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A new indanedione derivative alleviates symptoms of diabetes by modulating RAGE-NF-kappaB pathway in db/db mice

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ABSTRACT

Accumulating evidence indicates that a number of tissues are damaged due to build-up of abnormal amount of Advanced Glycation End products (AGEs) in several diseases including diabetes. Currently AGE inhibitors are scarce in clinical use indicating a need for development of new anti-AGE agents. The aim of the current study is to identify the new AGE inhibitors and to decipher their mechanism of action for alleviating symptoms of diabetes in mice. Among several derivatives, one of the derivatives of indanedione, IDD-24 demonstrated highest inhibition of AGE formation and AGE mediated reactive oxygen species production in HepG-2 and mature 3T3-L1 adipocytes. In mice treated with IDD-24, reduction in serum AGE formation and expression of Receptor for AGEs (RAGE) was seen in IDD-24 treated db/db mice. In vivo, glycogen synthesis was also increased in muscle tissue. In adipocytes, anti-AGE agent restored AGEs' induced diminished glucose uptake in fat cells. Mice treated with IDD-24 exhibited increased glucose tolerance, increaed serum adiponectin levels and decreased insulin resistance. Deciphering mechanism of IDD-24 in diabetic mice, it was observed that nuclear factor-κB (NF-κB) and serine phosphorylation of Insulin receptor substrate-1 (IRS-1) declined, while diminished activation of c-Jun NH2-terminal kinase (JNK) appears to be partly responsible for restoration of insulin signaling. We conclude that IDD-24 can be a possible treatment target to address symptoms of diabetes.

1. Introduction

Diabetes is the fastest growing chroninc condition all over the globe causing four million death, in 2017 [1]. Role of glycotoxins in development and progression of diabetes, renal faliur and cardiovascular diseases is well reported [2]. Hyperglycemia is the hallmark of the disease that facilitates buildup of AGEs. Diabetic patents exhibit 50% higher levels of serum AGEs as compared to normal individuals [3]. Additionally, ingestion of diet rich in AGEs significantly increases body's AGEs pool [4]. Accumulation of AGEs results in insulin insensitivity, beta cell dysfunction, consequently variety of organs and tissues are damaged, therefore, AGEs are also termed as glycotoxin [5–7]. At cellular level, activation of RAGE, initiates metabolic detoriation partly by enhancing reactive oxidation species and inflamation that bring about damage and destruction of muscular and vascular system [8]. Current research is probing potent AGEs resolver, however, few of the AGEs inhibitors were withdrawn from clinical trials due to serious side effects [9]. Because of the dearth of *anti*-glycotoxin agents in clinical use, there is a need to develop new AGEs inhibitors. Indanediones are β-diketonic in nature [10] and evidence exists for thier biological activities [11,12]. We have been developing antiglycation agents in the past few years [13] [14–16]. Here for the first time, we synthesized and studied therapeutic potential of a new glycotoxin inhibitor IDD-24, a derivative of indanedione, in genetic mice model of diabetes. The structure of the compound is shown in Fig. 1 A.

2. Materials and methods

2.1. Synthesis of benzylidine derivatives of indanedione

The synthesis of indane-1,3-dione derivatives was carried out as described [17]. In a 100 mL round-bottomed flask, indane-1,3-dione

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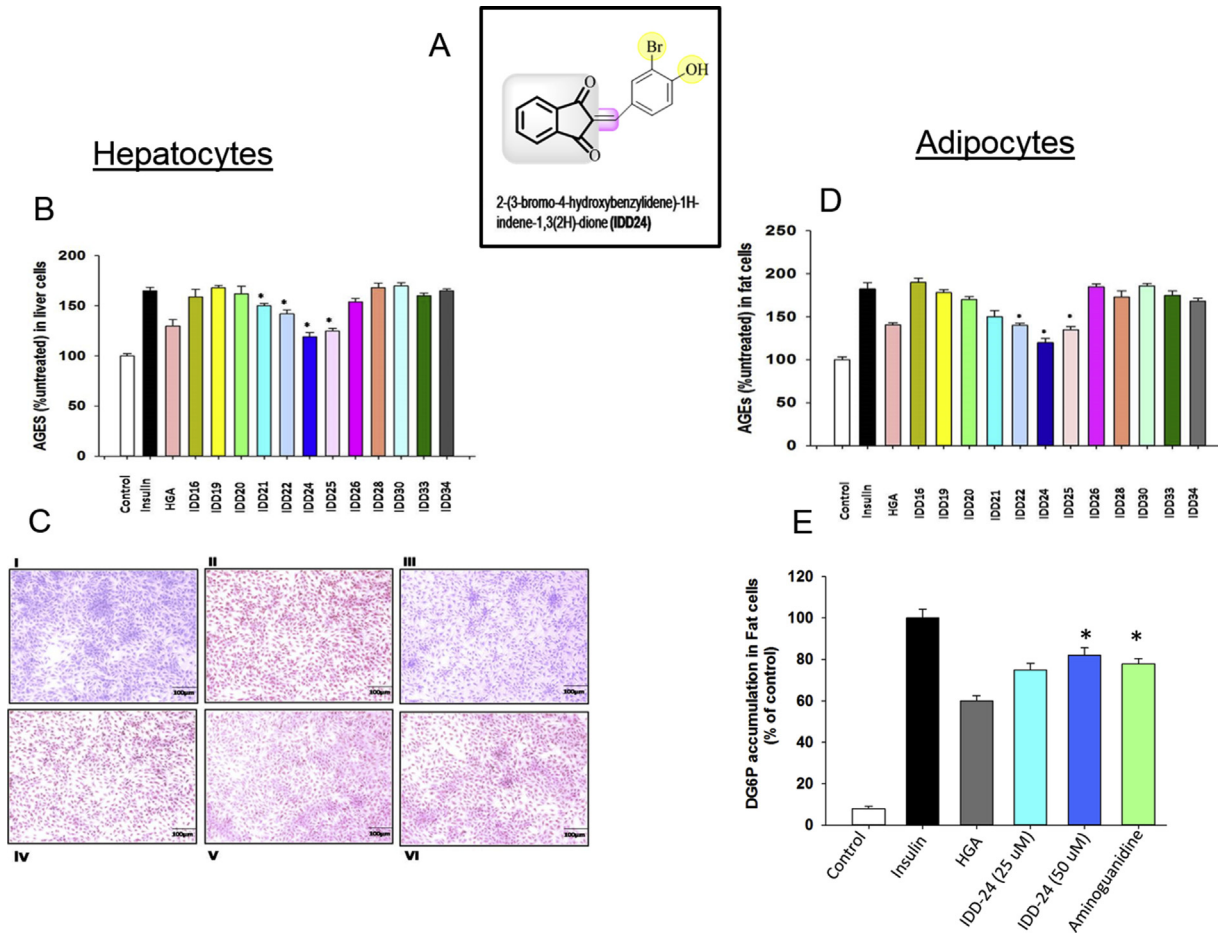

Fig. 1. Therapeutic effect of IDD-24 in insulin responsive cellular system (A) IUPAC name and chemical structure of indanedione derivative, IDD-24. (B) Measurement of intracellular AGEs in HepG-2 using fluorescence intensity assay (C) Glycogen synthesis measurements in human liver cells (I) Control (unstimulated cells) (II) Cells stimulated with 50uM insulin alone (III) Cells treated with HGA alone (IV) Cells treated with HGA and 25 μM IDD-24 (V) Cells treated with HGA and 50 μm IDD-24 (VI) Cells treated with 2 mM aminoguanidine alone. Scale bar 100 μm. (D) Measurement of intracellular AGEs in 3T3-L1 adipocytes cells. (E) Glucose uptake in adipocytes. Differentiated 3T3-L1 adipocytes were incubated with 20 ng/ml TNF-alpha and IDD-24 for 24 h followed by stimulation with 10 nM insulin for 1 h. In all above experiments results are mean ± SEMs, n = 6, *p < 0.05 cells stimulated with IDD-24 vs. cells stimulated with HGA alone.

(1 mmol) in pyridine (5 mL) was stirred for 5–10 min. Then, 1 mmol of the substituted benzaldehyde was added and the reaction mixture was refluxed for 1 h at 100 °C and progress of reaction was monitored through TLC. After cooling the reaction mixture, it was diluted slowly with ice cold water. The resulting precipitates were filtered and washed with water. The precipitates were crystallized from ethanol to form good yields of products. The structural determination was carried out by ^{1}H, ^{13}C NMR mass spectrometry, EI-MS, and HREI-MS.

2.2. Cell culture, cell lysis, immunoprecipitation and antibodies

Cell lines (HepG-2 and 3T3-L1) were grown in Dulbecco's modified Eagle's medium. Cells were stimulated with aminogunaidine (AG) as positive control and/or human glycated albumen (HGA) and/or IDD-24 and insulin. For *in vivo* investigation of signaling in muscle tissue, mice were injected with 5 IU insulin into the inferior vena cava. Liver, skeletal muscle and fat tissues were removed and homogenized at 4 °C (Stuart Homogeniser, UK) in lysis buffer, the tissues were then processed as described previously [18]. Antibodies against IRS-1 serine 307 and RAGE were acquired from Millipore, IRS-1 protein, PKC-α, phospho Tyrosine (p)100, phospho-NF-kappaB p65 (Ser536), NF-kappaB p65 protein from Cell signaling (Beverly, MA, USA), phospho- JNK (Thr183/Tyr185) and JNK protein antibodies from Abcam (MA, USA).

2.3. Cell viability assay

Cell lines were analyzed for viability against different derivatives of indanedione by MTT assay [19]. Cells were treated with different derivatives of indanedione followed by exposure to tetrazolium dye in a concentration of 0.25 mg/ml. Formazan formed during incubation was dissolved in dimethyl sulfoxide and quantified using spectrophotometer (Molecular devices SpectraMax 340, USA).

2.4. Anti-AGEs assay

Ability of indanedione derivatives in reducing intracellular glycative stress was measured by *anti*-glycation assay as described previously [20]. After stimulation of the cells with HGA and derivatives of indanedione for 24 h, 200 μL medium was collected and precipitated with absolute Trichloroacetic acid (TCA) the solution was then centrifuged and the pellet was washed with 5% TCA and was dissolved in PBS. 100 μL suspension was transferred to 96 well plate and absorbance was measured at 370 nm of excitation and 440 nm of emission using spectrofluorimeter (Molecular devices SpectraMax 340, USA).

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