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# Melatonin Increases the Expression of Insulin-like Growth Factor I in Rats with Carbontetrachlorid-Induced Hepatic Damage

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**Abstract:** The purpose of this study was to investigate, whether or not melatonin has a role in the prevention of $CCl_4$-induced hepatotoxicity in rat by means of IGF expression. The animals used in study were grouped as following: Control, $CCl_4$ injected group and $CC_4$ + melatonin injected group. Tissue samples were examined using immunohistochemistry and obtained data were evaluated statistically. IGF-I expression observed in hepatocytes was weakest in $CCl_4$ injection group. Melatonin administration increased IGF-I expression in hepatocytes. The HSCORE value of the $CCl_4$ injected group was lower than that of other groups. The highest HSCORE value belonged to $CCl_4$ + melatonin injected group. On the basis of the data in the present study, we suggest that melatonin secreted by the pineal gland may have preventative role in hepatic damage by increasing IGF-I releasing.

**Key words:** Carbon tetrachloride, hepatotoxicity, IGF-I, immunohistochemistry, melatonin, rat

## INTRODUCTION

Insulin-like Growth Factor (IGF-I) is a polypeptide with endocrine, paracrine and autocrine effects whose structure is 50% like that of insulin. Although, many tissues secrete it, >90% of circulating IGF-I is synthesized in the liver (Conchillo *et al*., 2007). In normal liver, IGF-I is synthesized at high levels in hepatocytes and in multiple non-parenchymal cell types including Hepatic Satellite Cells (HSCs). Normal hepatocytes express 5-20 fold higher levels of IGF-I than non-parenchymal cells and hepatocyte derived IGF-I is the major source of circulating IGF-I (Sanz *et al*., 2005). Recent studies have focused attention on role of IGF-I in liver disease and HSC biology. IGFs may play a significant role in hepatic fibrosis (Wang *et al*., 2005). Liver cirrhosis is a condition with IGF-I deficiency, which becomes more severe with disease progression, according to all reported studies. IGF-I levels are reduced in cirrhotic patients, whereas the Growth Hormone (GH) levels are increased (Conchillo *et al*., 2007). In cirrhosis the reduction of receptors for GH in hepatocytes and the diminished synthesis ability of the hepatic parenchyma cause a progressive fall in serum IGF-I levels. The clinical impact of the decreased in IGF-I production in advanced cirrhosis is largely unknown (Caufriez *et al*., 1991; Hattori *et al*., 1992; Moller *et al*., 1996). Recent studies in rats with carbon tetrachloride-induced cirrhosis have demonstrated that short courses of treatment with low doses of IGF-I are able to produce systemic beneficial effects (Castilla-Cortazar *et al*., 1997a, b, 1999, 2001; Cemborain *et al*., 1998; Pascual *et al*., 2000; Picardi *et al*., 1997) and are associated to hepatoprotective (Castilla-Cortazar *et al*., 1997b; Mirpuri *et al*., 2002) and antifibrogenic (Muguerza *et al*., 2001) effects (Garcia-Fernandez *et al*., 2005). Melatonin, a major secretory product of the pineal gland are known to be involved in a variety of physiological processes including the regulation of endocrine rhythms, antigonadotropic effects, neuroprotective effects and stimulation of the immune function, antioxidant and free radical scavenger effects (Kus *et al*., 2005; Oner and Ozan, 2003; Reiter, 1994). However, experimental studies have shown that light conditions, pinealectomy and also, long-term melatonin administration modify the synthesis and/or circadian release of GH and IGF-I as well as thyroid, adrenal cortex and testicular hormones (Oner and Ozan, 2003; Ostrowska *et al*., 2001, 2003). Melatonin results in a substantial increase in serum levels of IGF-I by stimulating IGF-I release (Pawlikowski *et al*., 2002; Schaeffer and Sirotkin, 1997; Vriend *et al*., 1990). The purpose of this study was to investigate, whether or not melatonin has a role in the prevention of $CCl_4$-induced hepatotoxicity in rat by means of IGF expression.

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## MATERIALS AND METHODS

**Animals and treatments:** Adult male Wistar albino rats (weighing 170-220 g) supplied by Firat (Euphrates) University Medical Faculty Experimental Research Unit were randomly divided into three groups with 8 animals per group. All animals received humane care in compliance with guidelines of Firat University Research Council's criteria. The rats were kept in plexiglas cages (4 animals cage$^{-1}$) where, they received standard chow and water *ad libitum* in an air-conditioned room with automatically regulated temperature (22±1°C) and lighting (07.00-19.00 h). All rats were allowed to acclimate for 1 week prior to experimentation. Control rats (group 1) received pure olive oil (1 mL subcutaneously (sc)) alone. Rats in group 2 were injected with $CCl_4$ (0.5 mL kg$^{-1}$ body weight per 1 mL olive oil sc; EM Science, Cherry Hill, NJ, USA) every other day for 1 month. Whereas, rats in group 3 received melatonin (25 mg kg$^{-1}$ body weight per 1 mL 10% ethanol sc; Sigma, St. Louis, MO, USA) with a subcutaneous injection of $CCl_4$ every other day for 1 month.

**Immunohistochemical procedure:** The liver tissue samples were fixed in Bouin's fixative for 8 h, embedded and then serial sections (5 μm) were collected on slides with polysine. After rehydrating, samples were transferred to 0.01 M citrate buffer (pH 6) and subsequently heated twice in a microwave oven for 5 min each time at 750 W for antigen retrieval. After cooling for 20 min at room temperature, the sections were washed with Phosphate Buffer Saline (PBS). To remove endogenous peroxidase activity, sections were kept in 3% $H_2O_2$ for 20 min and afterward washed with PBS. Sections were incubated with primary rabbit-polyclonal Insulin-like Growth Factor I (IGF-I) (NeoMarkers, Fremont, CA) for 1 h. Negative control sections were treated with nonimmune serum diluted in the same manner. Labeling was visualized using the Universal LSAB kit (Dako, Carpinteria, CA) according to the manufacturer's instructions. Staining was completed with DAB Chromogen (Dako, Carpinteria, CA) for 1-2 min and slides were counterstained with Harris's Hematoxylin, dehydrated and then cover-slipped with permount. All specimens were examined in Nikon-E600 light microscope.

**Quantitative evaluation:** Sections were evaluated with respect to IGF-I localization in a semiquantitative manner using a light microscope and selected areas were photographed.

Histological Score (HSCORE) values of IGF-I staining were obtained in a semiquantitative manner and included both intensity and distribution patterns of staining. Five different fields of five sections per specimen at 400× magnification were evaluated for the analysis of immunohistochemical IGF-I staining. Values were recorded as percentages of positively stained target cells in each of the four intensity categories which were denoted as 0 (no staining), 1 + (weak), 2 + (moderate), 3 + (strong). For each tissue, a HSCORE value was derived by summing the percentages of cells that stained at each intensity category and multiplying that value by the weighted intensity of the staining, using the equation:

$$HSCORE = \Sigma P_i(i + 1)$$

Where:

i = The intensity scores

$P_i$ = The corresponding percentage of the cells (Seval *et al*., 2004)

**Statistical analysis:** In comparing, HSCORE value belong to groups were analyzed by analysis of variance. When, the results were significant, Turkey's test (honestly significant difference) was performed to disclose differences between group means. The $p<0.05$ were considered to be statistically significant.

## RESULTS

In the light microscopic examination, liver section of control group exhibited a normal histological appearance. Immunohistochemical analysis of liver tissue sections revealed that the IGF-I expression, as brown granules in the cytoplasm and/or nuclei. The IGF-I labeling was moderate in the control group (Fig. 1). Livers of rats treated with $CCl_4$ showed degenerative changes. Connective tissue increasing in especially, portal areas and diffuse vacuolar degenerations were remarkable.

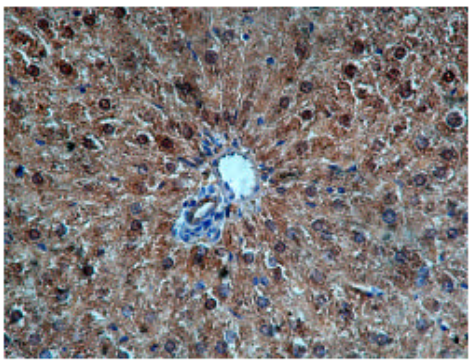

Fig. 1: Immunlocalization of IGF-I in the liver of control group ×20

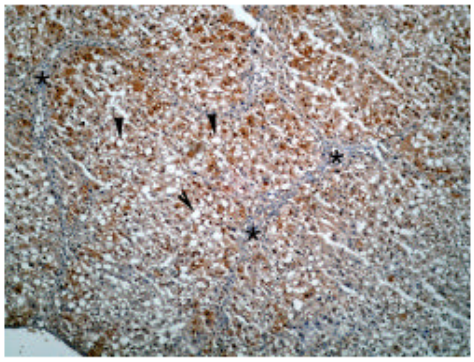

Fig. 2: Immunlocalization of IGF-I in the liver of $CCl_4$ injected group. Stars; connective tissue increasing, arrow heads; vacuolar degenerations ×10

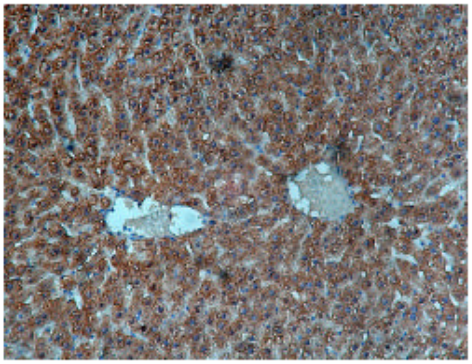

Fig. 3: Immunlocalization of IGF-I in the liver of $CCl_4$ + Melatonin injected group ×20

Table 1: HSCORE values (mean±SD) for IGF-I staining intensities in the groups

| Groups | HSCORE values |
|---|---|
| Control | 300.00±00 |
| $CCl_4$ injected group | 90.24±7.46* |
| $CCl_4$ + melatonin injected group | 380.00±40.82* |

*p<0.05 compared to the other groups

IGF-I labeling was apparently decreased with the development of hepatic degeneration in result of 1 month subcutaneous injections of $CCl_4$ (Fig. 2). The most strong staining was observed in $CCl_4$ + melatonin injected group. Histopathological changes observed after administration of $CCl_4$ were lost from rats treated with $CCl_4$and melatonin (Fig. 3).

HSCORE values of IGF-I immunostaining were shown in Table 1. The HSCORE value of the $CCl_4$ injected group was lower than that of other groups ($p<0.05$). The highest HSCORE value belonged to $CCl_4$+melatonin injected group and statistically was different from the other groups ($p<0.05$).

## DISCUSSION

In the present study, we investigated whether or not melatonin has a preventative role via IGF in $CCl_4$-induced hepatic damage. To the knowledge, this is the first report to examine the effect of melatonin on IGF expression in $CCl_4$-induced hepatic damage in rats.

$CCl_4$, which is an intrinsic hepatotoxin, was used for the purpose of inducing hepatic damage in this study, because carbon tetrachloride has previously been shown to exert its toxic effects on the liver. It has previously been reported that $CCl_4$ caused necrosis, fibrosis, mononuclear cell infiltration, steatosis and foamy degeneration of hepatocytes, increase in mitotic activity and cirrhosis in liver. Furthermore, it has also been reported that $CCl_4$ caused apoptosis in liver (Kus *et al*., 2005). Various studies related to role of IGF in hepatic damages have reported that serum IGF-I levels decreased during chronic liver disease (Friedman, 2000; Pinzani *et al*., 1990). Lipopolysaccharide administration decreases circulating levels of IGF-I both in humans (Lang *et al*., 1997) and in experimental animals (Fan *et al*., 1994; Soto *et al*., 1998). On the contrary, Scharf *et al*. (2004) have reported that a marked increase in IGF-I and IGFBP-1 mRNA levels very early after $CCl_4$ application. The study has shown that the IGF-I protein is mainly expressed in hepatocytes and that $CCl_4$-induced hepatic fibrosis is associated with reduced IGF-I expression. Earlier studies have reported that IGF-I plays an important role in the early stages of liver tissue repair. $CCl_4$ induced liver cirrhosis indicate that systemic administration of IGF-I improves nutritional status and liver function (Picardi *et al*., 1997) and reduces oxidative damage and fibrosis (Svegliati-Baroni *et al*., 1999). Transgenic mice over expressing IGF-I have accelerated liver regeneration after liver injury (Sanz *et al*., 2005). In addition, IGF-I treatment resulted in effective prevention of acute liver failure in rats induced by D-galactosamine and LPS (Inoue *et al*., 2003). This fact suggests a therapeutic potential for IGF-I in the prevention of acute liver failure.

Melatonin is a neurohormone released by the pineal gland. It is well known that melatonin is continuously synthesized and secreted during a period of continuous darkness and that its synthesis and secretion are inhibited during a period of continuous light (Reiter, 1994). Many studies indicate connection between pineal gland function and GH-IGF-I axis in mammals, however their results are not always synonymous. Vaughan *et al*. (1994) have shown significant decrease in circulating IGF-I concentrations in female hamsters and an increase in males after short photoperiods. A significant increase of GH and IGF-I concentrations have informed in Syrian male

hamsters after Melatonin administration at evening hours for the period of 10 weeks (Vriend *et al*., 1990). In the researcher's opinion the increase of IGF-I concentrations induced by exogenous melatonin administration is probably secondary to GH secretion. Our results concerning that melatonin increased IGF-I expression in hepatocytes were consistent with above results.

## CONCLUSION

On the basis of the data in the present study, we suggest that melatonin secreted by the pineal gland may have preventative role in hepatic damage by increasing IGF-I releasing.

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