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THE EFFECTS OF RECOMBINANT HUMAN IGF-1 (INSULIN-LIKE GROWTH FACTOR-1) INJECTION ON LIVER GROWTH IN RATS

KOCAMIS H, KARADAG SARI E, ASLAN S, GULMEZ N and DEPREM T

University of Kafkas, Faculty of Veterinary Medicine, Department of Histology and Embriyology, Kars, Turkey

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The purpose of the present study was to evaluate the effects of recombinant human (rh) IGF-1 administration on liver growth of rats. RhIGF-1 (100 ng/kg/day prepared in 0.01 M NaHCO3) was injected (s.c) daily to rats for seven days. Control groups received the same injection procedure with only 0.01 M NaHCO3. One day after the last injection, rats from both control and rhIGF-1 injected groups (n = 5 per group) were euthanized and liver tissue samples were collected (group I). Liver samples from both groups (n = 5 per group/collection day) were collected on week one (group II) and week two (group III) after the last injection. Tissue samples were immediately fixed in Bouin's solution and embedded in paraffin. Tissue sections were cut into 5-6 µ thickness and stained with Crossman's triple staining method. RhIGF-1 administration increased the number and the diameter of liver epithelial cells (hepatocytes) which in turn affected the liver growth of rats.

Key words: rat, liver, light microscopy, rhIGF-1

INTRODUCTION

IGFs are functionally and structurally similar to insulin, even though they are not solely produced by the pancreas. Insulin is established for its strong metabolic activity, whereas IGFs, particularly IGF-1, has a much stronger effect on growth than insulin (Murray *et al.*, 1993). The IGF family consists of IGF-1, IGF-2, IGF receptors and IGF binding proteins (Fu *et al.*, 2001). IGF synthesis in different tissues, particularly in the liver, is controlled by growth hormone (GH). Compared to IGF-1, IGF-2 synthesis is less affected by GH (Mc Murty *et al.*, 1994). IGFs are one of the most studied growth factors that have pivotal effects on tissue and organ growth (Stewart and Rotwein, 1996; Werner and Le Rotth, 1996).

IGF-1 (Humbel, 1990) has a strong effect on growth and development of several different cells and tissues *in vitro* and *in vivo* (Buyukkayhan *et al.*, 2003). IGFs regulate cell functions via their special receptors called IGF-R1 and IGF-R2 (Daughaday and Rotwein, 1989). IGF-1 mRNA expression is much higher in adult rat liver than in embryonic rat liver (Godfedson *et al.*, 1991). IGF knock-out mice exibit retarded growth and eventually postnatal death (Lui *et al.*, 1993).

The objective of the present study was to evaluate the effects of rhIGF-1 administration on liver growth in rats.

MATERIALS AND METHODS

Injection Procedure

In the current study, 30 female, 95 ± 5 g body weight rats (Wistar albino) were obtained from the Experimental Animal Unit of Istanbul University Medical Faculty. Rats were divided into two groups (n = 15 per group) and kept in separate cages. RhIF-1 (100 ng/kg/day prepared in 0.01 M $NaHCO_3$) was injected daily (s.c) for seven days. Control groups received the same injection procedure with only 0.01 M $NaHCO_3$. rhIGF-1 was kindly provided by Dr. A. F. Parlow (UCLA, CA, USA). Rats from both groups were kept under equal conditions (12h darkness, 12h light). Water and standard rat feed were provided *ad libitum*.

Histological Techniques

One day after the last injection (group I), rats from both rhIGF-1 injected and control groups (n = 5 per group) were euthanized with ether anesthesia and liver tissue samples were collected. Liver samples from both groups (n = 5 per group/ collection day) were collected one week (group II) and two weeks (group III) after the last injection. After being fixed in Bouin's solution, tissue samples were processed with alchol, methil benzoate and benzol, and then embeded in paraplast. Tissue sections from those paraplast embeded samples were cut into 5-6 μ thickness and stained with Crossmon's triple staining techniques (Crossman, 1937).

The number of liver epithelial cells (hepatocytes) and their cell and nuclei diameters were determined in each stained tissue section (at least 50 different locations of each tissue section). Micrometric measurements were carried out by a ocular micrometer attached to a microscope. The number of liver epithelial cells were calculated on a given area of 0.01 mm^2 on each stained tissue section. Those numbers were converted to micro meter values and then t- (Kabukcu, 1994) and f- (Minitab, 1994) tests were used to statistically compare the mean values.

RESULTS

The histological structure of rhIGF-1 rat liver tissue samples were found to be similar to that of control groups (Figure 1).

Histometric Findings

rhIGF-1 administration increased the number of hepatocytes in group I and II compared to that of the control ($P<0.001$), whereas they were decreased in group III. Additionally, the number of hepatocytes was found to be statistically different within each individual group (Figure 2). rhIGF-1 injection significantly increased hepatocyte cell diameters ($P<0.001$) in Group 1 and decreased in group II and III (Figure 3). However, it did not have any significant effect on nucleus

diameters of those cells in group I and III, but rhIGF-1 administration significantly reduced nucleus diameters of cells in group II (Figure 4).

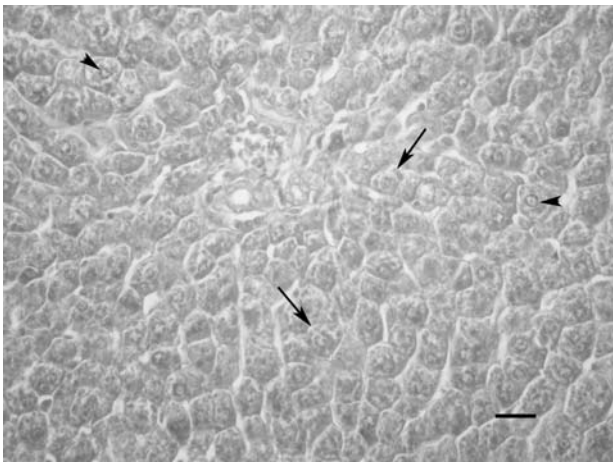

Figure 1. Light micrograph of a portion of the 7 day-old rat liver tissue. Arrows: Liver cells (hepatocytes), arrow heads: Cell nucleus. Bar: 50 μm

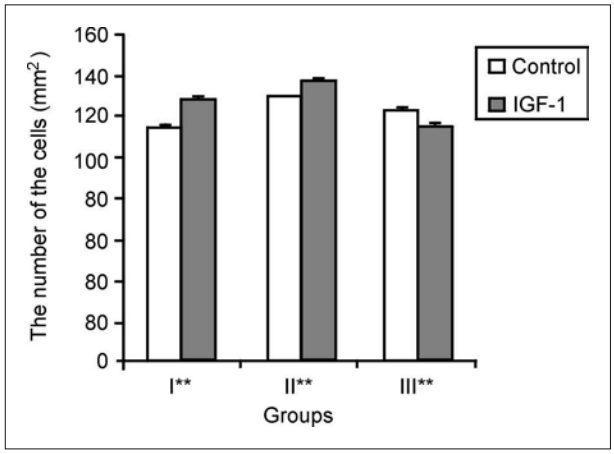

Figure 2. The number of liver cells (hepatocytes) in rhIGF-1 injected and control groups. ** $P<0.001$

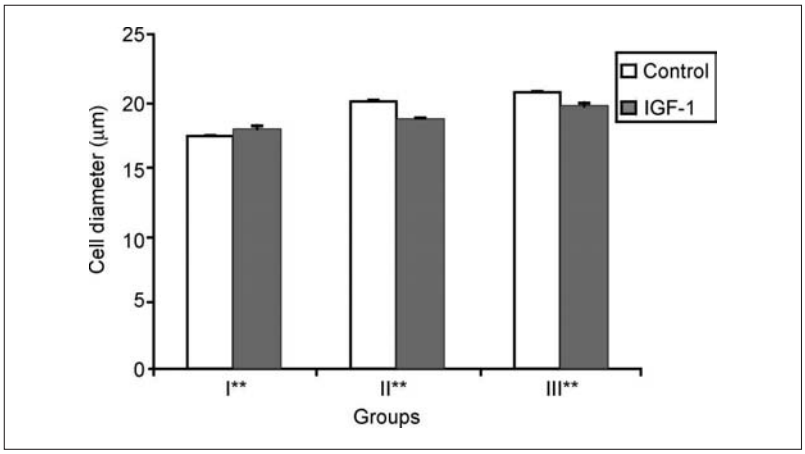

Figure 3. Liver cell (hepatocytes) diameters in rhIGF-1 injected and control groups.
**P<0.01

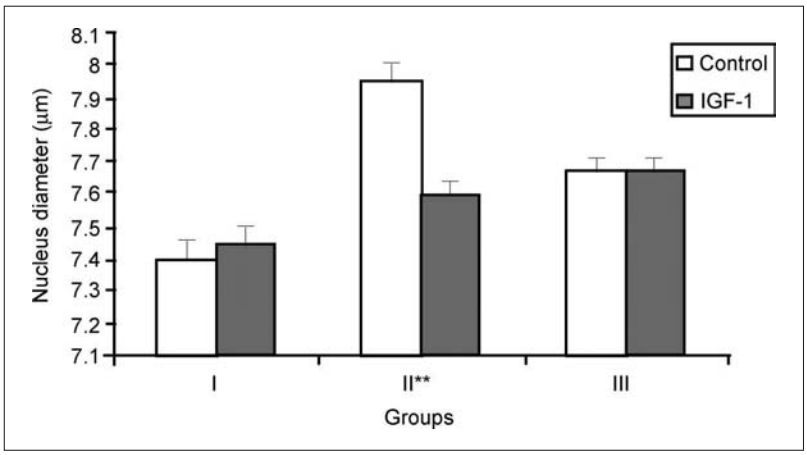

Figure 4. Liver cell (hepatocytes) nucleus diameters in rhIGF-1 injected and control groups.
**P<0.001

DISCUSSION

IGF-1 produced mainly by the liver has been widely accepted as one of the growth stimulant of many tissues in animal species. Several studies have shown that IGF-1 has postnatally very important effects on growth of mammals (Lui *et al.*, 1993; Yakar *et al.*, 1999) and poultry species. Biochard *et al.* (1992) Fu *et al.*(2001) demonstrated that IGF-1 mRNA level in the liver tissue increased duringn the rapid growth period of quails. Kocamis *et al.*, (1998) displayed that *in ovo* rhIGF-1

administration increased the posthatch growth rate of chicken. Additionally, IGF-1 administration during the embryonic development affected fetal growth of kidney, heart, lung and liver (Lok *et al.*, 1996).

IGF-1 has been demonstrated to stimulate organ formation and fetal growth of rats (Giddings and Carnaghi, 1992). In the present study, the number of liver epithelial cells was found to be suddenly decreased in rats from group III. This sudden decrease might be due to the increase in the diameter of these cells and their nucleus. We did not observe any decline fom either group I or II in terms of the number of liver epithelial cells. Furthermore, the number of liver epithelial cells, as well as the diameters of cells and nuclei from group I and II have been found to be higher than in group III.

IGF-1 mRNA expression has been found in the liver to be 40-100 times higher than in other tissues (Lund *et al.*, 1986). IGF-1 mRNA level has increased between embryonic day 11 and 13 of rats (Rotwein *et al.*, 1987). Additionally, IGF-1 level in plasma has been found to be low during birth, but has increased postnatally (Doughaday and Rotwein, 1989; Yakar *et al.*, 1999) have shown that IGF-1 in the circulation is one of the most important factors for postnatal growth of rats. Kikuchi *et al.* (1991) have demonstrated that IGF-1 gene seqence has a high similarity suggesting that IGF-1 has been well conserved throughout evolution.

In conclusion, rhIGF-1 administration increased the number and the diameter of liver epithelial cells (hepatocytes) which in turn affected liver growth of rats.

Address for correspondence:
Res. Assist. Dr. Ebru Karadag Sari
Department of Histology and Embriyology
Faculty of Veterinary Medicine, University of Kafkas
36100 Kars, Turkey
E-mail: ekaradag84@hotmail.com, ekarasari@yahoo.com

REFERENCES

1. *Biochard MT, Huybrechts LM, Decuypere E, Kuhn ER, Monvoisin JL et al,* 1992, Effectes of insulin-like growth factor-I (IGF-I). Infusion and dietary tri-iodothyronine (T_3) supplementation on growth, body composition and plasma hormone levels in sex-linked drawf mutant and normal chickens, *J Endocr*, 133, 101-10.
2. *Buyukkayhan D, Tanzer F, Erselcan T, Cinar Z, Yonem O,* 2003, Umblical serum Insulin Like Growth Factor-1 (IGF-1) in newborns, *Int Vit Nutr Res,* 73, 5, 343-6.
3. *Crossmonn G,* 1937, A modification of Mallory's connective tissue stain with a discussion of the principels involved, *Anat Rec*, 69, 33-8.
4. *Daughaday WH, Rotwein P,* 1989, Insulin-like growth factors I and II. Peptide, messenger ribonucleic acid and gene structures, serum, and tissue concentrations, *Endocr Rev*, 10, 68-91.
5. *Fu Z, Kubo T, Noguchi T, Kato H,* 2001, Developmental changes in the mRNA levels of IGF-1 and its related genes in the reproductive organs of japanese quails (*Coturnix coturnix japonica*), *IGF Res*, 11, 24-33.
6. *Giddings SJ, Carnaghi LR,* 1992, Insulin receptor gene expression during development. Developmental regulation of insulin receptor mRNA abundance in embryonic rat liver and yolk sac, developmental regulation of insulin receptor gene splicing, and comparison to abundance of insulin-like growth factor 1 receptor mRNA, *Mol Endocr*, 6, 10, 1665-72.

7. *Godfredson JA, Holland MD, Odde KG*, Hossner *KL,* 1991, Hypertrophy and hyperplasýa of bovine fetal tissues during development: fetal liver insulin-like growth factor I mRNA expression, *J Anim Sci*, 69, 1074-81.
8. *Humbel RA,* 1990, Insulin-like growth factor I and II, *Eu J Biochem*, 190, 445-62.
9. *Kabukcu MA,* 1994, Saglik, Sosyal ve Fen Bilimlerinde Uygulamali Istatistik. Selcuk Univ Ziraat Fak Tarim Ekonomi Bolumu, Konya.
10. *Kikuchi K, Buonomo FC, Kajimoto Y, Rotwein P,* 1991, Expression of Insulin-Like Growth Factor-I during chicken development, *Endocrinology*, 128, 1323-8.
11. *Kocamýs H, Kýrkpatrick-Keller DC, Klandorf H, Killefer J,* 1998, *In ovo* administration of recombinant human insulin-like growth factor-I (rh IGF 1) alter posnatal growth and development of the broiler chicken, *Poult Sci,* 77, 1913-9.
12. *Lok F, Owens JA, Mundy L, Robinson JS, Owens PC,* 1996, Insulin-like growth factor 1 promotes growth selectively in fetal sheep in late gestation, *Am J Physiol Regul Integr Comp Physiol*, 270, 1148-55.
13. *Lui JP, Baker J, Perkins AS, Robertson EJ, Efstratiadis A,* 1993, Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r), *Cell*, 75, 1, 59-72.
14. *Lund PK, Moats-Staats BM, Hynes MA, Simmons JG, Jansen M et al,* 1986, Somatomedin-C/insulin-like growth factor-I and insulin-like growth factor-II mRNAs in rat fetal and adult tissues, *J Biol Chem,* 261, 14539-44.
15. *Mc Murtry JP, Francis GL, Upton FZ, Rosselot G, Brocth DM,* 1994, Developmental changes in chicken and turkey IGF-1 studied with homologous radioimmunoassay for chicken IGF-1, *J Endocr,* 142, 225-34.
16. *Minitab,* 1994, Minitab Reference Manual. Release 10 for Windows Minitab Inc, USA.
17. *Murray RK, Mayes PA, Granner DK, Rodwell Victory W,* 1993, Harper'in Biyokimyasi, Baris Kitabevi, Istanbul, 595-614.
18. *Rotwein P, Pollock KM, Watso M, Milbrandt JD,* 1987, Insulin-like growth factor gene expression during rat embryonic development, *Endocr*, 121, 2141-4.
19. *Stewart CE, Rotwein P,* 1996, Growth, differentiation, and survival: multiple physiological functions for insulin-like growth factors, *Physiol Rev*, 76, 1005-26.
20. *Yakar S, Liu JL, Stannard B, Butler A, Accili D, Sauer B et al,* 1999, Normal growth and development in the absence of hepatic insulin-like growth factor-1, *Proc Natl Acad Sci*, 96, 7324-9.
21. *Werner H, LeRotth D,* 1996, The role of the insulin-like growth factor system in human cancer, *Adv Cancer Res*, 68, 183-223.

UTICAJ REKOMBINANTNOG INSULINU SLIČNOM FAKTORA RASTA 1 (IGF-1) NA RAST JETRE PACOVA

KOCAMIS H, KARADAG SARI E, ASLAN S, GULMEZ N i DEPREM T

SADRŽAJ

Cilj ovih istraživanja je bio da se utvrdi uticaj rekombinantnog, insulinu sličnom faktora rasta 1 (IGF-1), na rast jetre pacova. Ovaj hormon je aplikovan potkožno, pacovima težine 95 ± 5 g, u dozi od 100 ng/kg dnevno, rastvoren u 0,01 M $NaHCO_3$ tokom sedam dana. Kontrolna grupa životinja je dobijala samo

$NaHCO_3$ u istim vremenskim intervalima. Jedan dan nakon poslednje injekcije, žrtvovano je po pet životinja iz obe grupe i uzeti su uzorci tkiva jetre. Isti postupak je bio ponovljen nedelju dana i dve nedelje nakon poslednje injekcije. Uzorci tkiva su zatim bili fiksirani i ukalupljeni u parafin. Isečci debljine 5-6 μ su bojeni trostrukom metodom po Crossman-u. Aplikacija RhIGF-1 je imala za posledicu porast broja i dijametra hepatocita što je uticalo i na rast jetre.