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Influence on Plasma β-N-Acetyl-*D*-glucosaminidase of Reticuloendothelial Stimulation and Depression: An Experimental Study in Rats[1])

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Summary: Plasma levels of the lysosomal enzyme, β-N-acetyl-*D*-glucosaminidase (EC 3.2.1.30; 2-acetamido-2-deoxy-β-*D*-glucoside acetamidodeoxyglucohydrolase) were estimated 120 minutes after intravenous injection of zymosan in rats. Different groups of animals were investigated according to pretreatment with agents influencing reticuloendothelial activity. Pretreatment for two days with the reticuloendothelial stimulating agent, zymosan, or the reticuloendothelial suppressing agent, methyl palmitate, did not influence the basal plasma levels of β-N-acetyl-*D*-glucosaminidase.

In all groups after zymosan injection, plasma β-N-acetyl-*D*-glucosaminidase activity was increased in comparison with basal levels. The increase of enzyme activity was most pronounced after pretreatment with zymosan and differed significantly from enzyme activity after pretreatment with methyl palmitate. Alcohol administration in combination with zymosan did not cause a further rise of β-N-acetyl-*D*-glucosaminidase activity in normal rats nor in rats pretreated with zymosan.

The observations suggest that plasma levels of β-N-acetyl-*D*-glucosaminidase in rats after a standardized zymosan injection are related to the functional status of the reticuloendothelial system (RES).

Einfluß von Stimulation und Depression des reticuloendothelialen Systems auf die katalytische Konzentration der β-N-Acetyl-D-glucosaminidase im Plasma: Eine experimentelle Untersuchung an Ratten

Zusammenfassung: Die katalytische Konzentration des lysosomalen Enzyms β-N-Acetyl-*D*-glucosaminidase (EC 3.2.1.30; 2-Acetamido-2-desoxy-β-*D*-glucosid Acetamidodesoxyglucohydrolase) wurde 120 min nach intravenöser Injektion von Zymosan im Plasma von Ratten bestimmt. Verschiedene Tiergruppen wurden nach Vorbehandlung mit die reticuloendotheliale Aktivität beeinflussenden Stoffen untersucht. Vorbehandlung mit Zymosan als stimulierendem Agens oder Methylpalmitat als suppressivem Agens für zwei Tage beeinflußte die basale katalytische Konzentration des Enzyms nicht.

Nach Injektion von Zymosan war die β-N-Acetyl-*D*-glucosaminidase im Plasma im Vergleich zur basalen katalytischen Konzentration bei allen Gruppen erhöht. Der Anstieg war nach Vorbehandlung mit Zymosan sehr betont und unterschied sich deutlich von der katalytischen Konzentration nach Vorbehandlung mit Methylpalmitat. Gabe von Alkohol in Kombination mit Zymosan bewirkte keinen weiteren Anstieg des Enzyms bei normalen oder mit Zymosan vorbehandelten Ratten.

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Unsere Beobachtungen weisen darauf hin, daß die katalytische Konzentration von β-N-Acetyl-*D*-glucosaminidase im Plasma von Ratten nach standardisierter Zymosaninjektion zum funktionellen Zustand des reticuloendothelialen Systems in Beziehung steht.

Introduction

An increased serum level of β-N-acetyl-*D*-glucosaminidase has been found in patients with different diseases of the liver and in patients with acute ethanol intoxication (1, 2). The mechanisms leading to the elevated level of lysosomal enzymes are not clear. One possible reason could be an activation of body macrophages leading to an increased synthesis and secretion of lysosomal enzymes. Another reason could be a defective clearance from the blood of lysosomal enzymes, due to a decreased *Kupffer* cell function.

It has been proposed that serum levels of lysosomal enzymes could serve as markers for macrophage activation in mice (3). The release of lysosomal enzymes has been shown to be correlated with the process of phagocytosis (4).

Zymosan, a yeast product derived from the cell wall of *Saccharomyces cerevisiae*, has been demonstrated to activate macrophages (5) and to produce a marked hyperplasia and hyperfunction of the reticuloendothelial system (6, 7). Zymosan is phagocytosed by macrophages, and the injection of zymosan was followed by increased plasma levels of lysosomal enzymes (8, 9). Methyl palmitate is a well known suppressor of reticuloendothelial phagocytic activity (10).

The aim of the present study was to investigate the influence of reticuloendothelial zymosan-stimulation and methyl palmitate-depression on the activity of β-N-acetyl-*D*-glucosaminidase in plasma after a single dose of zymosan. The possibility that alcohol may influence the release of β-N-acetyl-*D*-glucosaminidase was also studied.

Materials and Methods

Twenty five male Wistar rats, weighing 154–192 g, were used. The animals were divided into five groups according to the administered agents. All animals were fed on standard food pellets and water ad libitum.

Methyl palmitate (Sigma Co.) was prepared as an emulsion by sonification for 10 min in 50 g/l dextrose and 1 g/l Tween 20 at a concentration of 400 g/l.

Zymosan (Sigma Co.) was suspended in 9 g/l saline at a concentration of 10 g/l.

Ten animals (groups A and E) did not receive any pretreatment. Pretreatment was given on two consecutive days with zymosan (groups B and D) and methyl palmitate (group C) by intravenous injection into the jugular vein under light ether anesthesia.

Before the first injection, the jugular vein was cannulated and heparin-plasma samples for basal levels of β-N-acetyl-*D*-glucosaminidase were collected. On the same day, zymosan was injected into all the animals and blood sampling (0.5 ml) was carried out 10, 30, 60, 90 and 120 min after the zymosan injection. This injection was preceded by an intravenous injection of alcohol in groups D and E. Plasma was analysed for β-N-acetyl-*D*-glucosaminidase catalytic activity concentration using a method described elsewhere (1). The coefficient of variation for the method was below 10%.

Results are given as mean ± SEM. When testing for differences, *Student*'s t-test and the nonparametric *Wilcoxon* rank sum test were used. Differences were regarded significant only when a p-value of less than 0.05 was obtained in both tests.

Results

β-N-Acetyl-*D*-glucosaminidase levels for the different groups are depicted in table 1. After the zymosan injection, a statistically significant increase of β-N-acetyl-*D*-glucosaminidase levels was seen in all groups. No deviation of basal levels of β-N-acetyl-*D*-glucosaminidase was found in animals pretreated with zymosan (B and D) or methyl palmitate (C), when compared with the non-pretreated groups (A and E).

Tab. 1. Activity of β-N-acetyl-*D*-glucosaminidase in serum before (basal) and after zymosan injection. Statistical significance for comparison with the basal activity in each group, using *Student*'s t-test, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

Group	β-N-Acetyl-*D*-glucosaminidase catalytic activity concentration (U/l)					
	Basal	10	30	60	90	120 minutes after zymosan injection
A	12.0 ± 0.6	12.7 ± 0.7	17.7 ± 0.6*	18.1 ± 0.7***	16.7 ± 1.2**	17.0 ± 0.4**
B	11.5 ± 0.7	14.5 ± 0.6**	18.7 ± 1.2**	21.1 ± 2.2**	21.7 ± 2.4**	18.7 ± 1.3**
C	11.4 ± 0.7	12.4 ± 1.4	15.3 ± 0.4*	15.7 ± 1.1*	16.4 ± 1.2**	15.3 ± 1.3**
D	11.8 ± 0.2	15.8 ± 1.3*	19.2 ± 2.0*	18.9 ± 2.1*	18.1 ± 1.3**	19.1 ± 1.8*
E	12.9 ± 1.0	15.2 ± 0.5*	19.7 ± 1.0**	22.9 ± 1.6**	22.6 ± 1.4**	20.5 ± 1.6**

After 60 min, the level of β-N-acetyl-*D*-glucosaminidase was significantly higher in the zymosan pretreated rats (B) than in the methyl palmitate pretreated rats (C) with a p-value of 0.05 (Fig. 1). After 90 min, the enzyme level of the zymosan pretreated rats was significantly higher than that in the control (A) and the methyl palmitate pretreated rats (C) with p-values of 0.05 in both comparisons (fig. 1).

The administration of alcohol (D and E) did not significantly influence the rise of β-N-acetyl-*D*-glucosaminidase activity when compared with the corresponding groups (A and B) where alcohol was not given.

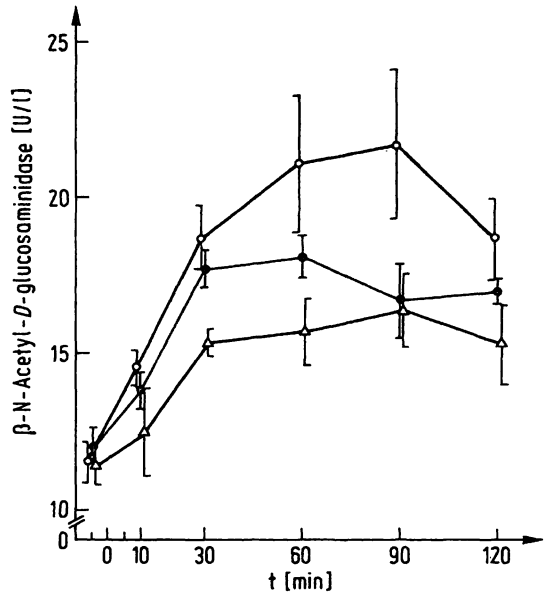

Fig. 1. β-N-Acetyl-*D*-glucosaminidase activity after zymosan injection into control animals (●—●), into animals pretreated with zymosan (○—○) and methyl palmitate (△—△).

Discussion

Several methods are available for measurements of reticuloendothelial function (11, 12). These methods are often based upon studies of the fate of injected colloidal particles and often require technical equipment such as a scintillation camera and computer system.

A simple laboratory test that could reflect the functional status of the RES would be most valuable. For this purpose lysosomal enzymes might be useful, since it has been suggested that serum levels of these enzymes could serve as markers for macrophage activation (3). Although resting macrophages release lysosomal enzymes into their extracellular environments, this release is increased during macrophage activation and phagocytosis (5, 8, 13, 14). On the other hand, increased serum levels of lysosomal enzymes after trauma have also been attributed to a depression of RES function (15).

Zymosan given as a single intravenous dose has been shown to increase the reticuloendothelial function in rats for at least 72 hours (7). Repeated administration of zymosan to rats has been shown to produce a marked hyperplasia of *Kupffer* cells and mononuclear granuloma formation in the liver (6, 7, 16).

Serum activity of certain lysosomal enzymes such as β-glucuronidase and acid phosphatase has been shown to be considerably increased as early as 20 min after the injection of zymosan in rats (8). The increase of serum lysosomal enzyme levels is, however, only transient and seems to exist only during the phagocytosis of zymosan (8). The rapid increase of lysosomal enzymes after zymosan injection probably results from the release of stored lysosomal enzymes (tissue forms). The turnover time of tissue forms of β-N-acetyl-*D*-glucosaminidase is known to be very short when purified preparations are intravenously injected into rats (15). This may explain why the rise of serum β-N-acetyl-*D*-glucosaminidase activity in rats after zymosan injection is of short duration. In the present study the serum levels of β-N-acetyl-*D*-glucosaminidase remained increased for at least 120 min over the basal levels, but were, however, normalized after 24 hours.

Increased plasma levels of cathepsin and acid phosphatase in rats after trauma have been attributed to a depressed RES function, i.e. a decreased plasma clearance by the *Kupffer* cells (15). In the present study, the basal enzyme levels for pretreated rats (zymosan or methyl palmitate) did not differ from those in control animals. Basal enzyme levels could thus not serve as markers for the degree of reticuloendothelial activation or depression.

The present study revealed significantly higher plasma levels of β-N-acetyl-*D*-glucosaminidase after zymosan injection in rats with an activated RES, when compared with rats with a suppressed RES. However, the enzyme response for the methyl palmitate pretreated animals did not differ significantly from that in controls. This observation suggests that plasma levels of β-N-acetyl-*D*-glucosaminidase after zymosan injection are more dependent on the degree of macrophage activation than on lysosomal enzyme clearance.

Alcohol administration did not significantly influence the rise of β-N-acetyl-*D*-glucosaminidase levels after zymosan injection in zymosan pretreated rats, nor in non-pretreated rats. These findings are not in accordance with earlier results concerning β-N-acetyl-*D*-glucosaminidase activity in acute alcohol intoxication in man (2). Those patients had, however, ingested excessive amounts of alcohol for several weeks.

In conclusion, isolated measurements of plasma β-N-acetyl-*D*-glucosaminidase activity in animals without concomitant macrophage challenge are of little value in predicting the status of the reticuloendothelial system. After a standardized zymosan injection, the level of plasma β-N-acetyl-*D*-glucosaminidase activity may be correlated with the degree of activation of the reticuloendothelial system.

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