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In Vitro Insulin Mimicking Action of Bis(maltolato)oxovanadium (IV)

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Bis(maltolato)oxovanadium (IV), an organic vanadyl complex was found to improve deranged glucose and lipid metabolism in experimental models of type 1 and type 2 diabetes. The mechanism of its antidiabetic action appeared to be its insulin sensitizing action *in vivo*. In the present investigation, the effect of bis(maltolato)oxovanadium (IV) at the two important target organs of insulin was studied *in vitro* using 3T3-L1 adipocytic cell line and C2C12 myoblasts, a skeletal muscle cell line. Bis(maltolato)oxovanadium (IV) both in the absence and presence of insulin was found to significantly increase triglyceride synthesis in 3T3-L1 adipocytes and transport of radiolabeled glucose in C2C12 myoblasts. The effect of bis(maltolato)oxovanadium (IV) in both these cell lines in the absence and presence of insulin was not significantly different from each other, indicating that bis(maltolato)oxovanadium (IV) acts as an insulin mimick rather than as an insulin sensitizer *in vitro*. The mechanism of the *in vivo* antidiabetic action of bis(maltolato)oxovanadium (IV) thus could be attributed to its insulin mimicking action at the insulin target organs like adipocytes and skeletal muscles.

Vanadium, a group Vb transition element ubiquitous in nature has been shown to be essential for normal cell growth and development in some mammalian species including rats and chicks[1,2] and in mammalian cell cultures[3]. However, the significance of this element in man is still unknown[4,5]. Vanadium has been reported to produce insulin like effects in several *in vivo*[6-9] and *in vitro*[10-12] models of diabetes mellitus. *In vivo* due to poor absorption from gastrointestinal tract, the dose of inorganic vanadium required to produce insulin like effect has been associated with dehydration and diarrhoea resulting in death of some animals[13,14]. In an attempt to increase potency and decrease toxicity bis(maltolato)oxovanadium (IV) (BMOV), an organic complex of vanadyl sulfate with maltol, was synthesized[15]. BMOV was found to be two to three times more potent glucose lowering agent than inorganic vanadium[16]. Earlier we have reported insulin-sensitizing action of BMOV on glucose and lipid homeostasis in neonatal streptozotocin (STZ)-induced type 2 diabetic rats[17]. The present investigation was undertaken to study the cellular mechanism of action of BMOV at two important insulin target organs namely adipocytes and skeletal muscles using *in vitro* cell culture techniques.

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MATERIALS AND METHODS

Measurement of adipocyte differentiation:

The 3T3-L1 fibroblasts (American Type Culture Collection, USA) were maintained in RPMI 1640 containing 5 % fetal calf serum (FCS), 100 U/ml penicillin and 100 µg/ml streptomycin, 0.5 µg/ml fungizone all procured from Gibco BRL, USA in a 75 cm^2 flask (Costar, USA) at 37° in a humidified 95 % air, 5 % CO_2 atmosphere[18]. Preadipocytes ($1x10^5$ cells/well) were cultured to confluency in 6 well plates (Costar, USA) for 2 d, then adipocyte differentiation was initiated by the method of Shibata *et al*[18]. Briefly, differentiation

was initiated by treating confluent preadipocytes with 1 μM/ml dexamethasone and 0.5 mM isobutylmethylxanthine (IBMX, Sigma Chemical Co. St. Louis, MO). After 2 d, the cells were given fresh medium containing BMOV (0-10 mM) in the presence and absence of 1 μg/ml insulin (Sigma Chemical Co. St. Louis, MO) and allowed to differentiate for additional 4 d. At the end of the experiment, cells in the plate were harvested using rubber policeman. BMOV was obtained from University of British Columbia, Vancouver, B.C., Canada as the gift sample. Scraped cells were transferred to 1.5 ml microcentrifuge tubes. The cell suspension was disrupted by sonication at maximum output for 15 s with a microtip. Intracellular triglycerides were determined using Ponte Scientific colorimetric estimation kit, USA. Cell layer protein content was estimated according to the method of Lowry *et al*[19]. Triglycerides were expressed as μg/mg protein.

Glucose transport in C2C12 myoblasts:

C2C12 murine myoblasts (American Type Culture Collection, USA) were cultured in 12 well plates in the Dulbecco's modified Eagle's medium (DMEM, Gibco BRL, USA) supplemented initially with 10 % fetal bovine serum (FBS, Gibco BRL, USA) containing 1 μg/ml gentamycin (Gibco BRL, USA) at 37° in a humidified 95 % air, 5 % CO_2 atmosphere. The medium was changed every 72 h. A day before the experiment, when the cells were confluent, they were incubated in DMEM without FBS. The glucose uptake by these cells was studied by the method of Hajduch *et al*[20] for BMOV (10 mM) in the presence and absence of insulin (6 pM) using 2-(1,2-H) deoxy-D-glucose (2 μCi/ml) for 30 min. The 2-(1,2-H) deoxy-D-glucose was obtained from NEN Life Science Products, Inc, MA, USA. At the end of 30 min incubation, the cells were washed thrice with ice cold saline and further incubated for 45 min. The cells were solubilized in 200 μl of 0.5 N NaOH for 40 min. The solubilized extracts were then processed for liquid scintillation counting using Tri-carb, Packard, USA.

Statistical analysis:

Results are presented as mean±SEM. Statistical differences between the means of the various groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's test. Data were considered statistically significant at P value<0.05 .

RESULTS

3T3-L1 cell differentiation:

After 4 d incubation of 3T3-L1 preadipocytes with BMOV (0-10 mM), cellular triglyceride accumulation normalized by protein levels was measured as an index of the differentiated adipocyte phenotype. Incubation of cells with BMOV alone significantly increased the intracellular triglyceride accumulation above that observed in the control cultures (no drug well) at 10 mM, however did not produce any significant effect at lower concentrations (fig.1). Simultaneous incubation of cells with BMOV and insulin significantly increased triglyceride synthesis at only 10 mM, however, the effect was not significantly different from that with BMOV alone (fig. 1).

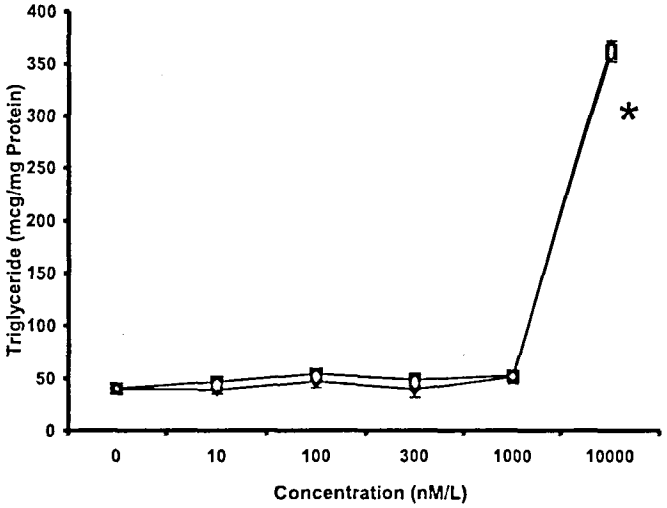

Fig. 1: Effect of BMOV and its interaction with insulin on triglyceride synthesis in 3T3-L1 adipocytes.

Data are mean±SEM of n=3.*Significantly different from no drug well ($p<0.05$). BMOV alone (-♦-) and BMOV +insulin (-■-).

Glucose transport in C2C12 myoblasts:

From the preliminary results and the earlier reports by Hajduch *et al.*[20], it was found that the maximum uptake of glucose by insulin occurs at a concentration of 6 pM at 30 min (fig. 2). BMOV alone as well as in the presence of insulin significantly increased uptake of radiolabeled glucose into C2C12 myoblasts compared to basal glucose uptake, however the difference between the effect of the BMOV in the absence and presence of insulin was not significant (fig. 2).

DISCUSSION

Insulin resistance could result from impaired glucose uptake by peripheral tissues (fat and muscle)[21]. Skeletal muscle is the chief site of insulin mediated glucose disposal. Thus the peripheral insulin resistance reflects mainly re-

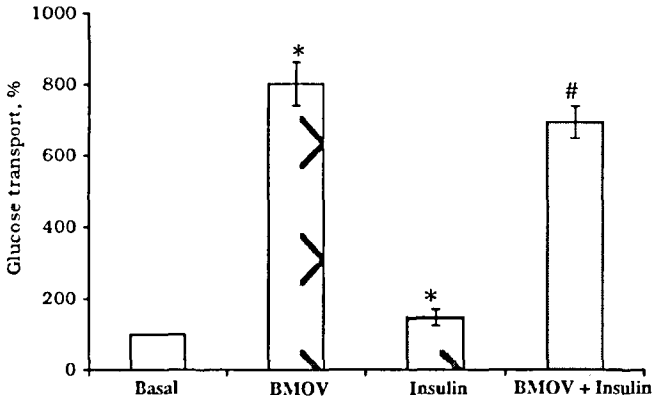

Fig. 2: Effect of BMOV on radiolabeled glucose transport in C2C12 myoblasts.

Data are mean±SEM of n=3. *significantly different from basal ($p<0.05$). #significantly different from insulin ($p<0.05$).

duced uptake by muscle after exposure to exogenous or endogenous insulin[22]. In addition to glucose metabolism and disposal, conversion of glucose into fat is another important metabolic pathway for the disposal of excess glucose. There is derangement of both glucose and lipid metabolism in insulin resistance and diabetes mellitus[21,23]. In earlier *in vivo* studies performed in our laboratory, BMOV was found to improve insulin sensitivity and normalize deranged glucose and lipid homeostasis of STZ-induced type 1 as well as neonatal STZ-induced type 2 diabetic rats. In the present investigation, the cellular effects of BMOV on adipocytes and skeletal muscles, two important insulin target organs were investigated.

3T3-L1 pre-adipocyte cell line is a well established cell line that responds to physiological doses of insulin with increase in glucose uptake, glucose oxidation, glycogen synthesis and lipogenesis under *in vitro* condition[24-26]. In the present investigation, it was observed that BMOV, both alone and in combination with insulin significantly increased intracellular triglyceride synthesis which was considered as an index of adipocyte differentiation. Studies carried out by various workers have reported activation of adipogenesis[27] in addition to enhancement of glucose transport and oxidation[28,29] and inhibition of lipolysis[10] in rat adipocytes. Triglyceride synthesis in response to BMOV treatment alone was not significantly different from that of co-incubation with insulin indicating that BMOV did not show synergism of insulin action. This suggests that BMOV acts as an insulinomimic agent rather than insulin enhancer. The insulinomimetic effects attributed to vanadium *in vitro* have been demonstrated to occur at higher concentrations (10^{-4}–10^{-3} M) than used for *in vivo* demonstration[30,31]. These observations are in accordance with the *in vivo* plasma lipid lowering effect of oral BMOV treatment. It appears that BMOV stimulates adipogenesis and inhibits lipolysis in fat cells mainly by activating lipoprotein lipase of adipose tissue capillary wall and inhibiting hormone sensitive lipases which inturn decreases circulating lipids as observed *in vivo*.

C2C12 mouse myoblasts is a frequently employed skeletal muscle cell line for studying the glucose transport[32,33]. Insulin produced a significant stimulation of glucose uptake by C2C12 myoblast cells. BMOV when incubated alone significantly increased the uptake of glucose by C2C12 myoblasts. However, when co-incubated with insulin did not potentiate the insulin action indicating that at 10 mM concentration vanadium acts as insulin mimic rather than insulin enhancer *in vitro*. These findings are in accordance with the observations of several earlier workers indicating enhancement of glucose transport and oxidation in rat skeletal muscle[11,34], thereby correcting the defect in insulin-stimulated muscle glycogen synthesis in pancretectomized rats[35]. BMOV may increase glucose transport into skeletal muscle and also fat cells by stimulating expression of GLUT 4 transporters and stimulating the enzymes involved in glycogen synthesis and glucose utilization.

In conclusion our data suggests that the insulin sensitizing action of vanadium complexes observed *in vivo* could be attributed to its insulin like effects at the peripheral target organs mainly adipose tissue and skeletal muscle.

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