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Effects of metformin on serum levels of sex hormone, leptin and insulin in ovariectomized Sprague–Dawley rats

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The aim of the present experiment was to study the effects of metformin (MF) on serum concentrations of sex hormone,leptin and insulin in ovariectomized (OVX) Sprague-Dawley (SD) rats. The serum estradiol (E_2), progesterone (P), leptin and insulin concentrations were measured with radioimmunoassay (RIA). Compared with SHAM operated rats, the serum concentrations of E_2 and P were significantly lower ($P < 0.01$), but leptin and insulin were significantly higher in OVX rats ($P < 0.01$). MF (135 mg kg^{-1} d^{-1}, ig, 30 d) had no evident effects on serum concentrations of E_2, P, leptin and insulin in OVX rats. MF (270 mg kg^{-1} d^{-1}, ig, 30 d) markedly increased serum concentrations of E_2 and P ($P < 0.05$) while decreased serum concentrations of leptin and insulin ($P < 0.05$ and $P < 0.01$) in OVX rats. We concluded that long-term treatment of high dose MF cannot only significantly decrease serum concentrations of leptin and insulin, but also increase E_2 and P in OVX SD rats.

1. Introduction

As an oral antidiabetic biguanide, metformin (MF) has not only been used for the treatment of the patients with NIDDM (non-insulin-dependent diabetes mellitus), but is also used for women with PCOS (polycystic ovary syndrome). Previous studies have shown that MF can contribute to recovery of ovulation and pregnancy in some women with PCOS (Zhao et al. 2003). But so far, there are only a few reports about the effects of MF on serum levels of sex hormone, leptin and insulin due to low E_2 in patients with ovarian dysfunction and hypofunction. In this context, the objective of the present investigation was to analyze the effects of MF on serum levels of sex hormone, leptin and insulin in ovariectomited (OVX) SD rats. So this may support clinical use of MF in gynecology.

2. Investigations and results

2.1. The serum levels of E_2 and P in experimental animals

The data confirm that serum levels of E_2 and P in OVX model group were significantly lower than that in the SHAM group ($P < 0.01$). Compared with the OVX model group, the serum levels of E_2 and P were not significantly different in the low dose MF group ($P > 0.05$), while in the high dose MF group the levels were markedly higher ($P < 0.05$) (Table 1).

Table 1: The effects of MF on serum levles of E_2 and P in OVX rats ($x \pm s$) n = 10)

Group	Dose (mg/kg)	E_2 (ng/L)	P (μg/L)
SHAM	–	20.08 ± 6.91	9.68 ± 3.05
OVX	–	$8.12 \pm 1.97^{\Delta\Delta}$	$4.08 \pm 2.53^{\Delta\Delta}$
MF	135	11.46 ± 2.85	6.14 ± 2.97
MF	270	$18.34 \pm 5.30^{*}$	$8.17 \pm 2.61^{*}$

$^{\Delta\Delta}P < 0.01$, compared with SHAM group; $^{*}P < 0.05$, compared with OVX model group

2.2. Serum levels of leptin, insulin and weight of experimental animals

The data confirm that the levels of leptin, insulin and weight were significantly higher than that in SHAM group ($P < 0.01$). In the high dose MF group, the levels of leptin, insulin and weight were significantly lower than that in the OVX model group ($P < 0.05$ and 0.01) (Table 2).

3. Discussion

As an oral antidiabetic biguanide, MF has not only been used for the treatment of the patients with NIDDM, but also for women with PCOS (polycystic ovary syndrome). Previous studies have shown that MF can contribute to the recovery of ovulation and pregnancy in some women with PCOS (Weerakiet et al. 2008; Zhao et al. 2003). It was discovered that ovarian dysfunction and low levels of E_2 and P are causes leading to infertility. Leptin is a peptide hormone encoded by obese gene and mainly produced by and secreted from adipose tissue. Leptin has a intimate relationship to obesity, infertility and insulin. Recently, it was considered that there is an adipose-leptin-insulin axis in the human body. Also it was reported that there was a bidirectional hormonal feedback loop between leptin and insulin.

Table 2: The effects of MF on serum levels of leptin, insulin and weight in OVX rats ($x \pm s$) n = 10)

Group	Dose (mg/kg)	Leptin (μg/L)	Insulin (mIU/L)	Weight (g)
SHAM	–	3.09 ± 1.67	24.28 ± 3.69	236.43 ± 21.31
OVX	–	$5.33 \pm 1.67^{\Delta\Delta}$	$37.96 \pm 3.71^{\Delta\Delta}$	$280.14 \pm 20.85^{\Delta\Delta}$
MF	135	4.86 ± 1.04	35.51 ± 2.24	261.71 ± 12.21
MF	270	$3.75 \pm 1.24^{*}$	$29.62 \pm 1.96^{**}$	$245.85 \pm 13.41^{*}$

$^{\Delta\Delta}P < 0.01$, compared with SHAM group; $^{*}P < 0.05$ & $^{**}P < 0.01$, compared with OVX model group

Under normal circumstances, on one hand, as fat stores increase, serum leptin concentrations rise, while leptin inhibits the secretion of insulin via pancreatic β-cells hyperpolarization. On the other hand, insulin has adipogenic function so it can stimulate adipose cells to secret leptin. Atamer et al. (2008) indicated that there were significantly higher serum levels of leptin and insulin in women with ovarian dysfunction than in healthy women. Furthermore, some investigations discovered that high levels of leptin and insulin in the body can cause ovulatory dysfunction. In the present study, we observed that serum levels of leptin, insulin and weight were higher in the OVX model group than in the SHAM group. Maybe following ovariectomy, rapidly reduced serum E_2 concentrations led to the activitity of neuropeptide Y (NPY). Therefore rats took more food and their weight quickly increased. Serum levels of leptin are directly proportional to body weight (Chu et al. 2006; Lambrinoudaki et al. 2003), so leptin concentrations increased. Under the obese pathologic condition, leptin receptor sensitivity decreased, pancreatic β-cells depolarized, were insulin was secreted, the inhibitory effects of leptin on insulin were reduced, insulin resistance and hyperinsulinemia finally occurred. After administration of high dose MF for 30 days, the serum levels of leptin, insulin and weight were remarkably decreased in comparison to the OVX model group. We supposed that the reduction of leptin concentrations may be related to the loss of body weight, decreasing of fat stores and reduced secretion of leptin. In addition, the result also may be dependent on the obviously decreased insulin concentrations after the treatment of MF. Subsequently, the levels of E_2 and P increased (Oztekin et al. 2005). Thus we suggest that these might be a gonad-leptin-insulin axis in rats body. MF can reduce the levels of leptin and insulin of rats with ovarian dysfunction and in order to raise the gonadal hormone levels. These result support treatment of infertility caused by ovarian dysfunction with MF.

4. Experimental

4.1. Drugs and agents

Metformin was provided by Beijing Zhonghui Medicine Limited Corporation, Beijing, China (No: 20080310). P, E_2 and insulin RIA Kits were obtained from the Weifang 3 V Bioengineering Limited Corporation, Weifang, Shandong Province, China. Leptin RIA Kit was obtained from the Beijing Puerweiye Biotechnology Limited Corporation, Beijing, China.

4.2. Equipment

TypeFT-613G Computer multiprobe γ-counter was made by the Nuclear Instrument Factory of Beijing Nucleus, Beijing, China.

4.3. Animals

Forty healthy adult virgin female SD rats weighing 200 ± 20 g were supplied by the Animal Research Center of Shandong University, Shandong Province, China (No: 20030004 SCXK).

4.4. Methods

Select forty healthy adult virgin female SD rats. All of them were randomly divided into four groups (each group:10):SHAM operation group, OVX model group, low dose MF ($135\ mg\ kg^{-1}\ d^{-1}$) group, high dose MF ($270\ mg\ kg^{-1}\ d^{-1}$) group. Besides SHAM group, the rats were bilaterally ovariectomized under 10% chloral hydrate anaesthesia ($350\ mg\ kg^{-1}$). Rats in the SHAM group were subjected to the same bulk lipid tissue under anaesthesia, but ovaries were not excised. After surgery, the rats were allowed 3 days injected penicillin to recover from surgery stress and prevent infection. Normal saline (NS) was given to the SHAM and OVX model groups, MF was given to the other two groups one week after the operation ig daily for 30 days. Twelve hours after the last ig, rats were anesthetized with chloral hydrate, and then blood samples were colleted from the carotid artery into heparinized glass tubes and centrifuged at 2500 rpm for 15 mins at 4 °C. Plasma was frozen at −20 °C until assayed. The level of serum E_2, P, leptin and insulin were measured by RIA.

4.5. Statistics

SPSS package programe was used for statistical analysis. All data were presented as mean ± standard deviation ($\bar{x} \pm s$). We evaluted the Gaussian distribution of each variable. *One-way ANOVA* followed by *Students-Newman-Keuls* (*SNK*) *test* for multiple comparisons was performed to compare the differences between the control and the other three groups. $P < 0.05$ was considered statistically significant.

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