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Protective effects of a PPARγ agonist pioglitazone on anti-oxidative system in testis of diabetic rabbits

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In the present study, modulation of oxidative stress by pioglitazone, a peroxisome proliferator-activated receptor gamma (PPARγ) agonist, was examined in testis of alloxan-induced diabetic rabbits. In diabetic animals, an increase in the activity of anti-oxidative enzymes: superoxide dismutase (Cu,Zn-SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and glutathione reductase (GSSG-R), and in the level of glutathione (GSH) but a decrease in the level of ascorbic acid (AA) were observed. These effects were accompanied by a significant increase in testicular lipid and protein oxidation. Pioglitazone affected the activity of Cu,Zn-SOD, normalized the activity of CAT, the level of AA as well as the levels of LPO and PCG without having any significant effect on blood glucose level.

1. Introduction

Under normal conditions potentially toxic reactive oxygen species (ROS) are efficiently neutralized by cellular anti-oxidative mechanisms. However, this balance can easily be broken leading to different cellular dysfunctions (Palmeira et al. 2001). In the testis, altered steroidogenesis by itself can generate free radicals. Hence, even slight alterations in ROS levels and their detoxification can substantially affect the cellular processes (Muralidhara 2007). Pioglitazone belongs to a new class of anti-diabetic drugs. Some of its effects can be mediated through activation of the peroxisome proliferator-activated receptor gamma (PPARγ) which are present in different tissues (Collino et al. 2006). Some of PPARγ agonists have been shown to be potent inhibitors of glycation and potent antioxidants (Pathan et al. 2006). Therefore, the present study was undertaken to examine the protective effect of pioglitazone on anti-oxidative system in testis of alloxan-induced diabetic rabbits.

2. Investigations and results

In diabetic animals, Cu,Zn-SOD and CAT activities were increased by 65 and 51% and by 67 and 77% as compared to control animals. There were also increases in GSH-Px activity by 72 and 27%, GSSG-R activity by 33 and 28%, as well as in GSH level by 22 and 17%. At the same time, AA level was diminished by 36 and 27%. Pioglitazone affected Cu,Zn-SOD activity by 25 and 20% as compared to diabetic non-treated animals. In addition, it restored CAT activity and AA level to values of the control healthy animals (Table). In diabetic testis, LPO levels were increased by 56 and 59%, and PCG level by 57 and 61% as compared to controls. With pioglitazone treatment, the levels of LPO and PCG were diminished to control values (Table).

3. Discussion

In the present study, the increase of some anti-oxidative parameters in diabetic tissue is rather unexpected because typical changes are reported as significant decreases (El-Missiry 1999). However, such increases have also been reported in diabetic testis (Muralidhara 2007; Palmeira et al. 2001). In the present study, diabetic animals treated with pioglitazone showed the activity of some anti-oxidative enzymes at the level of non-diabetic groups. This could mean that administration of pioglitazone could reduce excessive production of ROS with a resulting decrease in the anti-oxidative defense. Similar modulation of SOD and CAT activity by pioglitazone was demonstrated in the heart and liver of the alloxan-induced diabetic rabbits (Gumieniczek 2003, 2005). In the present study, the increases in LPO and PCG levels are similar to those of another study concerning diabetic testis (Muralidhara 2007). However, pioglitazone treatment significantly diminished lipid and protein oxidation. Such effects of the drug were observed in the kidney of diabetic rabbits (Gumieniczek 2003). Similarly to the present results, a marked fall in the level of AA has been reported in testis of diabetic animals (El-Missiry 1999). The present study showed that administration of pioglitazone brought the levels of ascorbic acid to near normal values. After pioglitazone, a similar effect was observed in the kidney of our diabetic rabbits (Gumieniczek 2003). In our hyperglycemic model, pioglitazone did not affect blood glucose and insulin concentrations. Therefore, the observed anti-oxidative effects could not be attributed to the drugs anti-hyperglycemic action. The reason for this could be that our diabetic rabbits were not insulin-resistant but insulin-deficient (Table). Similar lack of anti-hyperglycemic action of pioglitazone was observed in streptozotocin-induced diabetic animals (Pathan et al. 2006). On the other hand, its significant action on

Table: Effects of pioglitazone on the determined parameters in blood and testis of control and diabetic rabbits (all abbreviations are explained in the text)

Parameter	Weeks	Group C	Group CP	Group D	Group DP
Glucose (mmol/l)	4	5.7 ± 0.3	5.9 ± 0.3^{b}	24.9 ± 2.8^{a}	23.9 ± 1.8^{a}
	8	5.8 ± 0.3	5.7 ± 0.3^{b}	23.9 ± 0.8^{a}	24.4 ± 1.4^{a}
Insulin (mU/l)	4	13.30 ± 1.12	14.12 ± 0.98^{b}	2.79 ± 0.76^{a}	2.01 ± 0.34^{a}
	8	12.73 ± 1.26	12.72 ± 1.05^{b}	1.58 ± 0.21^{a}	1.67 ± 0.29^{a}
Cu,Zn-SOD (U/mg protein)	4	4.10 ± 0.13	4.48 ± 0.20^{b}	6.75 ± 0.21^{a}	$5.08 \pm 0.14^{a,b}$
	8	3.84 ± 0.10	$4.33 \pm 0.10^{a,b}$	5.79 ± 0.20^{a}	$4.65 \pm 0.28^{a,b}$
CAT (μmol H_2O_2/min/mg protein)	4	21.49 ± 0.47	$13.85 \pm 0.57^{a,b}$	35.90 ± 0.97^{a}	20.91 ± 0.64^{b}
	8	19.30 ± 0.90	$13.85 \pm 0.33^{a,b}$	34.08 ± 1.69^{a}	21.35 ± 0.22^{b}
GSH-Px (mU/mg protein)	4	11.93 ± 0.68	13.24 ± 0.59^{b}	20.48 ± 0.84^{a}	13.07 ± 0.44^{b}
	8	14.05 ± 0.52	14.67 ± 0.72^{b}	17.81 ± 0.68^{a}	16.36 ± 0.98^{a}
GSSG-R (mU/mg protein)	4	36.79 ± 0.85	41.39 ± 1.79^{b}	49.03 ± 0.93^{a}	47.59 ± 1.18^{a}
	8	41.89 ± 1.74	40.61 ± 1.28^{b}	53.62 ± 1.43^{a}	$43.98 \pm 0.14^{a,b}$
GSH (nmol/mg protein)	4	56.16 ± 2.38	68.33 ± 2.56^{a}	68.64 ± 1.63^{a}	64.85 ± 2.22^{a}
	8	59.39 ± 1.75	69.02 ± 1.08^{a}	69.60 ± 1.87^{a}	$77.96 \pm 2.37^{a,b}$
AA (μmol/g tissue)	4	0.89 ± 0.03	$1.20 \pm 0.04^{a,b}$	0.57 ± 0.01^{a}	0.78 ± 0.04^{b}
	8	0.88 ± 0.02	$1.03 \pm 0.05^{a,b}$	0.64 ± 0.03^{a}	1.02 ± 0.05^{b}
LPO (nmol/g tissue)	4	39.82 ± 0.47	40.48 ± 0.62^{b}	61.95 ± 4.42^{a}	39.12 ± 2.47^{b}
	8	38.54 ± 1.62	37.09 ± 2.37^{b}	61.17 ± 2.73^{a}	37.45 ± 3.39^{b}
PCG (nmol/mg protein)	4	1.20 ± 0.06	1.26 ± 0.11^{b}	1.88 ± 0.08^{a}	1.39 ± 0.07^{b}
	8	1.22 ± 0.01	1.27 ± 0.05^{b}	1.97 ± 0.08^{a}	1.40 ± 0.06^{b}

Values are mean ± SEM (n = 5).
[a] $P < 0.05$ versus Group C (normal control animals).
[b] $P < 0.05$ versus Group D (diabetic control animals).

hyperglycemia was noted previously in alloxan-induced diabetes (Chaudhry et al. 2007). However, it appeared at significantly lower glucose concentration and higher dose of pioglitazone.

In conclusion, in the present study some anti-oxidative properties of pioglitazone are confirmed *in vivo*. Although the precise mechanism of action of the drug is not clear from the present results, it is not primarily attributable to its anti-hyperglycemic action.

4. Experimental

The study was performed according to the protocol described earlier (Gumieniczek 2003, 2005). Male New Zealand rabbits of the mean body weight 3 kg were divided into four groups of 10 animals: normal control (Group C), normal treated with pioglitazone (1 mg/kg) (Group CP), diabetic control (Group D), and diabetic treated with pioglitazone (1 mg/kg) (Group DP). Pioglitazone was administered orally for 4 and 8 weeks, once daily in the morning, starting two weeks after the alloxan injection. The tissue was homogenized in phosphate buffer at pH 7.5 and centrifuged at 20,000 × g at 4 °C. Glutathione peroxidase (GSH-Px) and glutathione reductase (GSSG-R) activities were measured by the method of Paglia and Valentine (1978) and Mize and Langdau (1962), respectively. The data were expressed as mean ± SEM (n = 5). Statistical analysis was done using Kruskall-Wallis and Mann-Whitney U non-parametric tests, and Statistica (version 5.0) software.

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