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Clinica Chimica Acta 351 (2005) 105–113

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Effect of *N*-benzoyl-D-phenylalanine and metformin on carbohydrate metabolic enzymes in neonatal streptozotocin diabetic rats

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Received 30 June 2004; received in revised form 9 August 2004; accepted 9 August 2004

Abstract

Background: The effect of *N*-benzoyl-D-phenylalanine (NBDP) and metformin was studied on the activities of carbohydrate metabolic enzymes in neonatal streptozotocin (nSTZ) non-insulin-dependent diabetic rats.
Methods: To induce non-insulin-dependent diabetes mellitus (NIDDM), single dose injection of streptozotocin (STZ; 100 mg/kg body weight; i.p.) was given to 2-day old rats. After 10–12 weeks, rats weighing >150 g were selected for screening in NIDDM model, they were checked for fasting blood glucose concentrations to conform the status of NIDDM. NBDP (50,100 and 200 mg/kg body weight) was administered orally for 6 weeks into the confirmed diabetic rats.
Results: The activities of gluconeogenic enzymes were significantly increased, whereas the activities of hexokinase and glucose-6-phosphate dehydrogenase were significantly decreased in nSTZ diabetic rats. Both NBDP and metformin were able to restore the altered enzyme activities to almost control concentrations. Combination treatment was more effective than either drug alone.
Conclusion: The administration of NBDP along with metformin to nSTZ diabetic rats normalizes blood glucose and causes marked improvement of altered carbohydrate metabolic enzymes during diabetes.

Keywords: *N*-benzoyl-D-phenylalanine; Metformin; Glucose-6-phosphatase; Fructose-1,6-bisphosphatase; Hexokinase; Glucose-6-phosphate dehydrogenase; Diabetes mellitus

1. Introduction

Greater than 80% of individuals affected with diabetes mellitus have the type 2, non-insulin dependent, maturity onset form of the diseases [1]. Defects in insulin secretion and insulin action are universally present in type 1 diabetes and also in most type 2

Abbreviations: NBDP, *N*-benzoyl-D-phenylalanine; nSTZ, neonatal streptozotocin; NIDDM, non-insulin-dependent diabetes mellitus.

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doi:10.1016/j.cccn.2004.08.011

diabetes in both human patients and animal models, although it has become increasingly clear that diabetes is a heterogeneous disorder [2]. Hyperglycemia leads to the worsening of insulin secretion by β cells and impairment of insulin sensitivity in peripheral tissues, resulting in severe hyperglycemia. Thus, there are complex interrelationships among hyperglycemia and insulin action, all of which are involved in the pathogenesis of diabetes. Glucose homeostasis involves the coordinated regulation of several metabolic pathways, including gluconeogenesis and glycolysis, which is due to impaired carbohydrate utilization resulting from a defective or deficient insulin secretory response [3].

A number of oral hypoglycemic or antihyperglycemic agents have been developed for the treatment of type 2 diabetes with differing mechanism of action. The most recently developed group of compounds, which are structurally related to sulfonylurea and biguanides, is the nonsulfonylurea of which *N*-benzoyl-D-phenylalanine (NBDP; Fig. 1) is the member currently in use [4]. NBDP acts directly on the pancreatic β cell to stimulate insulin secretion that is rapid and short duration and depend upon the ambient glucose concentration. Generally D-phenylalanine derivative controls hyperglycemia, resulting in improved overall glycemic control in patients with type 2 diabetes.

Metformin is an oral hypoglycemic agent, which belongs to the class known as the biguanides. Chemically it is *N*-N-dimethylimidodicarbonimidic diamide (Fig. 2) [5]. Metformin is now widely used as one of the mainstays in the management of type 2

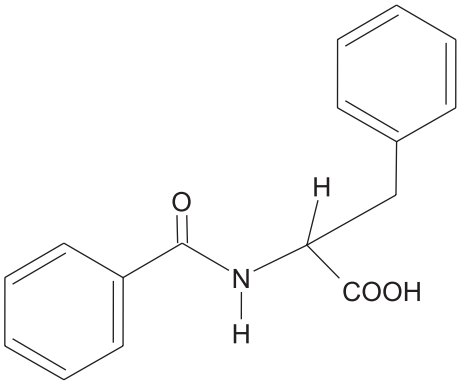

Fig. 1. Structure of *N*-benzoyl-D-phenylalanine.

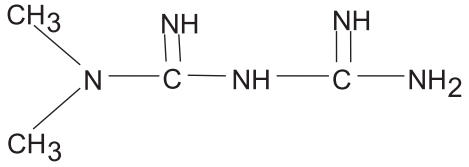

Fig. 2. Structure of metformin.

diabetes. Metformin reduces fasting plasma glucose concentration by reducing rate of hepatic glucose production via gluconeogenesis and glycogenolysis. Metformin improves glycemic control as monotherapy in combination with other oral antidiabetic agents, such as sulfonylureas and thiazolidine diones [6].

In the present study, we have evaluated the efficacy and safety of a combination of NBDP and metformin compared with either drug monotherapy in neonatal streptozotocin (nSTZ) diabetic rats. The study was designed to establish whether the combination of NBDP and metformin, with complementary pharmacological actions, would result in improved blood glucose concentration and the activities of hepatic key enzymes compared with control rats.

2. Materials and methods

2.1. Drugs and chemicals

All the biochemicals and chemicals used in this experiment were from Sigma, St. Louis, MO. The chemicals were of analytical grade.

2.2. Synthesis of *N*-benzoyl-D-phenylalanine

N-benzoyl-D-phenylalanine was synthesised in our laboratory [7], and the structure was confirmed by IR and ^{13}C NMR spectral studies.

2.3. Animals

Healthy albino Wistar strain rats were kept for breeding in the Central Animal House, Rajah Muthiah Medical College, Annamalai University, were used in this study. The rats were fed on pellet diet (Hindustan Lever, Mumbai, India) and water ad libitum. The animals were maintained in accordance with the guidelines of the National Institute of