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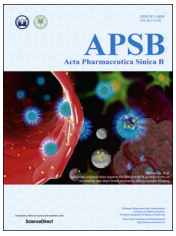

ORIGINAL ARTICLE

1,25-Dihydroxyvitamin D3 protects obese rats from metabolic syndrome *via* promoting regulatory T cell-mediated resolution of inflammation

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Abstract Vitamin D_3 has been found to produce therapeutic effects on obesity-associated insulin resistance and dyslipidemia through its potent anti-inflammatory activity, but the precise immunomodulatory mechanism remains poorly understood. In the present study we found that 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$], the biologically active form of vitamin D_3, significantly attenuated monosodium glutamate (MSG)-induced obesity and insulin resistance as indicated by body weight reduction, oral glucose tolerance improvement, and a glucose infusion rate increase as detected with hyperinsulinemic-euglycemic clamp. Moreover, 1,25$(OH)_2D_3$ not only restored pancreatic islet functions but also improved lipid metabolism in insulin-targeted tissues. The protective effects of 1,25$(OH)_2D_3$ on glycolipid metabolism were attributed to its ability to inhibit an obesity-activated inflammatory response in insulin secretory and targeted tissues, as indicated by reduced infiltration of macrophages in pancreas islets and adipose tissue while enhancing the expression of *Tgf-β1* in liver tissue, which was accompanied by

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increased infiltration of Treg cells in immune organs such as spleen and lymph node as well as in insulin-targeted tissues such as liver, adipose, and muscle. Together, our findings suggest that $1,25(OH)_2D_3$ serves as a beneficial immunomodulator for the prevention and treatment of obesity or metabolic syndrome through its anti-inflammatory effects.

1. Introduction

Obesity is associated with multiple adverse health outcomes collectively summarized as the "metabolic syndrome", consisting of insulin resistance, dyslipidemia, and cardiovascular diseases[1]. A linking role of inflammation between obesity and metabolic syndrome has been established which mostly refers to the release of various adipocyte products, such as cytokines, fatty acids, and oxygen free radicals from the white adipose tissue in obese individuals[2]. Many of these products function as molecules of damage-associated molecular patterns (DAMPs) to initiate chronic low-degree inflammation to promote the infiltration of adipose tissue with activated macrophages through the activation of pattern reorganization receptors (PRRs) such as Toll-like receptors (TLRs) expressed on immune cells and residual cells[3,4]. Indeed, accumulated evidence has indicated that both obesity and metabolic syndrome are inflammatory disorders and inflammatory responses are causally involved in insulin resistance by ultimately suppressing the insulin signaling pathway[5].

Epidemiological studies indicate that insufficient vitamin D status is a potential contributor to insulin resistance and obesity[6–8]. Data from a pilot study examining vitamin D deficiency in type 1 and type 2 diabetes suggests that vitamin D insufficiency is more common in type 2 diabetes than in type 1 diabetes, unrelated to age, sex, or insulin treatment[9]. However, studies on the administration of vitamin D supplements to vitamin D-sufficient patients with IGT or type 2 diabetes have yielded conflicting results. Some have reported an improvement, others no effect[10]. One study even showed a worsening of type 2 diabetes: supplementation in three British Asians with vitamin D deficiency and type 2 diabetes led to increased insulin resistance and the deterioration of glycemic control[11]. Interestingly, Wortsman et al.[12] reported that obesity-associated vitamin D deficiency is largely because of the decreased bioavailability of vitamin D_3 from cutaneous and dietary sources due to its deposition in body fat compartments. Furthermore, chronic feeding of mice with $1,25(OH)_2D_3$, the biologically active form, suppressed the inflammatory responses in a variety of animal models including experimental asthma, encephalomyelitis, and rheumatoid arthritis[13–15]. The potential immune modulating effects of $1,25(OH)_2D_3$ on the immune system have been initially derived from *in vitro* observations following the treatment of dendritic cells (DC) with $1,25(OH)_2D_3$ which could inhibit the maturation of myeloid DC *via* reducing secretion of IL-12 as well as the expression of co-stimulatory molecules. $1,25(OH)_2D_3$ also enhances the secretion of CCL22 by DC *in vitro*, which is a chemokine that attracts T cells into the skin[16]. IL-10-producing regulatory T cells can be induced *in vitro* by $1,25(OH)_2D_3$ in the presence of dexamethasone[17]. In addition, $1,25(OH)_2D_3$ can hamper the secretion of IFN-α from T cells and induce Th2 cell development with increased production of IL-4, IL-5, and IL-10[18]. Nonetheless, the immunoregulatory mechanism of $1,25(OH)_2D_3$ on obesity-associated insulin resistance and dyslipidemia is not fully understood.

In this study we have investigated the effects and mechanism of $1,25(OH)_2D_3$ in the regulation of insulin sensitivity and glucolipid metabolism in MSG-obese rats. We found that a short course of treatment with $1,25(OH)_2D_3$ improved insulin resistance and glucolipid metabolism disorder of MSG-obese rats by inhibiting the inflammatory responses in primary insulin-targeted tissues including adipose, liver, and muscle, which is largely due to increasing the infiltration of $CD4^+CD25^+FoxP3^+$ regulatory T-cells in these tissues. Our studies indicate that administration of $1,25(OH)_2D_3$ protects MSG-obese rats from the development of obesity and its related metabolic risks.

2. Materials and methods

2.1. Animal model

Wistar rats (newborn) were obtained from Vital River Laboratory Animal Technology (Beijing, China). All animal protocols conformed to the Guidelines for the Care and Use of Laboratory Animals prepared and approved by the Animal Care and Use Committee of the Chinese Academy of Medical Sciences and Peking Union Medical College. Newborn Wistar rats were s.c. injected with monosodium L-glutamate (MSG) at 4 g/kg/day for seven successive days as described previously[19]. In contrast to the normal rats, the MSG rats developed obesity with increased plasma TG, cholesterol, and free fatty acid contents as well as impaired insulin sensitivity in their adulthood[20]. Experimental protocols are outlined in Fig. 1. In protocol A (Fig. 1A), the four-week-aged MSG rats were given s.c. injections of 1 μg/kg $1,25(OH)_2D_3$ twice a week for 16 weeks, and insulin tolerance test (ITT) and the euglycemic hyperinsulinemic clamp were assayed at the end of the experiment to evaluate the incidence of insulin resistance. In protocol B (Fig. 1B), obese MSG rats were sorted into three groups according to body weights and fasting plasma glucose values, and then were treated with 1 μg/kg $1,25(OH)_2D_3$ (twice a week), 4 mg/kg/day rosiglitazone or vehicle for 8 weeks.

2.2. Oral glucose tolerance test

The OGTT and ITT were carried out as previously described[21]. In brief, after fasting for 6 h on the last treatment day, the animals were administrated an oral dose of 2 g/kg glucose. Blood samples were collected at 0, 30, 60, and 120 min after glucose loading. Blood glucose was analyzed by the glucose-oxidase method, and the area under the curve (AUC) was generated from the data of OGTT.