ORIGINAL ARTICLE

Scintigraphic evaluation of small intestinal transit in the streptozotocin induced diabetic rats

Durmus-Altun G[1], Vatansever U[2] , Arzu Vardar S[3], Altaner S[4], Dirlik B[1]

[1] Department of Nuclear Medicine, Trakya University, Medical Faculty,
[2] Department of Pediatrics, Trakya University, Medical Faculty,
[3] Department of Physiology, Trakya University, Medical Faculty,
[4] Department of Pathology, Trakya University, Medical Faculty, Edirne, Turkey.

Abstract:
Aim: Small intestine (SI) transit in the streptozotocin (STZ) induced diabetic rats were examined by using 99mTc-mebrofenin scintigraphy.
Materials and methods: Wistar albino rats (mean body weight: 220±12 g) were studied for both control (n=10) and diabetes mellitus (DM) (n=10) groups. Diabetes was induced by a single intraperitoneal injection of streptozotocin (50 mg kg(-1) body weight. SI transit time was assessed by measuring arrival times of 99mTc-mebrofenin from duodenum to caecum.
Results: The mean transit time of 99mTc- mebrofenin was 67.8±11 min in control group. The mean transit time of SI was prolonged in STZ induced diabetic animals with (111.9±12.5, p=0.01). There was significant correlation between small intestinal transit time and blood glucose level (r: 0.73, p=0.01).
Conclusion: We observed that SI transit was prolonged in diabetic animals using 99mTc- mebrofenin, and additionally this technique is a readily available method for the detection of transit abnormalities in animal experiment. Hippokratia 2011; 15 (3): 262-264

Key words: Small intestine, scintigraphy, 99mTc- mebrofenin, diabetes mellitus, streptozotocin

Corresponding author: Gulay Durmus Altun, Trakya University, Faculty of Medicine, Department of Nuclear Medicine, 22030 Edirne, Turkey, Tel: +90-284-2357641, +90-284-2355465, fax: +90-284-2353305, e-mail: gdurmusaltun@trakya.edu.tr or gulaydurmusaltun@gmail.com

Introduction

The techniques based on scintigraphy are suitable for medical investigation of gastrointestinal motility in physiology as well as in the investigation of specific problems encountered in pathophysiological conditions. Delayed gastric emptying and upper gastrointestinal symptoms were observed in about half of the diabetic patients by using a dual isotope test that measures emptying of solid and liquid meal components simultaneously[1]. The investigation of the motility of the small intestine (SI) is more complex because of its anatomical localization. Delayed gastric motility may interfere with evaluation of SI transient time when classic scintigraphic approach was used to evaluating whole gastrointestinal tract[1,2].

Streptozotocin (STZ) has been widely used to induce experimental diabetes in rats since it causes alterations similar to those found in diabetic humans[3,4]. In the animal studies, upper gastrointestinal transit was often measured with the nonabsorbable substants such as charcoal, Indigo Carmine containing diet, Evans methylene blue solution, FITC-dextran[5-8]. Despite the need for surgical intervention, and invasive, these methods are currently used.

A non-invasive biliary scintigraphic technique measuring the transit of contents through the SI, has been developed for human study[9]. The method has found use for quantitative studies of SI transit, in animals, especially rats, which have no gallbladder[10,11]. According to the data about diabetes mellitus (DM) and previous scintigraphic techniques, 99mTc-mebrofenin scintigraphy was examined for evaluating SI transit in the STZ induced diabetic rats.

Material and Methods

20 male Wistar albino rats, averaging 16 weeks old were utilised. Their weight ranged between 190 and 230 g with a mean of 220±12 g. The rats housed at the Animal Care and Research Unit was used for this study. Food and tap water were available *ad libitum*. In the windowless animal quarter automatic temperature (21 ± 1°C) and lighting controls (12 h light/12 h dark cycle) were performed. Relative humidity ranged from 55% to 60%. All animal experiments were conducted adhering to the Guide for the Care and Use of Laboratory Animals of U.S. National Institute of Health guidelines and were approved by Trakya University School of Medicine Animal Care and Use Committee.

The animals were divided into two groups. In the first experiment, one group of rats were served as control (n=10). In the second experiment, diabetes was induced by a single dose of STZ, (50 mg.kg–1 body weight, i.p.)[12]. STZ was dissolved in 0.1 mol/L citric buffer (pH 4.2). Fourty-eight hours after administration of STZ, the blood glucose concentration was determined in tail-nick blood samples by a Glucometer SureStep Plus (LifeScan, Johnson & Johnson India Ltd Company, US). Only ani-

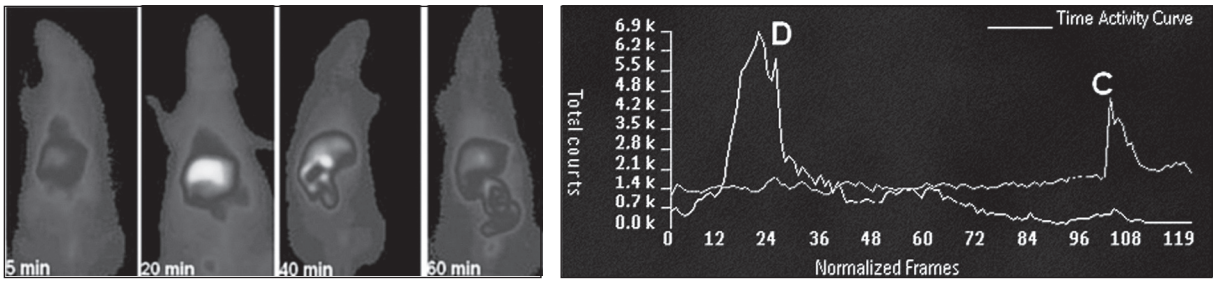

Figure 1: a) 99mTc-mebrofenin scintigraphy images of control animal, including hepatic and intestinal migration phases (5, 20, 40 and 60 min spot images). **b)** Time-activity curves ROIs over the duodenum (D) and the caecum (C). The respective time points for the appearance of the radioactivity in the separate ROIs were taken as the start and end-point of transit.

mals with blood glucose levels >300 mg/dl were utilized in the study. 48 hours after STZ injection, the diabetic rats were enrolled DM groups (n=10). Two weeks after STZ injection, 99mTc -mebrofenin (CIS bio international, Cedex, France) SI transit scintigraphy was performed on fasted state.

99mTc- mebrofenin small intestinal transit scintigraphy:

Imaging was carried out using a gamma camera (Orbiter; Siemens Corp., Iselin, NJ) equipped with a low-energy general-purpose collimator. The images were recorded on a 128x128 matrix. Immediately after administration of 1mCi (37 MBq) 99mTc-mebrofenin, dynamic 1-min image acquisitions were begun. Region of interests were outlined over the proximal duodenum and the caecum, and the count rate was evaluated against background activity. Small bowel transit time was determined from the difference in the arrival times of the radiopharmaceutical in the proximal duodenum and caecum (Figure 1.a-b).

Subjects were euthanized one day after imaging; histopathologic analysis of intestines was then performed. In macroscopically, the small and large bowels were seen regular. The tissues were fixed in 10% buffered formalin for 24 hours and embedded in paraffin. Sections 4 μm in thickness were prepareted and stained with hematoxylin-eosin. Sections were examined by light microscopy.

Results

In the control group, the mean transit time of 99mTc-mebrofenin was 67.8±11 min. The mean transit time of SI was prolonged in animals with STZ induced DM (111.9±12.5 min, p=0.01) (Figure 2).

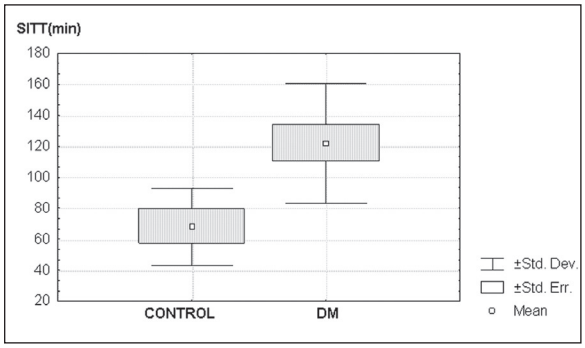

Figure 2: The mean transit time of SI (SITT) was prolonged in STZ induced diabetic animals.

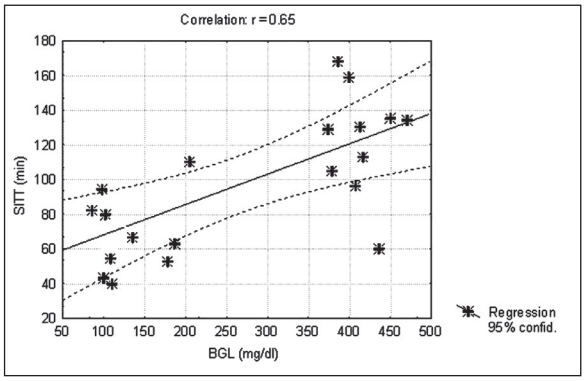

Figure 3: There was good correlation between small intestinal transit time (SITT) and blood glucose level (BGL) (r: 0.65, p=0.01).

The means of blood glucose level was significantly differ in both groups (99±8 mg/dl vs. 350±35 mg/dl, p: 0.0001). There was good correlation between SI transit time and blood glucose level (r: 0.65, p=0.01) (Figure 3).

In small intestinal sections of the control and diabetic groups, mucosa had columnar and regular epithelia of the surface and of the glands. The thicknesses of the mucosa in diabetic and control groups were equal. We found that the diameters of the vessels in the submucosa in the diabetic group were thicker than the ones in the control group. We saw that serosa was regular both in diabetic and control groups. The diameter of the small vessel wall in the mesentery in diabetic group was thicker than the diameter of the small vessel wall in the mesentery in control group. In addition, endothelia of the small vessel were irregular.

Discussion

The main findings of our study were first, mean transit time of SI was prolonged in animals with STZ induced DM; second, it was correlated with glucose level.

Diabetes mellitus is defined as a chronic disease characterized by metabolic disorders with fasting hyperglycaemia. The previous animal experiments show that diabetes leads to changes in the morphology and function of the SI [13-15]. Zhao et al.[16] reported that the viscoelastic behavior of intestinal wall changed during the development of diabetes in rat model with STZ induced DM. The

viscoelastic behavior is important for the motor function of intestine. In our study, small intestine motility was slower in DM groups than the control.

A physiological pathway to the intact gastrointestinal tract runs through the biliary tree technetium-99m analogs of iminodiacetic acid, including mebrofenin, and other radiopharmaceuticals developed for hepatobiliary scintigraphy are rapidly excreted in the bile after intravenous administration. In addition, no absorption, decomposition or enterohepatic circulation of these compounds has been found [10,11,17,18]. Scintigraphic evaluation of small intestine by using 99mTc-mebrofenin is neither invasive nor affected gastric empty or bacterial metabolisim, and these are considered the standard [9-11]. Recent reports indicate that acute hyperglycemia causes reversible impairment of motility in various regions of the gastrointestinal tract [19-21]. In the presented study, SI transit time is good correlated with blood glucose level. Lingenfelser et al.[22] reported that similar observations, which are the blood glucose concentration plays a role in the regulation of gastrointestinal motility and sensation.

The small intestine plays an important role in the digestion and absorption of many nutrients. There are morphological changes the SI morphology. Sanchez et al.[14] reported that the length of the entire SI was significantly increased in diabetic animals compared with control animals. The other study to evaluating SI morphology suggested that jejunal mucosal height in the free-feeding diabetic rats was significantly longer than that in the control rats [23]. In our study, contrast to Noda et al.[23], mucosal height in jejunum was not changed, but muscle thickness is increased in DM groups. We observed also thickness of submucosal vessels of SI in DM groups.

We concluded that 99mTc - mebrofenin small bowel transit scintigraphy is a readily available method for the detection of transit abnormalities in animal experiment. The prolonged transit time was determined in animal with DM, and its well correlated blood glucose level. Theoretically, transit measurements would be the most useful investigation because disordered propulsion is the clinically most important consequence of functional intestinal disorders. Therefore, 99mTc-mebrofenin intestinal scintigraphy for transit study might be used as a primary step in the work-up of a suspected gastrointestinal motor disorder.

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