}$TcS-colloid in acute hepatitis was 4.16 ± 1.53 min and differed significantly from control subjects (5.92 ± 1.55 min, p < 0.02) and patients with cirrhosis (7.51 ± 2.58 min, p = 0.005). There was no statistically significant difference in t/2 in patients with cirrhosis when compared to normals (0.05 < p < 0.10). When the t/2 of the 17 patients (8 with acute hepatitis and 9 with cirrhosis) was related to the plasma lysozyme of the same week a significant positive correlation was obtained (r = 0.66, p = 0.005) (Fig. 2).

**Discussion**

Lysozyme is secreted by macrophages and leucocytes. Elevated lysozyme levels can occur in a variety of blood, gastrointestinal and renal disorders and in patients
with tumours (9–13). Macrophages, when stimulated, secrete lysozyme in vitro [14] and in vivo [6]. Lysozyme has been proposed as an index of macrophage function [6]. The RES of the liver protects against infections and gut-derived endotoxins [2–4]. In alcoholism and in certain forms of liver disease RES function is known to be depressed [15–17]. The relationship between lysozyme level and RES function and the value of lysozyme in liver disease still remains to be settled.

In our study lysozyme levels in acute hepatitis were lower than in normals. The $t/2$ of injected colloid was also lower than in normals, indicating a more active phagocytosis. An increased RES function in acute hepatitis has also been reported by Cooksley et al. [16]. Possible explanations for the increased activity are a higher cellular activity and/or an increase in cell number [16,18].

In cirrhosis of the liver there are several factors which can reduce RES function, such as extra- and intrahepatic shunting, decreased availability of functional Kupffer cells and alcohol consumption [15,17,19,20]. In our patients with cirrhosis the $t/2$ was longer than in normals suggesting a worse RES function. We have found that cirrhotics with a mesocaval shunt have similar colloid $t/2$ to cirrhotics who do not have a surgical shunt [21]. As very few of our patients had recently consumed alcohol it is not possible to draw any conclusions about the role of shunting or alcohol-induced depression of RES function.

The plasma lysozyme levels in cirrhosis did not differ from normals. The $t/2$ of injected radio-labelled colloid in cirrhosis and acute hepatitis paralleled the lysozyme values. Thus the worse the RES function, the higher the lysozyme level. This seems to contradict the results of others [6,14]. Kokoshis and Di Luzio [6] found a more active RES to be associated with a higher lysozyme level after stimulating the RES with glucan. However, the lysozyme level is not only dependent on production but also on removal. It is known that several enzymes are cleared from the blood by the RES [22,23]. It is therefore possible that the changes in plasma lysozyme levels are related to changes in removal rates as well as changes in production rates.

In 8 of the 22 patients with acute hepatitis a temporary increase in the serum creatinine was present in the acute phase of the hepatitis. Endotoxemia could be demonstrated by the limulus test at that time [7]. The mild disorder in renal function is unlikely to be responsible for the depressed lysozyme levels as renal insufficiency results in an elevation of plasma lysozyme [11]. During catheterization studies it has been shown that only minimal amounts of lysozyme are cleared by the kidneys [24].

Of the acute hepatitis patients two had a rather high lysozyme level compared to the other hepatitis patients. These two patients had the highest lysozyme levels of the whole group and their acute liver damage was caused by paracetamol overdose or acute heart failure. They were admitted within 24 h of the initiating event. In these patients the higher lysozyme level might be due to decreased RES function instead of an increased RES activity such as is present at a slightly later stage of hepatitis in the other patients. In experimental hepatitis RES function is subnormal or normal during the first two days and increases thereafter [25]. In the two patients mentioned above plasma lysozyme on day 16 of admission was significantly lower than on admission. When the paired lysozyme values of the remaining fourteen patients were compared, excluding these two patients with a very acute hepatitis, a significant increase in plasma lysozyme is detectable ($2.40 \pm 1.61$ and $4.02 \pm 2.84$ mg/l, respectively, $p < 0.01$), although normal levels were not yet reached. These changes may reflect a return to normal of RES function in the hepatitis patients. Paired colloid clearance studies would be necessary to confirm this.
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