Leptin and Angiogenesis

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ABSTRACT

Leptin is a 16 KDa protein, consists of 167 amino acid residues. It has many functions including angiogenesis. Leptin either induces angiogenesis itself or influences the levels of other angiogenic factors. The aim of the present investigation was to study the effect of leptin on the levels of the angiogenic factors: vascular endothelial growth factor (VEGF) and thymidine phosphorylase (TP) enzyme activity in prepubertal female albino rats. Twenty prebubertal female albino rats were divided randomly into two groups; 1st group (group I); rats were injected intraperitoneally with saline alone and considered as control group. The 2nd group (group II); its rats were daily intraperitoneally injected with leptin (recombinant rat leptin (L5073), Sigma-Aldrich) in a dose of 3 µg/g. body weight in 100 µl saline for 10 days. Obtained results revealed that leptin increased significantly the serum levels of both VEGF levels and TP activity. In addition, there was a positive correlation between VEGF levels and TP activity.

INTRODUCTION

Leptin is a 16 KDa protein, discovered in 1994 in mutant obese mice[1] although its effects were discovered in 1950[2]. Leptin consists of 167 amino acid residues and although mainly produced by white adipose tissue cells, it is also produced by brown adipose tissue cells, placenta, ovaries, mammary epithelial cells, pituitary, bone marrow, liver and stomach[3]. Leptin has many physiological functions, mainly it binds to receptors in the hypothalamus to regulate energy balance and it plays a role in thermogenesis, reproduction, bone formation, hematopoiesis, and inflammation in addition to angiogenesis[4-7]. Leptin was found to influence the proliferative activity and vascular endothelial growth factor (VEGF) release from cultured mouse endothelial cells in vitro[8]. Leptin was found to act as a growth factor through several pathways, including mitogen-activated protein kinase (MAPK), Janus kinase 2–signal transducer and activator of transcription 3 (JAK2–STAT3) and phosphatidylinositol-3-kinase–protein kinase B (PI3K–AKT)[9].

Vascular endothelial growth factor (VEGF) is an endothelial cell specific growth factor. Five VEGF isoforms are generated as a result of alternative splicing from a single VEGF gene. These isoforms differ in their molecular mass and in biological properties[10]. The expression of VEGF is potentiated in response to hypoxia, by activated oncogenes, and by a variety of cytokines. VEGF induces endothelial cell proliferation,

promotes cell migration, and inhibits apoptosis. VEGF induces angiogenesis, increases the permeability of blood vessels and regulates vasculogenesis. Deregulated VEGF expression contributes to the development of solid tumors by promoting tumor angiogenesis and to the etiology of several additional diseases that are characterized by abnormal angiogenesis[10] .

Thymidine phosphorylase (TP, EC 2. 4 .2. 4) is an enzyme catalyzes the reversible conversion of thymidine to thymine[11]. TP is present in many tissues and cells in addition to blood plasma and serum and was found to play an important role in female reproductive cycle[12]. Platelet-derived endothelial cell growth factor (PD-ECGF) is a protein that induces endothelial cell migration in vitro and angiogenesis in vivo[12]. It was isolated in 1987 from human blood platelet[13]. PD-ECGF was found to have TP activity and the amino acid sequence of both proteins was identical[12].

The aim of the present investigation was to study the effect of leptin on the levels of the angiogenic factors: VEGF and TP in prepubertal female albino rats.

MATERIALS & METHODS

1- Animals:

The study included 20 prebubertal female Albino rats obtained from the Animal House of Faculty of Medicine, Assiut University. All animals were housed in Animal House, Sohag Faculty of Medicine under constant environmental conditions at 25^{o}C and 12-hours light dark cycle. Before study commencement; animals were adapted to diet and housing conditions for one week and had free access to standard laboratory chow and water during the study period. The rats were divided randomly into two groups; 1st group (group I), its rats were injected intraperitoneally with saline alone and considered as control group. The 2nd group (group II); its rats were intraperitoneally injected daily with leptin (recombinant rat leptin (L5073), Sigma-Aldrich) in a dose of 3 µg/g. body weight in 100 µl saline for 10 days. Leptin was expressed as lyophilized powder vial. The content of the vial was reconstituted by adding 1ml sterile tris HCL, PH 8.0, to prepare a working stock solution of 1 mg/ml. After reconstitution, the vial was stored at 2-8°C according to product instructions. At the end of the experiment, the rats were anaesthetized with ethanol and dissected, blood samples from the heart of each animal were collected for VEGF and thymidine phosphorylase activity assay. Blood samples were kept in 37^{o}C water bath for 30 minutes to enhance coagulation then centrifuged at 3000 r.p.m. for 10 minutes to separate serum which was kept in deep freeze at (-20 C^{o}) till the time of assay.

2- Assay of VEGF:

VEGF was assayed using rat vascular endothelial growth factor ELISA Kit, Cat. No., BEK-2109-2P according to the manufacture instructions.

3-Assay of thymidine phosphorylase activity:

Assay of TP activity was done based on the difference in extinction

coefficient between thymine and thymidine (obtained from Sigma, Cat. No. T1895) at A_{290} nm. One unit will convert 1.0 µM of thymidine and phosphate to thymine and 2-deoxyribose 1-phosphate per minute at pH 7.4 and 25°C[14]. The activity of the enzyme in the two groups was measured at different substrate concentrations and Linweaver-Burk plot was made by graphing 1/V versus 1/S.

STATISTICAL ANALYSIS:

Data were expressed as mean ± SD and analyzed using Mann Whitney test and Spearman correlation analysis.

RESULTS

1- VEGF levels:

Obtained results revealed that VEGF levels increased significantly in group II than in group I ($p<0.5$) as shown in fig. 1

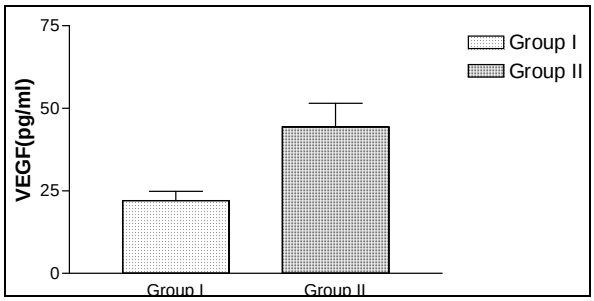

Fig.1. mean ± SD levels (pg/ml) of VEGF in the two groups of rats

2- TP enzyme activity:

Results showed that TP enzyme activity increased significantly in group II than in group I ($P< 0.05$). Fig.2. Linweaver-Burk plot of TP activity was shown in **(**Fig.5&6**).** There was positive correlation between TP activity and VEGF in both groups ($r = 0.83$ in group I & 0.87 in group II Fig.3&4).

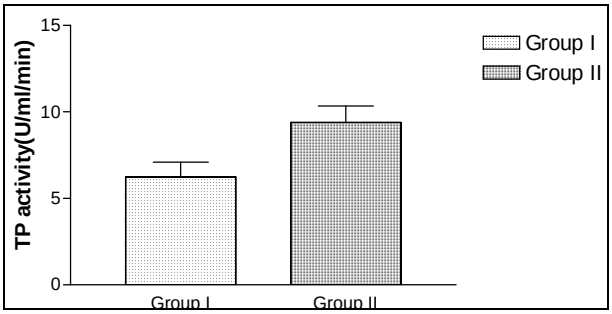

Fig.2. TP enzyme activity (U/ml/min) in the two groups of rats

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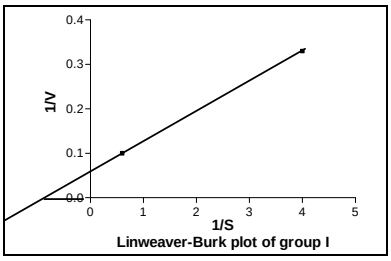

Fig.3. Correlation between TP activity and VEGF in group I

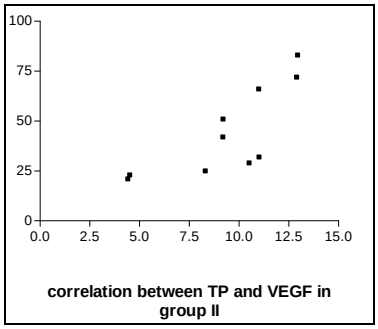

Fig.4. Correlation between TP activity and VEGF in group II

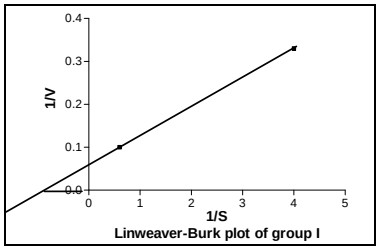

Fig.5. Linweaver-Burk plot of TP activity in group I

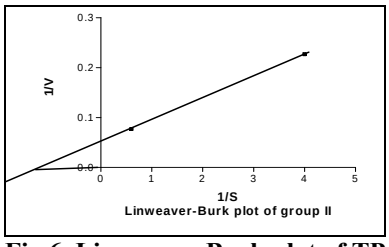

Fig.6. Linweaver-Burk plot of TP activity in group II

DISCUSSION

In the present investigation, the effect of leptin on the levels of the angiogenic factors; VEGF and TP in prepubertal female albino rats was studied. Obtained results revealed that leptin injection significantly increased VEGF levels and TP activity. Angiogenesis is defined as the formation of new blood vessels from preexisting ones. It may be pathological or physiological[15]. However, angiogenesis is generally a quiescent process in the healthy adult male, in the female it is highly regulated and turned on for brief periods of time in selected organs such as the ovary and the endometrium. Examples of such regulated angiogenesis include follicular growth, corpus luteum formation, endometrium differentiation and repair, and embryo implantation and development[16]. Leptin was found to generate a growth signal via a tyrosine kinase-dependent pathway and promote angiogenic processes via activation of the leptin receptor (Ob-R) in endothelial cells[17]. In-vivo and in-vitro assays revealed that leptin has an angiogenic activity and that vascular endothelium is a target for leptin[18]. In contrast to the leptin-deficient ob/ob mice, where no vascular fenestrations are detected, capillary fenestrations are found in leptin producing adipose tissue in lean mice. Thus, leptin plays a critical role in the maintenance and the regulation of vascular fenestrations in adipose tissue. Furthermore, leptin, fibroblast growth factor (FGF)-2 and VEGF synergistically stimulate angiogenesis[19]. Our results regarding

the increase of VEGF in rats injected by leptin were in accordance with those obtained by other investigators[20-23]. Janus kinase (JAK) and phosphatidylinositol -3- kinase (PI3K) signaling seem to be crucial to leptin-induced upregulation of the expression of VEGF and its receptor VEGFR-2, since treatment with specific inhibitors of these pathways blocks the upregulatory effects exerted by leptin on VEGF and VEGFR-2. The mitogen-activated protein kinase (MAPK) pathway also mediates leptin-enhanced VEGFR-2 expression and is partially involved in the induction of VEGF expression[9].

TP and PD-ECG are synonyms that refer to the same identical protein. TP induces endothelial cell migration in vitro and angiogenesis in vivo and in the chicken chorio-allantoic membrane (CAM) assay[12]. Several studies revealed an association between the expression of VEGF and thymidine phosphorylase[24-29]. The TP gene is localized on chromosome 22q13 and is composed of ten exons dispersed over a 4.3 kb region. The TP promoter lacks a ‘‘TATA’’ and a ‘‘CCAAT’’ box, sequences recognized by RNA polymerase II, prevalent in most eukaryotic genes. However, it contains a cluster of six to nine SP1-binding motifs, just upstream of the transcription start site. The transcription factor SP1 is activated by protein kinase A, which is in turn activated by cyclic adenosine monophosphate (cAMP). SP1 sites are also involved in the transcription of VEGF[30]. Interferon-γ (INF- γ), tumor necrosis factor-α (TNF- α) and interleukins induce the expression of TP. INF- γ and TNF- α induce the expression of TP by activation of JAK-STAT pathway, which also activated by leptin. In conclusion, the intraperitoneal injection of leptin in premature albino rats caused an increase in VEGF levels and TP activity, indicating an additional angiogenic effect of leptin and as angiogenesis is an important process in tumor development, so disrupting the leptin signaling pathway may help to treat tumors.

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اللبتن وعملية تكون الأوعية الدموية

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اللبتن عبارة عن بروتين يتكون من ١٦٧ حمض امينى. وهو يقوم بعدة وظائف ضمنها عملية تكون أوعية دموية جديدة. في هذه الدراسة، قمنا بدراسة تأثير اللبتن على مستوى عاملين من العوامل التي تعمل على تكون أوعية دموية جديدة وهما العامل الذي يعمل على نمو بطانة الأوعية الدموية وإنزيم الثيميدين فسفوريلاز في إناث الجرذان قبل مرحلة البلوغ. اشتملت الدراسة على عدد ٢٠ من اناث الجرذان، قسموا عشوائيا إلى مجموعتين، تم حقن المجموعة الأولى بمحلول ملحي عادى والمجموعة الثانية تم حقنهم بجرعة من اللبتن تقدر بثلاثة ميكروجرامات/ جرام من وزن الجسم وذلك من خلال الغشاء البريتونى لمدة عشرة أيام. وأظهرت النتائج أن اللبتن تسبب في زيادة ذات دلالة احصائية في مستوى العاملين كما لوحظ وجود علاقة ارتباط ايجابية بين العامل الذي يعمل على نمو بطانة الأوعية الدموية وإنزيم الثيميدين فسفوريلاز.