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Salmon calcitonin and omega-3 fatty acids exhibit antagonistic and non-additive effects in experimental diabetes

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ABSTRACT

Because optimising therapy for the management of diabetes mellitus remains challenging, the study investigated the effects of salmon calcitonin (Sct) and/or omega-3 fatty acids (N-3 – eicosapentaenoic acid and docosahexaenoic acid-3:2), compared to metformin, on selected biochemical parameters in male Wistar rats, in an experimental model of diabetes. Forty rats were used for this study. They were divided into eight groups of five rats each, which included: Normal control; Diabetic (D) control; D + N-3; D + low dose Sct (Sct. Lw); D + high dose Sct (Sct. Hi); D + N-3 + Sct.Lw; D + N-3 + Sct.Hi; and D + metformin. Diabetes was induced in overnight fasted rats by the administration of streptozotocin (65 mg/kg b.w., i.p.), 15 min after the administration of nicotinamide (110 mg/kg b.w., i.p.). Nine days later, Sct was administered at 2.5 and 5.0 IU/kg b.w./day (i.m.), while N-3 and metformin were administered at 200 and 180 mg/kg b.w./day (p.o.) respectively, for four weeks. Sct, N-3, and metformin significantly reduced total cholesterol, LDL-C, cortisol, c-telopeptide of type 1 collagen, and collagen type 2 alpha-1. The combined administration of Sct and N-3 had more favorable effects on triglyceride and HDL-C than either monotherapy. Unlike metformin and Sct. Hi, N-3 significantly reduced alkaline phosphatase activity. Moreover, N-3 significantly suppressed the hypocalcaemic, hyperglycaemic, and insulin resistance provoking actions of Sct. Furthermore, N-3 contradicted the hepatic glycogen depletion and inhibition of nitric oxide synthesis brought about by Sct. In conclusion, N-3 demonstrated antagonistic and non-additive actions with Sct. Moreover, the effects of the combined administration of Sct and N-3 were comparable to that of metformin; therefore, they might be considered as therapeutic alternatives in diabetes.

1. Introduction

The growing prevalence of diabetes mellitus (DM) in the various countries of the world remains a public health challenge. It has been estimated that by the year 2030 the number of patients living with DM would be about 366 million [1]. This is quite significant compared to the 117 million that was recorded in year 2000 [1]. Although there are different types of DM, type 2 DM is the most prevalent. It has a global occurrence of about 90–95% of all known cases of diabetes [2].

Diabetes is characterised by hyperglycaemia, which results from insulin resistance and/or the destruction of pancreatic beta cells. The disease is also accompanied with imbalance in carbohydrate, protein, and fat metabolism [3,4], and altered integrity of bone and cartilage tissues. Type 2 diabetic subjects have been reported to have normal or increased bone mineral density [5], and reduced osteoblast population [6]. Moreover, there are incongruent reports in literature on the effect of DM on bone resorption [7]. Advanced glycation end products (AGEs) formed as a result of hyperglycaemia act on the receptor for AGEs (RAGE) to bring about cartilage degradation [8].

The deliberate initiation of DM is impermissible in human. Therefore, experimental animals are used to study the aetiopathogenic mechanisms underlying the initiation and progression of this disease, and to investigate the effects of various promising therapeutic interventions. Although there are different models for the induction of type 2 DM in animals, the streptozotocin-nicotinamide model introduced by Masiello and colleagues [9] is one of the most widely used methods. Despite extensive research on the possible pharmacological approaches that can be used to care for diabetic subjects, the optimal therapy for the management of this disease remains a controversial issue [10].

Even though metformin has been recommended by various professional bodies as the first - line treatment in the management of type 2 diabetes [11], it administration is associated with gastrointestinal side effects [12] and impaired renal function [11].

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Furthermore, metformin causes poor vitamin B_{12} status [13] and hence impaired cognitive function [14]. Therefore, there is the need for an alternative therapeutic intervention in type 2 diabetic state. Unlike metformin, calcitonin has direct effect on bone and cartilage tissues due to the presence of its receptor [15]. Salmon calcitonin is the most widely used form of calcitonin in clinical practice. This is because of its 40–50 times higher intrinsic potency compared to human calcitonin, and its enhanced analgesic action [16]. The drug is commonly administered via intramuscular, intravenous or subcutaneous route. However, the oral form is under clinical trial. Generally, calcitonin is a hyperglycaemic hormone with no reported diabetogenic action even after chronic treatment [17]. Like calcitonin, N-3 have beneficial effects on bone and cartilage tissues [18]. Moreover, there are incongruent reports in literature on the effects of N-3 in diabetic condition [19]. Therefore, this study investigated the effects of salmon calcitonin and/or omega-3 fatty acids (eicosapentaenoic acid and docosahexaenoic acid – 3:2), relative to metformin, in streptozotocin + nicotinamide-induced diabetes mellitus in male Wistar rats.

2. Materials and methods

2.1. Drugs and chemicals

Salmon calcitonin, streptozotocin and nicotinamide were purchased from Sigma Aldrich, St. Louis, Missouri, USA. Omega-3 fatty acids and sodium pentobarbital were purchased from Gujarat Liqui Pharmacaps Pvt. Ltd., Vadodara, Gujarat, India, and Nicholas Piramal Ltd., Thane, Maharashtra, India, respectively. In addition, metformin was purchased from Jiangsu Ruinian Qianjin Pharmaceutical Company, Chuanbu Yixing Economic Development Zone, Jiangsu, China.

2.2. Experimental animals and care

Forty (40) adult male Wistar rats (weight range: 180–220 g) were used for this study. They were purchased from the Animal Holding Unit of the Biochemistry Department, University of Ilorin, Ilorin, Nigeria, and were housed in wooden cages maintained under standardised conditions (12 h light/dark cycle, 27–30 °C, 50–80% relative humidity). After one week of acclimatisation, but, prior to the administration of various chemical agents that were used in this study, five rats were randomly allotted to each of the groups. The rats were given standard pelletised diet (Ace Feed PLC Ibadan, Oyo, Nigeria) and tap water ad libitum daily, and were weighed weekly.

The animals were cared for according to the criteria outlined in the National Institutes of Health "guide for the care and use of Laboratory animals" (NIH Publications No. 8023, revised 1978) and approved (Approval Number: UERC/ASN/2016/655) by the Ethical Committee of the University of Ilorin, Ilorin, Nigeria.

2.3. Experimental design

The forty rats that were used for this research were randomly distributed into eight groups of five rats each. These included: Normal control; Diabetic (D) control; D + N-3; D + Low dose of salmon calcitonin (Sct.Lw); D + High dose of salmon calcitonin (Sct.Hi); D + N-3 + Sct.Lw; D + N-3 + Sct.Hi; and, D + Metformin (Metfm).

Salmon calcitonin was administered intra-muscularly once daily, at a low and high dose of 2.5 and 5.0 IU/kg b.w. respectively. Metformin was administered at 180 mg/kg b.w./day (*p.o.*) [20], while omega-3 fatty acids were administered at 200 mg/kg b.w./day (*p.o.*) [21]. Treatments started nine days after the induction of diabetes mellitus, and they lasted for 28 days.

2.4. Induction of diabetes mellitus

Diabetes mellitus was induced in overnight fasted rats by the single intraperitoneal injection of streptozotocin (STZ) (65 mg/kg b.w.) that was dissolved in freshly prepared cold citrate buffer solution (pH – 4.5), and nicotinamide (NAD) (110 mg/kg b.w.) in normal saline. Streptozotocin was administered 15 min after the administration of NAD [22]. Three days after the induction of diabetes mellitus, the blood glucose of the animals was determined using a glucometer (Accu – Check Active, manufacturer – Roche Diagnostics, Pvt Ltd., Mumbai, Maharashtra, India), and the rats with a blood glucose concentration of more than 250 mg/dl were considered diabetic and were used for the study.

2.5. Preparation of injectable form of salmon calcitonin

Salmon calcitonin powder was dissolved in 0.9% of sodium chloride solution in order to achieve the desired doses [23]. The solution was stored in a refrigerator at 2 °C–8 °C for the maintenance of the viability of the hormone.

2.6. Biochemical measurements

Twelve (12) hours after administration on the 28th day of treatment, the rats were anaesthetised with sodium pentobarbital (40 mg/kg b.w., *i.m.*). Afterwards, the animals were dissected in order to collect blood by cardiac puncture. The whole blood for the determination of serum insulin was collected in plain bottles, and was left undisturbed for 30 min to clot. However, the blood for the determination of the other biochemical parameters was collected in heparinised sample bottles, which were centrifuged at 4000 revolutions per minute, for 15 min, at −4 °C, using a cold centrifuge (Model 8881, manufacturer – Centurion Scientific Ltd, United Kingdom). The separated serum and plasma samples were collected into separate plain bottles and were stored at −20 °C prior to the biochemical analyses.

The diagnostic kits for the determination of calcium ion (Ca^{2+}), cortisol, triglyceride (TG), total cholesterol (TC), and high density lipoprotein cholesterol (HDL-C) were purchased from Fortress Diagnostics Limited, Belfast, Northern Ireland, United Kingdom, while the analytic kits for the quantification of insulin, c-telopeptide of type 1 collagen (CTX-1), collagen type 2 alpha – 1 (C2M), and total alkaline phosphatase (TALP) were purchased from Elabscience Biotechnology Company Ltd., Wuhan, Hubei, China. Non-enzymatic colourimetric assay kit purchased from Oxford Biomedical Research, Inc., Rochester Hills, Michigan, U.S.A was used for the determination of nitric oxide (NO). Moreover, Hassid's and Abraham's method was used for the determination of hepatic glycogen content [24]. The analyses were performed according to the manufacturers' instruction.

2.7. Determination of insulin resistance

Insulin resistance was calculated according to the formula:

$$\text{Fasting blood glucose (mmol/l)} \times \text{Fasting serum insulin (pmol/l)} / 22.5 [25]$$

2.8. Determination of low density lipoprotein cholesterol (LDL-C)

Low density lipoprotein cholesterol was determined using the formula below

$$\text{LDL-C(mg/dl)} = \text{TC} - (\text{HDL-C} - \text{TG}/5) [26]$$

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