



Original article

The effect of insulin-loaded trimethylchitosan nanoparticles on rats with diabetes type I



Mohammad Jamshidi^a, Nasrin Ziamajidi^a, Iraj Khodadadi^a, Arash Dehghan^b, Giti Kalantarian^a, Roghayeh Abbasalipourkabir^{a,*}

^a Department of Biochemistry, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran

^b Department of Pathology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran

ARTICLE INFO

Keywords:

Insulin
Diabetes
Trimethyl chitosan nanoparticles
Pyruvate kinase
Glucokinase

ABSTRACT

Objective: The aim of this study was to explore the efficacy of insulin-loaded trimethylchitosan nanoparticles on certain destructive effects of diabetes type one.

Materials and methods: Twenty-five male Wistar rats were randomly divided into three control groups (n = 5) and two treatment groups (n = 5). The control groups included normal diabetic rats without treatment and diabetic rats treated with the nanoparticles. The treatment groups included diabetic rats treated with the insulin-loaded trimethylchitosan nanoparticles and the diabetic rats treated with trade insulin. The experiment period was eight weeks and the rats were treated for the last two weeks.

Result: The livers of the rats receiving both forms of insulin showed less severe microvascular steatosis and fatty degeneration, and ameliorated blood glucose, serum biomarkers, and oxidant/antioxidant parameters with no significant differences. The gene expression of pyruvate kinase could be compensated by both the treatment protocols and the new coated form of insulin could not significantly influence the gene expression of glucokinase ($p < 0.05$). The result of the present study showed the potency of the nanoparticle form of insulin to attenuate hyperglycemia, oxidative stress, and inflammation in diabetes, which indicate the bioavailability of insulin-encapsulated trimethylchitosan nanoparticles.

1. Introduction

Diabetes mellitus is a chronic medical condition which, based on forecasts, will increase in epidemic dimensions to affect up to 380 million people by 2025 [1]. This metabolic disorder is caused by a blend of insulin resistance and inappropriately functioning pancreatic beta cells, resulting in the inability of the body to produce insulin in type1 diabetes and further complications in type2 diabetes. Despite extensive research to develop alternative treatment, to date, insulin therapies are the most reliable way to control the blood glucose level [2]. The challenge for people with type 1 diabetes and also, for many with type 2 diabetes, is the subcutaneous injection of insulin, sometimes up to four times a day [3]. The route of parenteral administration is the main way because of the low bioavailability and inadequate stability of this compound in the gastrointestinal tract when administered by oral delivery [4]. This is because of the incomplete and/or erratic absorption due to the mucus layer and the tight junctions, degradation by the acidic pH, and the hydrolytic enzyme activity [5]. The complications of parenteral administration include low patient

compliance, lipodystrophy, and hypertrophy of the skin, pain, trauma as well as a high risk of infection [6,7]. In addition, even though the target organ of this compound is the liver and the pattern of the physiological mechanism of insulin secretion is by portal circulation, the parenteral administration of insulin mainly causes peripheral circulation. Thus, an oral administration could mimic endogenously secreted insulin in the portal circulation [8,9]. This fact makes the oral route of administration the most desired route of administration [10].

Based on the importance of the oral route of insulin delivery and yet, the limitations of this kind of delivery route, several drug delivery systems have been made in order to overcome the challenges and protect the protein following oral administration [11]. One of the drug delivery systems is via nanoparticles, which were widely investigated for the enhancement of the permeability and consequently, the bioavailability of these hydrophilic macromolecular peptides [12,13].

Chitosan is a natural cationic polysaccharide macromolecule obtained by the alkaline deacetylation of the natural polysaccharide chitin [14]. This is the hydrophilic, biocompatible, and biodegradable macromolecule, which is found as the most abundant polymer in nature

* Corresponding author at: Department of Biochemistry, School of Medicine, Hamadan University of Medical Sciences, 65176 Hamadan, Iran.

E-mail addresses: rpourkabir@hotmail.com, abbasalipourkabir@umsha.ac.ir (R. Abbasalipourkabir).

Table 1

The characteristics of primers of, Glucokinase, Pyruvate Kinase and Beta actin genes.

Genes	Primers	Primer length	Amount of uses (μl)	GC%	Tm (	Product length (bp)
Glucokinase	Forward: TTCACCTTCTCCTCCCTGT	20	1	50	57.88	108
	Reverse: TCCTACGATGTTGTTCCTTC	21	1	47.62	57.40	
Pyruvate Kinase	Forward: AACCTCCCACTCAGCTACA	20	1	55	60.18	114
	Reverse: CCCTTCACAATTTCCACCTC	20	1	50	56.31	
Beta-actin	Forward: ATCAGCAAGCAGGAGTACGAT	21	1	47.62	59.24	94
	Reverse: AAAGGGTGTAAACGCAGCTC	21	1	47.62	59.39	

after cellulose [15]. The interaction between this cationic compound with the anionic components of the glycoproteins on the epithelial cell surface on one side and the changes it induces in the cytoskeletal F-actin in another side can open the tight junction of the intestinal epithelium and increase the permeability of this delivered protein [16]. In addition, the other features of this Food and Drug Administration (FDA)-approved compound, such as high molecular weight and no systemic side effects, make chitosan an object of interest as an excipient for use in drug formulations [17].

The direct link between oxidative stress and the pathogens of diabetes related to β -cell dysfunction in the development of diabetic liver injury was reported; it also stimulates other diabetes-related consequences, such as cardiovascular complications, endothelial dysfunction, and atherosclerosis [18,19].

Based on the advantages of chitosan, the main goal of our study is to use this nanoparticle delivery system to support insulin administered orally compared with traditional parenteral administration of insulin.

2. Methods

2.1. Nanoparticles preparation

The trimethylchitosan nanoparticles were taken from Dr G. Kalantarian.

2.2. Preparation of streptozotocin (STZ) and induction of diabetes

For induction of type 1 diabetes, the rats were intraperitoneally injected with a single dose of streptozotocin (Santa Cruz Biotechnology, Inc. Dallas, USA) at the dose of 60 mg/kg body weight. The STZ was freshly prepared by dissolving the STZ powder in 0.1 M sodium citrate buffer (pH = 4.5). The fasting blood sugar (FBS) was measured by the blood obtained from the tail of the animal and by One Touch Basic Glucometer (Arkray Glucocard G meter, Japan). The FBS level was assayed before the STZ injection, after 48 h of STZ administration and at the sixth and the eighth days after diabetes induction. Fasting blood glucose of above 200 mg dL⁻¹ concurrent with glycosuria were considered as diabetes [20].

2.3. Experimental design

Male Wistar rats (n = 25) weighing an average of 200 g were housed in standard cages (33 × 23 × 12 cm³) under the controlled condition of temperature (25 ± 2 °C), lighting (12-h light/dark cycles), and free access to commercial rat chow diet and tap water *ad libitum* throughout the acclimatization and experimental periods. The study was approved by the local ethics committee of our faculty state and the national medical board, in accordance with the ethical standards of the Principles of Laboratory Animal Care. After one week of acclimatization, all 25 rats were randomly divided into five groups (each group contains 5 rats): control normal (C), diabetic rats without treatment (DM), diabetic rats treated with nanoparticle (DM + N, 1 ml by gavage), diabetic rats treated with nano-insulin (DM + NINS, 1 ml by gavage) and diabetic rats treated with trade insulin (DM + INS,

0.2 ml by subcutaneous injection). The experiment period was eight weeks long and the rats were treated over the last two weeks. The animals were weighed before and after the STZ injection and every two weeks until the day of their dissection. At the end of the experiment, the rats were euthanized by using chloroform and blood was drawn from the abdominal aorta. The sera samples were separated by 10 min centrifugation at 3000 × g and were kept at -20 °C until analysis. The liver sample was dissected, washed rapidly with cold PBS, and one sample was kept in formalin for histopathologic examination while the other samples were immediately submerged in liquid nitrogen and stored at -75 °C for gene expression. All the experiments were performed in accordance with the procedures approved by the Animal Care Center, Hamadan University of Medical Sciences (Res: IR.UMSHA.REC.1395.197).

2.4. Real-Time PCR

RNX-Plus (Sinaclon, Tehran, Iran) was used to extract the RNA from the samples. The extracted RNA was then quantified by Nanodrop One (Thermo Fisher Scientific) and qualified by agarose 1.2% (Sigma-Aldrich Co. Ltd., Dorset, UK) in a TBE (Tris/Borate/EDTA) buffer. Then, an equal amount of RNA of each sample was reverse to the complementary DNA (cDNA) by a fermentas cDNA synthesis kit (Thermo Scientific, Vilnius, Lithuania) [21]. The Pyruvate Kinase gene expression was amplified and quantified by real-time PCR using the SYBR premix Ex TaqTMII (TaKaRa Biotechnology, Shiga, Japan) on a Roche LightCycler[®] 96 System (Roche Diagnostics Corporation, Indianapolis, USA). The gene expression was calculated via a 2^{-ΔΔCt} method described by Livak and Schmittgen [22]. The forward and reverse primer sequences are listed in Table 1.

2.5. Analysis of serum biochemical parameters

The serum samples were used to analyze the serum biomarkers of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) by commercially available kits (Pars Azmoon Diagnostics, Iran).

2.6. Antioxidant analysis

The ferric reducing ability of plasma (FRAP) reagent was used to measure the total antioxidant capacity (TAC) in serum. This method was done on the basis of the antioxidant capacity in samples which reduced a ferric ion (Fe³⁺) complex found in the 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) to the ferrous ion (Fe²⁺) and was expressed as μmol/L [23]. The total oxidative status (TOS) assay was evaluated on the basis of the measurement of the ferric ion obtained by the ferric-xylenol orange 1 (FOX1) reagent. This is based on the oxidation of ferrous ion to ferric ion in the presence of various oxidant species in acidic medium and is expressed as μmol/L [24]. The byproduct of the lipid peroxidation, thiobarbituric acid reactive substances (TBARS), was measured using 1.1.3.3-tetraethoxypropane as the standard, after the precipitation of the serum protein by the trichloroacetic acid (10%) according to the Yagi method (1984). This was expressed in terms of

Download English Version:

<https://daneshyari.com/en/article/8526461>

Download Persian Version:

<https://daneshyari.com/article/8526461>

[Daneshyari.com](https://daneshyari.com)