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The Investigation of Recombinant Human IGF-I (Insulin-Like Growth Factor-I) Injection on Rats Liver Malondealdehyde and Reduced Glutathione Levels

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Summary

The effect of insulin-like growth factor-I (IGF-I) administration on lipid peroxidation and reducted glutathione in liver of rats was studied. Total of 30 female rats (Wistar albino), weighing 95±5g, were divided into two groups and kept in two separete cages. The second group (n=15) was rh IGF-I (100 ng/kg/day) in 0.01 M NaHCO3) was subcutananeusly injected tor at for a 7 days period. Also, first group (control group (n=15)) was subcutananeusly received 0.01 M $NaCHO_3$ in same manner with rh IGF-I treated group. At the end of injection period, at 8th, 15th and 22nd days, rats have been weighed and liver tissues were removed. At the 7th injection day, removed liver tissues from control group has been examined and has not been seen any significant changes statistically. At the 15th injection day, has seen that MDA levels are increased ($P<0.01$) and GSH levels are decreased ($P<0.01$) statistically. On the other hand at the 22nd day has been recorded that both MDA and GSH levels are decreased. These results showed that IGF-I has been important metabolic effect on the MDA and GSH.

Keywords: Insulin like growth factor I, lipid peroxidation, reduced glutathione, rat

Recombinant Human IGF-I (Insülin-Like Growth Factor-I) Enjeksiyonu Yapılan Ratlarda Karaciğer Malondialdehit ve Redükte Glutatyon Düzeylerinin Araştırılması

Özet

Bu çalışmada deney hayvanı olarak, ağırlıkları 95±5 g arasında değişen 30 adet dişi rat (Wistar albino), kontrol ve deney olarak 15'er hayvanlık iki gruba ayrıldı ve ayrı kafeslere alındı. Deney grubundaki ratlara 7 gün boyunca her 24 saatte bir 100 ng/kg/gün dozunda rekombinat human Insülin-Like Growth Factor-I (rh IGF-I), kontrol grubuna ise sadece 0.01 M $NaHCO_3$ subkutan olarak enjekte edildi. Yedi günlük enjeksiyonu takip eden 8, 15 ve 22. günlerde ratlar tartılarak karaciğer dokuları alındı.

Enjeksiyonun 7. gününde kontrol grubundan alınan karaciğer örneklerinde malondialdehit (MDA) ve redükte glutatyon (GSH) düzeylerinde istatistiksel olarak önemli bir değişim görülmedi. Onbeşinci günde MDA düzeylerinde istatistiksel olarak önemli bir artış görülürken, GSH düzeyinde aynı oranda bir azalış görüldü. Yirmiikinci günde hem MDA hem de GSH düzeylerinde önemli bir azalma kaydedildi ($p<0.01$). Bu sonuçlar IGF-I'in MDA ve GSH üzerinde metabolik bir etkiye sahip olduğunu göstermektedir.

Anahtar sözcükler: İnsülin benzeri büyüme faktörü-I, lipit peroksidasyonu, redükte glutatyon, rat.

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INTRODUCTION

Oxidative stress, resulting from the increased production of free radicals and reactive oxygen species and/or a decrease in antioxidant system, causes severe damage to biologic macromolecules and dysregulation of normal metabolism and physiology[1,2]. Oxygen radicals are known to produce membrane peroxidation and MDA formation[3], which are both detrimental to cellular function. Peroxidation can increase membrane permeability, whereas MDA can inactivate membrane transporters[3] by forming intra and intermolecular cross links[3,4]. Such events may be more damaging in the long term[5]. To minimize free radical damage, there is a complex antioxidant system, which includes the interception of free radicals with antioxidants to form less reactive compounds. Antioxidants prevent the organism from the harmful effects of free radicals by scavenging or inhibiting their formation. Cells maintain their vital functions against oxidative damage with the help of a system that involves antioxidant enzymes.

It is generally known that the GSH-related thiols participate in many important biological reactions, including the protection of cell membranes against oxidative damage[6]. The endogenous antioxidant glutathione (GSH) is the most abundant antioxidant in the body and is responsible for neutralizing reactive oxygen species produced in the mitochondria[7,8]. GSH scavenges super oxide radicals and hydrogen peroxide resulting in the formation of the disulfide glutathione (GSSG). GSSG is subsequently reduced by GSSG reductase and several other antioxidant enzymes are used to process and detoxify reactive oxygen species ROS[8]. However, the effect of IGF-I on the anti-oxidative enzymes, especially in liver tissue, should be clarified.

The insulin-like growth factors (IGF-I and IGF-II) compare a family of polypeptide hormones structurally related to insulin with multifunctional metabolic and anabolic properties[9]. Growth hormone released from the anterior pituitary gland increases IGF-I synthesis and secretion from the liver and, at the cellular level, promotes the synthesis of DNA, RNA and protein. Although these actions are most commonly associated with long bone growth and the increase in lean body mass that occurs during normal mammalian development, studies in our laboratory and others have focused on a possible relationship between oxidative stress and IGF-I and liver[10,11].

Insulin growth factor-I is a growth factor with anabolic effects[12], which is mainly produced by hepatocytes. Patients with advanced liver cirrhosis show low serum concentrations of this hormone[13]. Previous works have shown beneficial effects of low doses of IGF-I on experimental cirrhosis including liver function tests and oxidative liver damage[14]. To better understand the possible pathways behind the beneficial effect of IGF-I, the aim of this work is to investigate several parameters involved in oxidative damage in hepatic tissue.

MATERIALS and METHODS

Animals

Wistar albino female rats, weighing 95±5 g, were fasted for 12h, but allowed free access to water before the experiments. The animals were kept in wirebottom cages, in a room at a constant temperature (22±2°C) with 12-h light and dark cycles, and were fed a standart rat chow (Table 1) and tap water ad libutum. The rats were randomly divided into the two groups of fifteen each. The second group was rh IGF-I (100 ng/kg/day) in 0.01 M $NaHCO_3$) was subcutaneous injected for a 7 days period. Also, control group (n=15) was subcutaneous received 0.01 M $NaCHO_3$ in the same manner with rh IGF-I treated group.

Tablo 1. Diyet bileşimi
Table 1. Diet composition

Ingredients	%
Wheat	10
Corn	23
Barley	15
Wheat bran	8
Soybean	26
Fish flour	8
Meat-bone flour	4
Pelted	5
Salt	0.8
Vitamin mineral mix*	0.2

* *Vitamin A, D_3, E, K_3, B_1, B_2, B_6 and B_{12}, nicotinamide, folic acid, biotin, Mn, Fe, Zn, Cu, I, Co, Se, antioxidants (butyldroxytoluol) and Ca.*

Biochemical Analysis

Analysis of malondialdehide in the liver

Lipid peroxidation in the liver tissue was assessed by estimation of malondialdehyde (MDA) according to the method of Ohkawa et al[15]. Reduced glutathione (GSH) was measured estimated in the liver tissue. GSH was assayed by the method of Beutler et al[16]. GSH concentrations were calculated for 1 g protein content from the 13000 g supernatant fraction and protein concentration, in supernatants were determined by Folin-phenol reagent with bovine serum albumin as the standard[17].

Statistical Analysis

The collected values as mean±SE were statistically analyzed (student's t-test) (IFFC, 1987) and evaluated using the SPSS 6.0 (1993) softwove programe. P values< 0.01, 0.05 were considered significant18.

RESULTS

Lipid peroxidation is thought to be an important mechanism of liver injury and malondialdehyde (MDA) is one of its end products. Thus, measurment of MDA can be used to assess lipid peroxidation.

Tablo 2. rh-human IGF-I verilen ratlarda karaciğer MDA ve GSH düzeyleri
Table 2. Liver MDA and GSH levels in rats after rh-human IGF-I administration

Time after administration (day)	n	Control		Treatment	
		MDA (nmol/g prot)	GSH (nmol/g prot)	MDA (nmol/g prot)	GSH (nmol/g prot)
7	5	794.9±32.2	3.32±0.3	789.4±32.2	3.27±0.3
15	5	792.3±28.4	3.25±0.2	804.7±26.8*	2.56±0.4*
22	5	794.8±27.4	3.33±0.3	776.8±32.6*	2.94±0.3*

* $p<0.01$

Table 2 shows the biochemical values, GSH and MDA levels of IGF-I-tretatment animals and normal control subjects. GSH and MDA levels in liver did not differ significantly in the liver tissues of IGF-I-treated groups (7th day), compared to those of controls group. On the 15th day it has been seen that MDA level has been incresed statistically on the other hand, GSH level has been decreased at the same ratio with MDA. On the 22th day it has been seen that both MDA and GSH levels have decreased.

DISCUSSION

Antioxidants prevent new radical species formation by converting existing free radical species into less harmful molecules or by preventing transformation of free radicals from other molecules. To avoid from ROS-induced injury to tissue, a complex antioxidant system, consisting of both enzymatic and non enzymatic defenses, has evaluated. Traditionally, antioxidants have been defined as substances that prevent the formation of ROS or other oxidants, scavenge them, or repair the damage that they cause[19-22]. There is a considerable evidence that IGF-I decreases ROS and free radicals production both in vivo and invitro, and depressed IGF-I synthesis in rat is considered to be responsible for enhanced oxidative stress[14,23]. Liver damage has been associated with oxidative stress and lipid peroxidation[16-24]. Recent studies from laboratory in rats with carbon tetrachloride-induced cirrhosis have demonstrated that short courses of treatment with low doses of IGF-I are associated to hepatoprotective[11,23,24].

In order to give a better insight into the pathways by which IGF-I seems to exert its the hepatoprotective actions, this study was aimed to analyze several parameters involved in oxidative stress in the liver, such as MDA, GSH both in IGF-I treated and untreated rats.

In order to find a relationship between the studied parameters and oxidative liver damage, MDA levels, an index of lipid peroxidation, were evaluated. Hepatic levels of MDA were increased in untreated rats compared with control group as it was previously reported in cirrhotic rat similar protocols[10-11]. The same result is shown in our study in Table 2. However, the reduction in the antioxidant defense systems

recovered to control levels at 22nd day, the evaluated MDA and GSH decreased with the recovery of radical scavenging enzymes ($p<0.01$) compared to those of control group. GSH is known to be counter-regulatory to a number of cytokines including IGF-I, which is being investigated as a possible surrogate marker for breast cancer risk. GSH conjugation of IGF-I is a major pathway of excretion of IGF-I[25].

These data indicate that the antioxidant defense system against oxidative damage was impaired after IGF-I–induced. In addition, the recovery of this system in the liver is mainly caused by the degradation of lipid peroxide, resulting in a decrease in MDA. Those findings support the hypothesis that IGF-I enhances the activity of the pathway and impairs the antioxidant status, particularly glutathione redox cycle, resulting in a higher defense against oxidative stress[10,24].

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