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# Attenuation of macrophage accumulation and polarisation in obese diabetic mice by a small molecule significantly improved insulin sensitivity

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ABSTRACT

Accumulation and polarization of anti-inflammatory M2 to proinflammatory M1 macrophage in the adipose tissue of obese diabetic mice is an important pathogenic signature. It worsens lipid induced inflammation and insulin resistance. Here we demonstrate that a small molecule, a peroxyvanadate compound i.e. DmpzH [$VO(O_2)_2$ (dmpz)] or dmp, could robustly decrease macrophage infiltration, accumulation and their polarization in high fat diet (HFD) induced obese diabetic mice. In searching the underlying mechanism it was revealed that SIRT1 level was strikingly low in the inflamed adipose tissue of HFD mice as compared to mice fed with standard diet (SD). Administration of dmp markedly increased SIRT1 level by inducing its gene expression with a consequent decrease in macrophage population. Elevation of SIRT1 coincided with the decrease of MCP1, Fetuin-A (FetA) and IFNγ. Since MCP1 and FetA drive macrophage to inflamed adipose tissue and IFNγ promotes M2 to M1 transformation, both recruitment and M1 induced inflammation were found to be significantly repressed by dmp. In addressing the question about how dmp induced excess SIRT1 could reduce MCP1, FetA and IFNγ levels, we found that it was due to the inactivation of NFκB because of its deacetylation by SIRT1. Since NFκB is the transcriptional regulator of these molecules, their expressions were significantly suppressed and that caused sharp decline in macrophage recruitment and their polarity to M1. This effected a marked fall in proinflammatory cytokine level which significantly improved insulin sensitivity. dmp is likely to be the first molecule that rescues inflammatory burden contributed by macrophage in obese diabetic mice adipose tissue which causes significant increase in insulin sensitivity therefore it may be a meaningful choice to treat type 2 diabetes.



## 1. Introduction

A few crucial events in obesity induced insulin resistance and type 2 diabetes create considerable impediment for therapeutic intervention. This is primarily due to remodelling of adipose tissue (AT) because of oversupply of lipid. This remodelling includes formation of hypertrophied inflamed adipocytes, extrusion of fatty acid causing ectopic lipid deposition, lipotoxicity and infiltration of macrophages into the adipose tissue. All these effect a low grade chronic inflammation in obese diabetic mice [1,2]. Adipose tissue macrophage (ATM) is closely linked to this inflammatory condition which leads to insulin resistance [3,4]. Increase in adipose tissue inflammation is associated with the alteration of ATM phenotypic

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switch i.e. from anti-inflammatory or alternatively activated or M2 macrophage to proinflammatory or classically activated or M1 macrophage [5], which greatly contributes to aggravate inflammation and insulin resistance [4]. Inflamed adipocyte generate plethora of negative signals among which two chemoattractants i.e. MCP1 and FetuinA (FetA) are primarily responsible for driving macrophage to adipose tissue [6,7]. Because of the participation of both inflamed adipocyte and M1 macrophage, an intricate pathogenic condition is created with excess of proinflammatory cytokines i.e. TNFα, IL-6 and IL-1β secretions [8] and that intensify insulin resistance.

On the background of this scenario, Sirtuin 1 (SIRT1), the mammalian homologue of silent information regulator 2 (SIR2) gene in yeast, which encodes a NAD dependent deacetylase [9] showed a promise as its increased expression reduces macrophage recruitment [10]. How it performs this is yet unclear. Major problem here is the significant fall in SIRT1 level in inflamed adipose tissue with a consequent increase in M1 macrophage population [10] because of IFNγ elevation [11] creates a crucial problem. IFNγ promotes M2 to M1 transformation and since M1 macrophage is a powerful contributor to adipose tissue inflammation and insulin resistance, enhancement of SIRT1 and suppression of IFNγ is expected to rescue this inflammatory burden.

In this report, we have described that dmp, a water soluble orally active small molecule (Mw. 300) could effectively reduce adipose tissue inflammation in obese diabetic mice by inhibiting IFNγ and overexpressing SIRT1 which effected decrease in ATM with a decline in M1 phenotype and these together could significantly improved insulin sensitivity.

## 2. Materials and methods

### 2.1. Chemicals and reagents

All cell culture materials were obtained from Gibco-BRL/Life Technologies, USA. We purchased primary antibodies from abcam, UK and Santa Cruz Biotechnology Inc., CA, USA. Alkaline phosphatase-conjugated secondary antibodies and qPCR primers were procured from Sigma Chemical Co, St. Louis, USA. All other chemicals were purchased from Cayman Chemical, USA and Sigma Aldrich, USA.

### 2.2. Animal treatment and cell culture

Control BALBc male mice aged 12–18 weeks were conditioned with 12-h light/12-h dark cycle at 25 ± 2 °C and divided into three groups, mice received standard diet (SD) ad libitum, high fat diet (HFD) for 16 weeks [12] and HFD mice orally administered with dmp at a dose 300 μg/kg body weight. Adipocytes, 3T3L1 adipocytes, stromal vascular fraction (SVF) and mouse macrophage cell line RAW 264.7 were processed and cultured following our previous descriptions [13]. All experiments with mice were done following the guidelines prescribed by and with the approval of the Animal Ethics Committee of Visva-Bharati.

### 2.3. Immunoblotting

Immunoblot analysis was performed as described in earlier publication [14].

### 2.4. Quantitative PCR

Total RNA was extracted and cDNA was synthesized from 3T3L1 adipocytes, mice adipocyte and SVF using previous protocol and qPCR was performed using specific primers [15].

### 2.5. Cell migration assay

The RAW264.7 cell migration assay was performed in a Boyden chamber system (Millipore QCM 24-well colorimetric cell migration assay kit) by adding incubation media of SD, HFD and dmp treated HFD adipocytes in the lower chamber. The details of this procedure has been described by us previously [7].

### 2.6. ChIP assay

ChIP was performed by using a ChIP assay kit (Upstate Biotech Millipore) by using anti-Foxo1 antibody and primers for mouse SIRT1 promoter by following our previous procedure [15].

### 2.7. Immunofluorescence

Adipose tissue section was incubated overnight with anti-CD11c antibody (dilution 1:100) followed by FITC-conjugated goat anti-mouse secondary antibody (dilution 1:1000) for 2 h. Cells were counterstained with DAPI and observed with a confocal microscope (Leica DMi8).

### 2.8. Histological studies

The adipose tissue from SD, HFD and dmp treated HFD mice were fixed, dehydrated and processed for hematoxylin and eosin staining followed by microscopic observation using Axio microscope (Carl Zeiss Pty Ltd, 25 Khartoum Rd, Macquarie Park, NSW 2113).

### 2.9. [14C] 2-DOG uptake

Insulin (100 nM) was added to 3T3L1 adipocytes for 30 min which was followed by the addition of [14C] 2-DOG (0.4 nmol/ml) for 5 min and then treated with ice-cold KRB buffer containing 0.3 mM phloretin. Cells were treated with 1% NP-40. [14C] 2-DOG uptake was determined in a liquid scintillation counter (PerkinElmer Tri-Carb 2800 TR, Waltham, Massachusetts, USA).

### 2.10. GLUT-4 translocation assay

GFP-Glut 4 translocation assay was performed with 3T3L1 adipocytes by following a previous description from our laboratory [16].

### 2.11. Fatty acid oxidation assay

Cellular beta oxidation rate of SD, HFD and dmp treated HFD mice was measured by the method described previously [17].

### 2.12. FACS

SVF cells from SD, HFD and dmp fed HFD mice were isolated, processed and analysed by flow cytometry according to previous description [7].

### 2.13. Statistical analysis

Data were analysed by one-way ANOVA where P value indicated significance, means were compared by post hoc multiple range test. All values are means ± S.E.M. We considered at least p values of $<0.05$ as statistically significant.