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Elevated serum β-glucuronidase reflects hepatic lysosomal fragility following toxic liver injury in rats

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Abstract

The level of serum β-glucuronidase increases in various pathological conditions, including liver disorders. The aim of this investigation was to study the changes in liver lysosomal membrane stability during experimentally induced hepatic fibrosis that may result in the elevation of serum β-glucuronidase. Liver injury was induced by intraperitoneal injections of *N*-nitrosodimethylamine (NDMA) in adult male albino rats over 3 weeks. The progression of fibrosis was evaluated histopathologically as well as by monitoring liver collagen content. Lipid peroxides and β-glucuronidase levels were measured in the liver homogenate and subcellular fractions on days 0, 7, 14, and 21 after the start of NDMA administration. Serum β-glucuronidase levels were also determined. A significant increase was observed in β-glucuronidase levels in the serum, liver homogenate, and subcellular fractions, but not in the nuclear fraction on days 7, 14, and 21 after the start of NDMA administration. Lipid peroxides also increased in the liver homogenate and the lysosomal fraction. The measurement of lysosomal membrane stability revealed a maximum lysosomal fragility on day 21 during NDMA induced fibrosis. In vitro studies showed that NDMA has no significant effect on liver lysosomal membrane permeability. The results of this investigation demonstrated that lysosomal fragility increases during NDMA-induced hepatic fibrosis, which could be attributed to increased lipid peroxidation of lysosomal membrane. In this study, we also elucidated the mechanism of increased β-glucuronidase and other lysosomal glycohydrolases in the serum during hepatic fibrosis. *(Biochemistry and Cell Biology 2008; 86: 235-243).*

Introduction

Hepatic fibrosis is a dynamic process that involves the interplay of different cell types in the hepatic tissue. The pathogenesis of hepatic fibrosis is mediated through oxidative stress and hepatocyte injury and is always accompanied by impaired hepatic metabolism and deposition of connective tissue components, especially collagen and hyaluronan in the liver.

Lysosomes are a distinct group of cell organelles that contain a variety of acid hydrolases. Changes in lysosomal stability have been reported in various spathological conditions as well as connective tissue disorders. However, lysosomal membrane stability has not been examined in any liver disorder including hepatic fibrosis. The aim of this investigation was to study the changes in liver lysosomal stability during NDMA-induced hepatic fibrosis in adult male albino rats. Because β-glucuronidase is used as a reporter gene to monitor gene expression and is also very rich in liver lysososmes compared with all other glycohydrolases, we selected it as a marker enzyme to study changes in lysosomal membrane stability during NDMA-induced hepatic fibrosis.

Materials and Methods

Hepatic fibrosis was induced by intraperitoneal injections of NDMA in 1 µL doses per 100 g body mass. The injections were given on 3 consecutive days of each week over a period of 3 weeks. Treated and control animals were sacrificed on days 7, 14 and 21 from the beginning of exposure.

The clinical indices of hepatic fibrosis were evaluated histopathologically as well as by quantifying total collagen content in the liver. The liver sections were stained with hematoxylin and eosin, examined using an Olympus BH2 microscope, and photographed. Lysosomes were prepared from the liver and β-glucuronidase activity was determined in various subcellular fractions. The rate of release of β-glucuronidase from the lysosomal fraction was taken as a measure of lysosomal membrane stability.

Figure 1

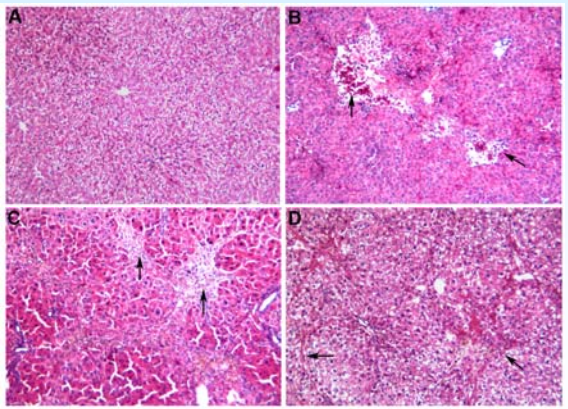

Figure 1. Hematoxylin and eosin staining of rat liver during the progression of NDMA-induced hepatic fibrosis (× 40). **(A)** Normal liver. **(B)** Day 7 of NDMA treatment. Massive hepatic necrosis, multifocal collapse of the liver parenchyma (arrows), severe centrilobular congestion and dilatation of sinusoids with focal hemorrhage. **(C)** Day 14 of NDMA treatment; note well developed fibrosis and early cirrhosis with multifocal hepatocyte necrosis and neutrophilic infiltration (arrows). **(D)** Day 21 of NDMA treatmetn; note well developed cirrhosis with collagen fibers (arrows).

Table 1. Subcellular distribution of liver β-glucuronidase during the progression of N-nitrosodimethylamine-induced hepatic fibrosis in rats.

Fraction	Control (n=9)	Day 7 (n=9)	Day 14 (n=7)	Day 21 (n=6)
Whole liver (total activity)	85.30 ± 3.44	157.28 ± 4.08**	215.96 ± 6.71**	178.71 ± 5.92**
Nuclear	28.25 ± 1.12	31.74 ± 1.29	32.39 ± 1.65	32.45 ± 1.71
Lysosomal	122.73 ± 4.14	223.75 ± 6.39**	268.46 ± 8.90**	241.12 ± 7.62**
Soluble	60.86 ± 2.62	125.14 ± 3.92**	210.84 ± 6.98**	230.48 ± 7.65**
Ratio of soluble (free) to lysosomal (bound) activity	0.496	0.559*	0.785**	0.955**
Ratio of soluble (free) to total activity	0.713	0.795*	0.976**	1.289**

Note: Enzyme activities for the fractions are expressed as µmol *p*-nitrophenol liberated/(h/100 mg protein). The values represent the means ± SE vs.– the control.
Statistical significance was determined with an ANOVA (*p < 0.05 and **p < 0.001).

Table 2. Effect of NDMA on liver lysosomal membrane permeability and the rate of release of β- glucuronidase from liver lysosomes during the progression of NDMA induced hepatic fibrosis in rats.

Group	Total lysosomal activity	Rate of release β-glucuronidase, % of total activity				
		0 min	15 min	30 min	45 min	60 min
Control (n=9)	122.73 ± 4.14	15.36 ± 0.72	17.54 ± 0.93	20.62 ± 0.98	22.62 ± 1.06	23.32 ± 1.15
Control +NDMA (n=9)	128.46 ± 5.65	13.21 ± 0.68	18.36 ± 0.88	21.26 ± 1.08	23.13 ± 1.15	25.36 ± 1.31
NDMA day 7 (n=9)	223.75 ± 6.39	16.62 ± 0.76	20.26 ± 1.24	25.61 ± 1.56	28.17 ± 1.96*	36.85 ± 2.37**
NDMA day 14 (n=7)	314.46 ± 10.90	15.31 ± 0.81	23.95 ± 1.35**	32.56 ± 2.21***	43.39 ± 2.58***	49.48 ±2.87***
NDMA day 21 (n=6)	261.12 ± 8.62	17.92 ± 0.92	26.86 ± 1.56***	35.72 ± 2.42***	48.16 ± 2.72***	56.26 ± 3.16***

The total lysosomal activity is expressed as µmoles of *p*-nitrophenol liberated/(h/100 mg protein).
Values represent the means ± SE vs.– the control
Statistical significance was determined with an ANOVA (* p<0.05, ** p<0.01, and *** p< 0.001).

Figure 2

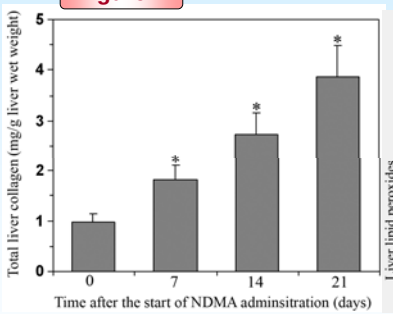

Figure 2. Total collagen content in the liver during NDMA-induced hepatic fibrosis in rats. The values given are mean ± standard deviation (*p < 0.001 with an ANOVA). The collagen content in the liver tissue was determined by estimating the level of hydroxyproline, a characteristic imino acid present in collagen. The total collagen content was calculated by multiplying the hydroxyproline content by the factor 7.46.

Figure 3

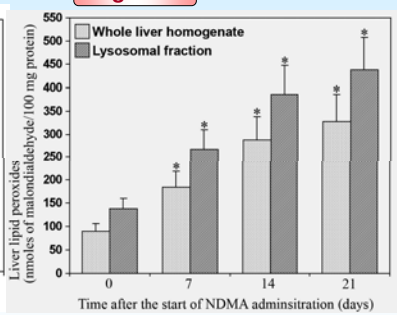

Figure 3. Lipid peroxides in the whole liver homogenate and lysosomal fraction during NDMA-induced hepatic fibrosis in rats. The data represent the mean ± SD (*p < 0.001 with an ANOVA compared with control values). Lipid peroxides were determined by the thiobarbituric acid reaction method using tetramethoxypropane as a standard.

Figure 4

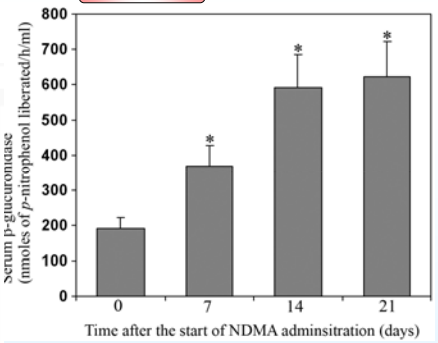

Figure 4. Serum β-glucuronidase levels during NDMA-induced hepatic fibrosis in rats. Values represent the means ± SD (*p < 0.001 with an ANOVA). Serum β-glucuronidase activity was determined by spectrophotometry using *p*-nitrophenyl β-D-glucuronide as a substrate.

Conclusions

- Treatment with NDMA produced well developed fibrosis in rat liver within 21 days.
- NDMA induced hepatic fibrosis resulted in increased levels of serum β-glucuronidase.
- Hepatic lysosomal fragility was increased during NDMA induced hepatic fibrosis.
- The increased hepatic lysosomal fragility was associated with increased oxidative stress and lipid peroxidation.
- Increased lysosomal fragility is the mechanism of elevation of serum β-glucuronidase in hepatic fibrosis.

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