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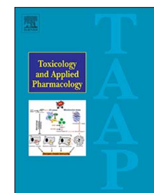

# Atorvastatin and insulin equally mitigate brain pathology in diabetic rats

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ABSTRACT

Although insulin and atorvastatin have been shown to exert glycemic control and could improve brain function, the effects of atorvastatin or insulin as well as the combination of atorvastatin plus insulin on brain pathology in diabetes mellitus type 1 (T1DM) are unclear. Therefore, this study investigated the effect of atorvastatin, insulin or combined drugs on brain pathology in streptozotocin-induced diabetic rats. Thirty-six male rats were divided into two groups, a control group (n = 12) and a diabetic or experimental group (n = 24). Diabetic rats were further divided into four groups (n = 6/group) and the groups received either a vehicle (normal saline), atorvastatin (10 mg/kg/day), insulin (4 U/day) or a combination of the drugs for 4 weeks. The control group rats were divided into two groups (n = 6/group) to receive either just the vehicle or atorvastatin for 4 weeks. We found that streptozotocin-induced diabetic rats developed hyperglycemia, showing evidence of increased brain oxidative stress, impaired brain mitochondrial function, increased brain apoptosis, increased tau protein expression, increased phosphorylation of tau protein expression and amyloid beta levels, and decreased dendritic spine density. Although atorvastatin and insulin therapies led to an equal reduction in plasma glucose level in these diabetic rats, the combined drug therapy showed the greatest efficacy in decreasing plasma glucose level. Interestingly, atorvastatin, insulin and the combined drugs equally mitigated brain pathology. Our findings indicate that the combined drug therapy showed the greatest efficacy in improving metabolic parameters. However, atorvastatin, insulin and the combined drug therapy shared a similar efficacy in preventing brain damage in T1DM rats.

## 1. Introduction

Diabetes mellitus type 1 (T1DM) is an irreversible disorder with severe complications involving damage to multiple organs, including heart, kidney, nerves and retina (Sochett and Daneman, 1999; Melendez-Ramirez et al., 2010). In addition, impaired cognitive function, increased brain apoptosis, increased tau protein expression, impaired brain mitochondrial function and decreased dendritic spine density have been found in T1DM mice, rats and patients (Kroner, 2009; Ho et al., 2013; Semaming et al., 2015). It has been shown that insulin therapy not only prevents organ damage in T1DM (American Diabetes, 2017), but also improves cognitive function in subjects with comorbid diabetes and Alzheimer's disease (Dhamoon et al., 2009; Morris and Burns, 2012). Although insulin had beneficial effects as regards the improvement of glycemic control and cognitive function, hypoglycemia can still be a serious adverse effect following insulin therapy (American Diabetes, 2017). Recently, it has been suggested that the combination of insulin therapy with other therapies, including statins, in the treatment of T1DM may be used to decrease cardiovascular risks in diabetic patients (American Diabetes, 2017).

Atorvastatin is one of many lipid-lowering agents which leads to the reduction of cholesterol biosynthesis via the inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase enzyme (Stancu and Sima, 2001). Atorvastatin has been shown to exert glycemic control (Suzuki et al., 2005; Tanaka, 2011), reduce oxidative stress (Sugiyama et al., 2005; Kishi et al., 2008; Li et al., 2010), and attenuate brain

*Abbreviations:* T1DM, diabetes mellitus type 1; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; T2DM, diabetes mellitus type 2; C, control rats; DM, diabetic rats; DMI, insulin-treated diabetic rats; DMS, atorvastatin -treated diabetic rats; DMIS, combined drug-treated diabetic rats; MDA, malondialdehyde; ROS, reactive oxygen species; HPLC, high performance liquid chromatography; TCA, trichloroacetic acid; TBA, thiobarbituric acid solution; BCA, bicinchoninic acid assay; DCFHDA, dichlorohydrofluoresceindiacetate; ECL, enhanced chemiluminescence; Aβ, amyloid beta; STZ, streptozotocin

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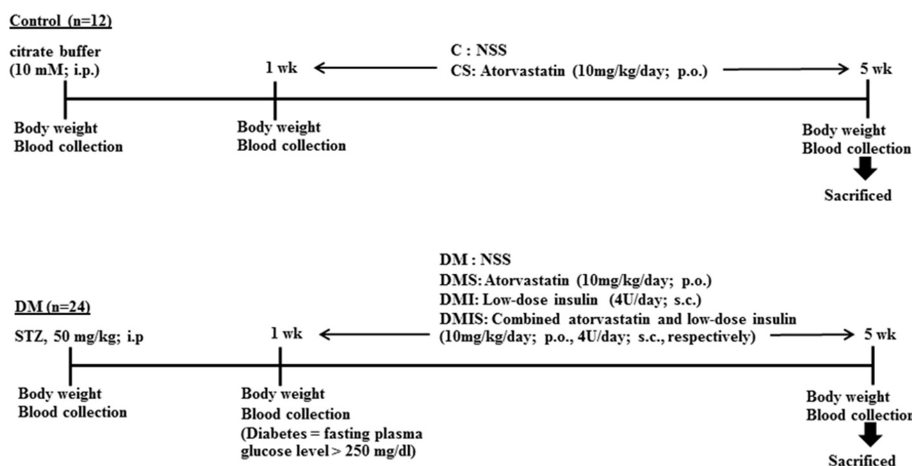


**Fig. 1.** The experimental protocol of the study. STZ = streptozotocin; C = control rats; DM = diabetic rats; DMI = insulin-treated diabetic rats; DMS = atorvastatin-treated diabetic rats; DMIS = combined drug-treated diabetic rats.

oxidative stress and cognitive impairment in rats with ischemic stroke (Kishi et al., 2008; Yang et al., 2015). Interestingly, it has been reported that the combination of insulin therapy and atorvastatin provided greater benefits than either of the 2 drugs as a monotherapy on the improvement of glycemic control and the reduction of liver oxidative stress in diabetes mellitus type 2 (T2DM) rats (Matafome et al., 2009). There is some evidence that the benefits of atorvastatin may be due to its effects on mitochondrial biogenesis (Bouitbir et al., 2012) and also the restoration of mitochondrial enzyme complex activities in rat and mice brains (Kumar et al., 2012a,b). In addition, a previous study showed that either atorvastatin, insulin or combination of the 2 drugs led to reduced cardiac mitochondrial swelling in T2DM rats with cardiac ischemia/reperfusion injury (Matafome et al., 2008).

Despite these reports on the benefits of atorvastatin and insulin therapies in cases of diabetes, the effects of either atorvastatin or insulin therapy, as well as the combination of atorvastatin and insulin therapy on brain pathology, including brain mitochondrial function, brain apoptosis, brain oxidative stress, tau protein, amyloid beta and dendritic spine density, in the T1DM model have never been investigated. Our hypothesis was that the administration of either atorvastatin or insulin attenuates brain pathology in streptozotocin-induced diabetic rats, and that the combination of these 2 drugs provides the greatest benefits on brain protection in these diabetic rats.

## 2. Materials and methods

### 2.1. Animal models and experimental protocols

All experiments were conducted in accordance with the approved protocol from the Faculty of Medicine, Chiang Mai University Institutional Animal Care and Use Committee and were in compliance with NIH guidelines. Thirty-six male Wistar rats weighing 200–220 g (aged ~ 6 weeks old) were obtained from the National Animal Center, Salaya Campus, Mahidol University, Thailand. All animals were individually housed in a temperature-controlled environment with a 12:12 light-dark cycle. Rats were given ad libitum access to food and water. Body weight and food intake were recorded weekly. Rats were divided into a control group (n = 12) and a diabetic or experimental group (n = 24). In the control group, rats were injected intraperitoneally with citrate buffer (10 mM). Then, these control rats were divided into two subgroups (n = 6/subgroup) to receive either a vehicle (normal saline: C) or low-dose atorvastatin (10 mg/kg/day; p.o.; CS) for 4 weeks. In the diabetic group, rats were injected intraperitoneally with streptozotocin to induce diabetes (a single dose of STZ, 50 mg/kg). Diabetes was indicated by hyperglycemia (fasting plasma glucose level > 250 mg/dl) (Semaming et al., 2015). After the development of the diabetic condition, rats were divided into four subgroups (n = 6/subgroup) to receive either vehicle (normal saline: DM), low-dose atorvastatin (10 mg/kg/day; p.o.: DMS), low-dose insulin (4 U/day; s.c.: DMI) or combined low-dose atorvastatin and low-dose insulin (10 mg/kg/day; p.o., 4 U/day; s.c., respectively; DMIS) for 4 weeks. The reason for using low-dose insulin therapy and low-dose atorvastatin therapy in the study was to prevent hypoglycemia, which is a serious side effect of drug treatment for diabetic patients. At the end of the experimental protocol, animal were fasted for 5 h. After that, the animals were put into deep anesthesia using 2–3% isoflurane. Blood was collected to determine metabolic parameters (insulin, glucose, total cholesterol levels). Then, their brains were rapidly removed and separated to right and left hemispheres. The right hemisphere was used to determine the dendritic spine density using Golgi staining. The left hemisphere was separated to upper and lower parts. The upper part was used to determine brain mitochondrial function by measuring ROS production, membrane potential changes and mitochondrial swelling. The lower part was used to determine levels of brain oxidative stress by measuring MDA levels, brain apoptosis by measuring Bax and Bcl2 protein expressions, Alzheimer's disease markers by measuring Aβ42 level, tau protein expression and phosphorylation tau protein expressions. The experimental protocol is shown in Fig. 1.

### 2.2. Biochemical analysis for assessment of insulin, glucose and cholesterol levels

Fasting plasma glucose and cholesterol concentrations were determined by colorimetric assay using commercially available kits (ERBA diagnostic, Mannheim, Germany). The fasting plasma insulin levels were measured using Sandwich ELISA kits (LINCO Research, Missouri, USA).

### 2.3. Determination of malondialdehyde (MDA) levels

Malondialdehyde (MDA) level, an indicator of oxidative stress, was determined using a high performance liquid chromatography (HPLC) method (Candan and Tuzmen, 2008). Briefly, brain homogenates were mixed with 10% trichloroacetic acid (TCA) containing butylated hydroxytoluene (BHT), incubated at 90 °C for 30 min, and centrifuged at 6000 rpm for 10 min. The supernatant was mixed with $H_3PO_4$ and thiobarbituric acid solution (TBA) and incubated at 90 °C for 30 min. MDA levels were measured via absorbance detection at 532 nm by the HPLC system, and were determined directly from the standard curve, and reported as an MDA equivalent concentration (Pratchayasakul et al., 2017).

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