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Research Article

The Effect of Diethylnitrosamine on the Levels of Sialic Acid, Lipid-Bound Sialic Acid and Enzyme Activities of Transferase in Rat Serum*

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Abstract: The aim of this study was to investigate the effects of a chemical carcinogen, Diethylnitrosamine (DENA), on these markers, sialic acid (SA), lipid-bound sialic acid (LBSA) and the liver enzymes aspartate aminotransferase, alanine aminotransferase, and gamma-glutamyltransferase.

In this research 180 male Wistar albino rats were used. Rats were divided equally into three groups. Intraperitoneal (i.p.) injection of saline was given to rats in the control group. Treatment group I was given i.p. 100 mg/kg-body weight DENA dissolved in saline. Treatment group II was given i.p. 200 mg/kg-body-weight DENA dissolved in saline. These doses were given to each group just once. Blood samples were taken from fifteen rats, which were chosen randomly from each group per month during the four months. Levels of SA and LBSA and activities of ALT, AST and GGT were determined in serum. Significant elevations in the serum levels of SA and LBSA and activities of ALT, AST and GGT were observed in treatment groups when compared with the controls.

In this study, the high levels of enzyme activities, sialic acid, and lipid-bound sialic acid led to hepatocellular degeneration. The results suggest that combined evaluation of these markers (SA and LBSA) and liver enzyme activities can be useful in predicting malignant change.

Key Words: Diethylnitrosamine, sialic acid, lipid-bound sialic acid, liver enzymes, rats.

Dietilnitrosamin'in Sıçanlarda Serum Siyalik Asit, Lipid-Bağlı Siyalik Asit ve Transferaz Enzim Aktivitelerine Etkisi

Özet: Bu çalışmanın amacı kimyasal bir karsinojen olan Dietilnitrosamin (DENA) 'in serum siyalik asit (SA) ve lipid-bağlı siyalik asit (LBSA) ile alanin transaminaz (ALT), aspartat transaminaz (AST) ve gama-glutamiltransferaz (GGT) enzim düzeylerine etkisini araştırmaktır.

Çalışmada 180 dişi sıçan kullanılmıştır. Sıçanlar kontrol ve deneme grupları olarak üçe ayrılmıştır. Her grup 60'ar hayvandan oluşmuştur. Kontrol grubu olarak kullanılan ratlara periton içi (i.p) serum fizyolojik verilmiştir. Deneme-I grubuna serum fizyolojik içinde çözülmüş 100 mg/kg-vücut ağırlığına DENA, Deneme-II grubundaki sıçanlara ise 200 mg/kg-vücut ağırlığına DENA i.p. olarak verilmiştir. Tüm gruplara DENA tek doz olarak verilmiştir. Kan örnekleri dört ay boyunca her ay her bir gruptan rastgele seçilen sıçanlardan sağlanmıştır. Serumda SA, LBSA düzeyleri ve ALT, AST, GGT enzim aktiviteleri ölçülmüştür. Deneme-I ve Deneme-II gruplarının çalışma süresince serum SA, LBSA, ALT, AST, GGT ortalama değerleri kontrol grubunun değerleriyle karşılaştırıldığında, farklılıklar istatistiki olarak anlamlı bulunmuştur.

Çalışma sonuçları değerlendirildiğinde, enzim aktiviteleri ile SA, ve LBSA düzeylerindeki artışlar, karaciğer hücre dejenerasyonundan kaynaklanabileceğini düşündürmektedir. Sonuçlar, tümör markırlarının (SA, LBSA) ve karaciğer enzim aktivitelerinin beraber değerlendirilmesinin tümöral değişimlerin teşhisinde faydalı olabileceğini önermektedir.

Anahtar Sözcükler: Dietilnitrosamin, siyalik asit, lipide bağlı siyalik asit, karaciğer enzimleri, rat.

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Introduction

Diethylnitrosamine (DENA), which has carcinogenic and toxic effects, can be found in air, water, soil, workplaces, tobacco smoke, processed meats and different food (1-3). It may also be derived from the metabolism of some therapeutic drugs (4).

Administration of DENA to animals has been shown to cause cancer in liver and, at lower incidences, in other organs as well (5,6). In liver cancer research with experimental animals, DENA is used either as a complete carcinogen or as an initiator in multistage models (7). When used as a tumour initiator, DENA is usually given at a single dose of 200 mg/kg to induce pronounced liver necrosis and presumably certain gene mutations in some hepatocytes (8,9). Dunsford et al. (6) used DENA opportunistically to improve cancer development in liver cells with enhanced multiplication caused by hepatocyte necrosis.

The administration of carcinogenic substances may bring about changes in enzyme levels arising from clonic proliferation, so it is of some importance to analyse enzyme activity variation quantitatively in order to understand the processes involved (9,10). DENA has effects on the serum and tissue-specific enzymes. Some research has indicated that it increases the activity of ALT, AST, GGT, B-glycuronidase glycose-6 phosphate dehydrogenase (G-6-PDH), and hexokinase and decreases the activity of lactate dehydrogenase (LDH), glycose-6 phosphatase (G.6.P.az), malate dehydrogenase (MDH), adenosin triphosphatase (ATPaz), cytochrome reductase and uridine diphospho-glucuronyl transferase (11-14).

In the research of Guiliani and Zaki (15), i.p. DENA were given to rats for 5 days in dose of 2 mg/kg body weight. It was determined that serum ALT and AST enzyme activity increased. Özkurt (16) determined that the activities of LDH increased and the activities of citrate synthetase decreased in the first and third months after DENA was given to rats. Bakır et al. (12) observed that the activities of glucose-6-phosphate-dehydrogenase and hexokinase increased, the activities of malate dehydrogenase decreased in the livers of rats given DENA as a single dose of 100 mg/kg body weight. Moor et al. (11) followed the results of their experiment, in which a single dose of i.p. DENA (0.1, 0.2, 0.4 mmol/kg-body weight) was given to mice, during 32 weeks. According to their results, liver damage was observed in the 8th week but hepatic nodules were determined in the 8th month.

Biological markers can be used to monitor cancer, predict the therapeutic response and prognosis of cancer, and in some certain situations even diagnose cancer. These markers, referred to as tumour markers, naturally occur or are modified molecules that can be measured in serum, plasma, or other body fluids and their concentration changes in the presence of cancer (2,17,18).

N-acetylneuraminic acid (sialic acid) is an N-acyl derivative of neuraminic or acid amino sugar derivative, derived from N-acetylmannosamine and pyruvic acid. It is an important constituent of glycoproteins and glycolipids. N-acetylneuraminic acid occurs in many polysaccharides, glycoproteins, and glycolipids in animals and bacteria (17,19). The correlation between levels of sialic acid and sialic acid-containing glycolipids (gangliosides) in tumours and serum with the growth characteristics of the tumours was investigated in transplantable hepatomas and squamous cell carcinomas initiated with carcinogenic agents in vivo and in tissue culture (19). Research on the changes of the glycoprotein and sialoglycoprotein metabolisms on the surface of tumour cells led to the use of SA enzymes of glycosil transferase as tumour markers. Serum SA and LBSA are considered important criteria in the development of a tumour and its treatment, particularly to follow up metastasis formation (20-22). Neoplasms often have an increased concentration of SA on the tumour cell surface. Sialoglycoproteins and/or gangliosides are shed or secreted by some of these tumour cells increasing the concentration of SA and lipid-bound sialic acid (LBSA) in the blood (21).

Increased serum levels of SA have been reported in patients suffering from cancer (22), and alcoholism (23), and in patients with bacterial infections (20,24), rheumatoid arthritis (24,25), and various other diseases (20).

The acute toxicity of DENA to the liver has been well documented in the literature but whether DENA also affects the sialic acids, and lipid-bound sialic acid has been not addressed in these studies.

The aim of this research is to study the toxic effect of DENA. For this purpose, the level of SA and LBSA, which are widely considered tumour markers, and the activities of the enzymes namely ALT, AST and GGT, are examined.

Materials and Methods

In this research, 180 male Wistar albino rats, which were 28 days old, were used throughout the study. The animals were kept under standardized conditions (light from 06 00 hr to 18 00 hr, 21 ± 2 °C humidity 55%) with standard rat pellets and water supplied ad libitum.

Rats were randomly divided into three groups comprised of three to 60 rats per group. At 5 weeks of age, rats received an intraperitoneal (i.p.) injection of DENA (Sigma Chemical Co.) or saline. The first one was the control group and the other two groups were the treatment groups. Control group: i.p. saline was given to this group while the treatment groups were given DENA.

Treatment group I: A single dose of DENA (100 mg/kg body weight) dissolved in saline was given i.p. to rats. Treatment group II: A single dose of DENA (200 mg/kg body weight) dissolved in saline was given i.p. to rats. After the beginning of the injection of DENA blood samples were taken from 15 rats in every group for biochemical parameters once a month during the four months. Levels of SA and LBSA and activities of ALT, AST and GGT were determined in the sera. SA and LBSA analyses were used the Warren thiobarbiturate method (26). ALT, AST and GGT enzyme reagents were supplied by Sigma Diagnostics (Sigma Chemical Co. Ltd., Poole, Dorset, UK) and spectrophotometric assays were performed. From the beginning of the research, 5 rats were taken from each group in every 4 weeks, they were euthanised with sulphuric acid and a systemic autopsy was carried out.

Statistical analysis: All data from each treatment groups and control group were analysed using Student's t-test.

Results

The levels of serum SA indicate significant statistical differences in the first and second treatment groups compared to the control group ($P < 0.001$). There is also a significant statistical difference ($P < 0.001$) with respect to the levels of serum LBSA between the control group and treatment groups I and II (Table). When the activities of serum AST in the first treatment group are

Table. Changes in enzyme activities and Sa and LBSA levels in DENA administrated rats.

Para Meters	Groups N = 15	I. Month X ± SE	II. Month X ± SE	III. Month X ± SE	IV. Month X ± SE
	Control	23.45 ± 0.13	26.89 ± 0.39	30.80 ± 0.38	43.10 ± 0.34
SA	Treatment-I	29.90 ± 0.40***	35.0 ± 0.49***	40.2 ± 0.93***	46,00 ± 0.33*
(mg/dl)	Treatment-II	33.65 ± 0.65***	41.0 ± 0.41***	59.5 ± 1.14***	73.3 ± 1.75***
	Control	17.48 ± 0.29	18.40 ± 0.35	20.70 ± 0.64	21.10 ± 0.55
LBSA (mg/dl)	Treatment-I	22.0 ± 1.00***	38.19 ± 0.40***	41.4 ± 0.46***	49,5 ± 1.00***
	Treatment-II	23.4 ± 1.12***	39.6 ± 0.40***	43.3 ± 0.60***	54.36 ± 0.73***
	Control	16.71 ± 1.22	17.73 ± 0.59	21.60 ± 1.02	27.88 ± 0.85
AST (IU/l)	Treatment-I	19.91 ± 2.62	23.03 ± 1.43	34.7 ± 1.30***	48.8 ± 2.09***
	Treatment-II	21.01 ± 1.58*	29.6 ± 2.42***	38.3 ± 1.11***	58.2 ± 2.39***
	Control	51.48 ± 1.73	58.05 ± 1.94	60.04 ± 1.31	63.34 ± 1.93
ALT (IU/l)	Treatment-I	61.84 ± 2.53**	72.98 ± 3.10**	84.0 ± 2.93***	94.3 ± 1.76***
	Treatment-II	77.8 ± 2.98***	81.1 ± 1.94***	89.7 ± 1.92***	97.6 ± 1.33***
	Control	1.6 ± 0.16	1.7 ± 0.15	1.8 ± 0.20	2.0 ± 0.21
GGT (IU/l)	Treatment-I	1.9 ± 0.23	2.2 ± 0.20	2.6 ± 0.22**	3.5 ± 0.43**
	Treatment-II	2.1 ± 0.25	2.8 ± 0.25**	3.7 ± 0.38***	4.5 ± 0.58***

*, **, *** Significantly ($P < 0.05$, $P < 0.01$, $P < 0.001$) different from control group.
X= mean SE= Standard Error

compared with the activities in the control group, it is determined that the activities of enzyme increased. While this increase was insignificant in the statistical sense in the first and second month, it was significant in the third and fourth ($P < 0.001$) months. It is also determined that the activities of serum AST in treatment group II increased compared to the control group. These increases were $P < 0.05$ in the first month, $P < 0.001$ in the second, third and fourth months. The activities of serum ALT in treatment group I increased compared to the control group. In the statistical sense, this increase was significant in the first ($P < 0.01$), second, third and fourth months ($P < 0.001$). It is determined that there was a significant difference in activities of serum ALT between treatment group II and the control group. The difference continued ($P < 0.001$) throughout the 4 months. The activities of GGT in treatment group I were increased compared to the activities in the control group. This increase was statistically insignificant in first and second months but it was significant in third ($P < 0.01$) and fourth ($P < 0.001$) months. It is also determined that were increases in the levels of serum GGT in treatment group II compared to the control group. This increase was statistically insignificant in the first month but was significant in the second ($P < 0.01$) third and fourth ($P < 0.001$) months.

Discussion

In this research after i.p. DENA injection, the activities of ALT, AST and GGT, and levels of SA and LBSA increased in the two treatment groups compared with the control group. These statistically significant increases continued during the experimental period.

It is determined that the enzyme activities of serum ALT, AST and GGT increased in the treatment groups compared to the control group in the course of 4 months. These increases are statistically significant and the increase in the enzyme activities in treatment group II, in which a high dose of DENA was used, is higher than in treatment group I (Table).

Hepatospecific enzymes were increased when hepatocellular damage and these enzymes are activated in hepatoma (14). AST, ALT, GGT and ALP exhibit high levels in the abnormally functioning liver, thus establishing them as an index of liver function recovery degree (27,28). DENA is not only carcinogenic, as a potent alkylating agent, but also inhibits protein synthesis. After this inhibition, changes in the activity of enzyme may occur (1,15). It is observed that DENA causes an increase in some enzyme activities and decreases in some other enzyme activities (11-13,15,16).

AST and ALT activities in blood serum are generally accepted as an index of liver damage and this tendency is also known to be distinct in rodents (14). ALT is recognised to be a highly liver specific enzyme. Also the measurement of serum GGT is a frequently used parameter of liver diseases (29). In this study the activities of serum ALT in treatment group I increased compared to the control group. This increases was found significant in the first ($P < 0.01$), second, third and fourth months ($P < 0.001$). It is determined that there was a significant difference in activities of serum ALT between treatment group II and the control group. The difference continued ($P < 0.001$) throughout the 4 months. AST is not only a specific index to the liver but also it was distributed in the liver, heart, kidney, skeletal muscles and brain. However, when the activities of serum AST in the treatment groups are compared with the control group, it is determined that the activities of enzyme increased during the four months. GGT activities in the serum were also increased, and the highest activity was observed in the fourth month. These findings are in agreement with those of Woo-song et al. (14) and Guiliani and Zaki (15).

This study was designed to evaluate the clinical application of serum total sialic acid (TSA) in the diagnosis of liver damage. We thus investigated the effects of DENA on serum SA and LBSA levels in this study. In many cancer studies, it is also found that increases in the levels of SA and LBSA point out the beginning of tumoral formation as well as metastases formation (22,24,30). The increases in the levels of LBSA may indicate that neoplastic deformation occurred in the cells. When the amount of precursors of ganglioside and of neutral glycolipid increase, the amount of complex glycolipid decrease and this event indicates deformation in the cells (21,30). According to many studies, increases in the levels of SA and LBSA can be defined as an indicator of a change in the structure of glycolipid in cell membrane (20,30).

The levels of serum SA and LBSA were higher in treatment group II in comparison to the levels in

treatment group I. These results may emanate from the difference in doses given to the first and second treatment groups.

Before macroscopic findings, following up the levels of SA and LBSA, and the levels of enzymes ALT, AST and GGT can give an idea about the liver damage caused by the toxic effect of DENA, which is hepatotoxic and hepatocarcinogenic. However, this kind of research can be supported by histopathological examinations in order to provide in-depth analysis of liver damage.

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