Biochemical Study of Anti-Inflammatory Effect of Osteoblastic Activator in Experimental Pancreatic Degeneration in Rat

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A B S T R A C T

A total number of 160 Wister female rats, 12 -wk. old were , weighed and divided into five experimental groups to investigate the effect of streptozotocin (STZ) injection (30 and 60 mg/kg B.W) with and without zoledronic acid (0.15 mg/kg B.W) on some blood serum constituents such as interleukins (IL-2, IL-6), immunoglobulin E (IgE) and cortisol. The results indicated that it's paralleled to the published data on the hyperglycemia and inflammatory condition in experimentally pancreatic degeneration. The current results showed that, injection with STZ was associated with a statistically significant elevation in serum IL-2, IL-6, IgE and cortisol but zoledronic treatment with STZ resulted in a decrease in serum IL-2, IL-6, IgE and cortisol. These results indicated that ZOL acid has beneficial effect to slow pancreatic carcinoma induction when treated with STZ at once in experimental animals.

Keywords: *STZ, Zoledronic acid, pancreas.*

(http://www.bvmj.bu.edu.eg) **(BVMJ-35(1): 164-170, 2018)**

1. INTRODUCTION

Pancreatic degeneration creates a highly immunosuppressive tumor microenvironment though the production of inhibitory cytokines and the recruitment of immunosuppressive cells (Lechner et al., 2010), including T regulatory cells.

Bisphosphonates (BP) are currently the most important class of inhibitors of osteoclast-mediated bone resorption and are used extensively for the treatment of skeletal diseases, such as Paget's disease, (Silverman, 2008) postmenopausal osteoporosis (Black et al., 2007) and tumor induced osteolysis. (Coleman, 2004). Some studies showed that zoledronic acid (ZOL) demonstrates antitumor activity in several human neoplasms such as myeloma, colon (Sewing et al., 2008) and pancreatic cancers (Tassone et al., 2003). Furthermore, ZOL has been reported to inhibit proliferation and induce apoptosis of tumor cells through the mevalonate pathway (Benford et al., 1999) by preventing the translocation of small GTPase Ras to the plasma membrane. Recently, Li et al. (2011) published a report showing the ability of ZOL to inhibit invasion and migration through down regulation of vascular endothelial growth factor (VEGF) and matrix metalloproteinase (MMP) in human nasopharyngeal carcinoma cells in vitro. Therefore, the objective of this study aimed to investigate the potential effect of

zoledronic acid on some inflammatory parameters of degenerated pancreas by using STZ.

2. Materials and methods

This study was carried out at biochemistry Dept., faculty of veterinary medicine, Benha University. One hundred and Sixty Wister female rats, 12 -wks-old were divided into five experimental groups. Rats were housed in separate metal cages, then the five experimental groups were arranged as, the first as a control group (un-treated), the second group injected with 30 mg STZ/ kg BW, the third group injected with 60 mg STZ/kg, the fourth group injected with 30 mg STZ plus 0.15 mg ZOL/kg and the fifth group injected with 60 mg STZ plus 0.15 mg ZOL/ kg BW to studying their effects on some sera contents such as interleukins (IL2&IL6), immunoglobulin E (IgE) and cortisol at 14 and 28 day after treatment.

Experimental partial degenerated pancreas was induced by a single intraperitoneal injection of streptozotocin with 30 mg/kg and completely degenerated pancreas was induced by a single intraperitoneal injection of streptozotocin by 60 mg/kg. Streptozotocin (Sigma Chemicals Co, St. Louis, MO, USA). It was dissolved in cold 0.01 M citrate buffer, pH 4.5 and always freshly prepared for immediate use within 5 min. STZ injections were given intraperitoneally and the doses were determined according to the body weight of animals (*Hamilton et al., 1998).* Zoledronic acid was purchased it from Global Napi pharmaceuticals Co. (Metadronic vial) and injected by 0.15 mg / kg BW through the tail vein.

Data collection and estimated parameters:

At 14 and 28 day after treatment start, blood samples were collected in centrifuge tubes from four rats per each treatment without anticoagulant and kept at room temperature for one hour to clot. The samples were centrifuged at 3500 rpm for 15 minutes to separate clear serum. After that, serum IL-2 and IL-6 (Robb , 1984), IgE (Bergstrand and Scand , 1956) and serum cortisol were determined using available commercial Kits.

Statistical analysis:

Data obtained were statistically analyzed using the general linear model of SAS (2004), as follows: $Yik = \mu + Ti + + eik$ where : Yik = an observation; μ = Overall mean; T = Effect of treatment; i = (1, 2,.. and 5) ; and eik = Random error. Significant differences among treatments means were tested by Duncan's multiple range test (Duncan, 1955).

3. RESULTS

A significant differences ($P\leq 0.01$) were observed in serum interleukin 2 (IL-2) content among experimental groups at 14 and 28 day after treatment (Table 1). Serum IL-2 was significantly ($P\leq 0.01$) elevated for rats injected by STZ with or without ZOL than the control. However, ZOL injection plus STZ at the same time resulted in a significant decrease in serum IL-2 content than those injected with STZ only at 14 or 28 day after treatment. The group treated with 30 mg STZ with or without ZOL recorded the lower value of IL-2 than those treated with 60 mg STZ at 14 or 28 day after treatment.

Results of Table 2 shows a significant differences ($P\leq 0.01$) in serum interleukin 6 (IL-6) content among experimental groups at 14 and 28 day after treatment. Serum IL-6 was significantly ($P\leq 0.01$) elevated for rats injected by STZ than the control. However, ZOL injection plus STZ at the same time resulted in a significant decrease in serum IL-6 content than those injected with STZ only at 14 or 28 day after treatment. The group treated with 30 mg STZ with or without ZOL recorded the lower value of IL-6 than those

treated with 60 mg STZ at 14 or 28 day after treatment. Generally, serum IL-6 content was elevated for all treated groups at 28 day after treatment as compared with their levels at 14 day for the same treated groups especially the group treated with both STZ dosses. The injection with Zol plus STZ resulted in more decrease in IL-6 at 28 day than 14 day after treatment.

Serum IgE and was significantly (P≤0.01) elevated for all treated groups by STZ with or without ZOL than the control group (un-treated) at 14 and 28 day after treatment (Table 3). Serum IgE was significantly higher of rats injected by 60 mg STZ/ kg as compared with those treated with 30 mg STZ, while it was significantly (P≤0.01) lowered for treated groups with ZOL plus STZ than those treated with STZ only. Rats treated with ZOL plus 30 mg STZ had significantly lower value of serum IgE as compared to other treated groups.

Serum cortisol was significantly (P≤0.01) elevated for all treated groups by STZ with or without ZOL than the control group (un-treated) at 14 and 28 day after treatment (Table 4). Serum cortisol content was higher for rats injected with 60 mg STZ/ kg than those treated with 30 mg STZ. Injection with ZOL plus STZ resulted in a decrease in serum cortisol than those treated with the same STZ dose only at 14 or 28 day after treatment. Generally, serum cortisol was elevated for all treated groups at 28 day than 14 day after treatment.

Table 1: Effect of streptozotocin (STZ) injection with or without zoledronic (ZOL) on serum interleukin 2 (IL-2) content at 14 and 28 day after treatment.

Experimental groups	Period after treatment, day	
	14	28
	$\bar{X}$±SE	$\bar{X}$±SE
T1 (Control, -ve)	0.65 ± 0.10 ^{d}	0.65 ± 0.10 ^{c}
T2 (30 mg STZ)	3.16 ± 0.15 ^{b}	4.42 ± 0.13 ^{a}
T3 (60 mg STZ)	4.14 ± 0.10 ^{a}	5.1 ± 0.32 ^{a}
T4 (30 mg STZ + ZOL)	1.37 ± 0.26 ^{c}	2.35± 0.23 ^{b}
T 5 (60 mg STZ + ZOL)	1.68 ± 0.20 ^{c}	2.87 ± 0.30 ^{b}
Significant	**	**

a,b,c… d :means in the same column within each item bearing different superscripts are significantly different (P ≤ 0.05), SE = stander error; ** = significant at P≤ 0.01

Table 2: Effect of streptozotocin (STZ) injection with or without zoledronic (ZOL) on serum interleukin 6 (IL-6) content at 14 and 28 day after treatment.

Experimental groups	After the beginning of treatment, day	
	14	28
	$\bar{X}$±SE	$\bar{X}$±SE
T1 (Control, V^{-})	6.86 ± 0.19 ^{c}	6.86 ± 0.19 ^{c}
T4 (30 mg STZ)	9.30 ± 0.30 ^{b}	22.20 ± 1.75 ^{a}
T 5 (60 mg STZ)	12.45 ± 0.50 ^{a}	25.26 ± 1.80 ^{a}
T4 (30 mg STZ + ZOL)	7.01 ± 0.20 ^{c}	10.92± 0.70 ^{bc}
T 5 (60 mg STZ + ZOL)	8.71 ± 0.27 ^{b}	15.63 ± 2.49 ^{b}

a,b,c… d :means in the same column within each item bearing different superscripts are significantly different ($P \leq 0.05$), SE = stander error

Table 3: Effect of streptozotocin (STZ) injection with or without zoledronic (ZOL) on serum IgE content at 14 and 28 day after treatment.

Experimental groups	After the beginning of treatment, day	
	14	28
	$\bar{X}$±SE	$\bar{X}$±SE
T1 (Control, V⁻)	23.71 ± 2.32 [d]	23.71 ± 2.32 [d]
T2 (30 mg STZ)	92.30 ± 7.06 [b]	108.35 ± 9.07[b]
T3 (60 mg STZ)	139.75 ± 5.62 [a]	167.85 ± 8.88 [a]
T4 (30 mg STZ + ZOL)	56.63 ± 3.38 [c]	71.36 ± 5.11[c]
T 5 (60 mg STZ + ZOL)	87.89 ± 6.26 [b]	95.56 ± 7.39 [b]

a,b,c… d :means in the same column within each item bearing different superscripts are significantly different ($P \leq 0.05$), SE = stander error

Table 4: Effect of streptozotocin (STZ) injection with or without zoledronic (ZOL) on serum cortisol content at 14 and 28 day after treatment.

Experimental groups	After the beginning of treatment, day	
	14	28
	$\bar{X}$±SE	$\bar{X}$±SE
T1 (Control, V⁻)	6.98 ± 0.94 [c]	6.98 ± 0.94 [c]
T2 (30 mg STZ)	17.86 ± 1.22[ab]	34.75 ± 5.93 [b]
T3 (60 mg STZ)	23.03 ± 1.92 [a]	51.03 ± 3.36 [a]
T4 (30 mg STZ + ZOL)	14.22 ± 2.30 [b]	30.62 ± 3.29 [b]
T 5 (60 mg STZ + ZOL)	22.67 ± 2.77 [a]	39.50 ± 7.14 [ab]

a,b,c… d :means in the same column within each item bearing different superscripts are significantly different ($P \leq 0.05$), SE = stander error

4. DISCUSSION

During the first two or four weeks after STZ injection, glycemia rose in all treated rat groups with 30 or 60 mg STZ /kg BW, suggesting similar acute STZ toxic effects on the endocrine pancreas. These results may be due to pancreatic β-cells were degenerated or necrosis by STZ treatment, leading a decrease in insulin secretion and an increase in blood glucose concentration. Generally, the hyperglycemia of rats which induced by treatment with 30 or 60 mg STZ/kg resulted in a significant increase in some serum interleukins (IL-2 & IL-6) at 14 and 28 days after treatment than un-treated group. These results are in agreement with those obtained by Tang et al. (2011) who found that plasma levels of IL-6 increased in rats with STZ-induced diabetes. Monocyte interleukin (IL6) levels are significantly elevated in type 1 diabetic subjects (Devaraj et al., 2006). Zolderonic treatment plus STZ at once caused a significant decrease in serum interleukins (IL-2& IL-6) than un-treated group at 14 and 28 day after treatment may be due to zoledronic acid has a direct effect on the primary tumor and plays a major role as anti-tumor activity (Tassone et al., 2003 ; Marten et al., 2007). Also, ZOL injection at the initial treatment of pancreatic degeneration by STZ may resulted in a delay of induce pancreatic carcinoma. Also, ZOL injection may resulted

in a decrease of STZ capacity to induce pancreatic tumor for β-cells because ZOL by inhibit a key enzyme, farnesyl diphosphonate (FPP) synthase, in the biosynthetic mevalonate pathway (Zekri et al., 2014). These data suggest that zoledronic acid inhibits the expression and secretion of IL-6 normally produced in high levels in PC-3 cell lines. Also, zoledronic acid play anti-tumor agent which could be effected on IL-6 signaling pathways in pancreatic cells. In the literature the first data on cytotoxic effect of zoledronic acid was produced by Aparicio et al. (1998) in myeloma cell lines. Then several studies (Tassone et al., 2000; Mundy et al, 2001; Lee et al, 2002) have suggested direct anti-proliferative and pro-apoptotic effects of the bisphosphonates on myeloma cancer cells.

The hyperglycemia of rats which induced by injection STZ with 30 or 60 mg STZ/kg BW resulted in a significant increase in serum IgE at 14 day after treatment than un-treated group. This means that, serum IgE was positively associated with higher glucose and lower insulin in experimental diabetic rats. Our findings in line with previous studies, a nested case-control design and logistic regression analysis of 135 patients with CHD and 135 control subjects, serum IgE levels were higher in CHD patients than in control subjects (Erdogan et al., 2003). Also, Wang et al (2011) provided the first evidence that increased plasma levels of mast cell proteases and IgE may serve as important risk factors for type 2 diabetic patients, particularly when hs-CRP or other common diabetes mellitus risk factors are considered.

A significant increase in serum cortisol of rats which injected with 30 or 60 mg STZ/kg BW at 14 days after treatment than un-treated group. Generally, the interaction between several genetic and environmental factors results in a heterogeneous and progressive disorder with variable degrees of insulin resistance and pancreatic β-cell dysfunction (Stumvoll et al., 2005). Our results show that within the range of physiological normal, increasing cortisol is strongly associated with increasing diabetic pathophysiology, particularly in the variables that depend on hyperglycemia. It seems probable that the stimulatory effect of cortisol on these variables is a consequence of the direct action of cortisol on hepatic gluconeogenesis (Khani and Tayek, 2001). While, ZOL injection plus STZ at once had beneficial effects on serum cortisol, which resulted in a decrease of serum cortisol as compared with those treated with STZ only. These data suggest that zoledronic acid inhibit the expression and secretion of corticoids hormones, or ZOL may be appear to improve pancreatic ß-cell function directly, that it decrease the risk of impaired glucose metabolism. So that, ZOL injection improve insulin production in pancreatic ß-cell , which decrease serum glucose, then low serum cortisol levels which are a mixture of direct and indirect effects of ZOL plus STZ .

5. Conclusion

Based on the present data, treatment by zoledronic acid (0.15 mg/kg) combined with STZ (30 or 60 mg/kg) might be attributed to an inhibition of invasion and proliferation of cancer cells of pancrease. So, the therapy with zoledronic may show a great promise for the treatment of pancreatic cancer at the further.

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