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ORIGINAL ARTICLE

EFFECT OF ACARBOSE ON *IN VITRO* INTESTINAL ABSORPTION OF MONOSACCHARIDES IN DIABETIC RATS

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Background – Acarbose is known to lower blood glucose concentration by functioning as an á-glucosidase inhibitor in the intestine. It is also suggested that acarbose may directly arrest the intestinal absorption of hexoses. The purpose of the present study was to further elucidate the normal intestinal absorption of hexoses and the effect of acarbose on the rate of intestinal absorption of monosaccharides in normal and streptozocin-induced diabetic rats.

Methods – Segments of small intestine, as everted sacs, from normal and diabetic rats were incubated in solutions of various concentrations of monosaccharides, with and without acarbose, at 37° C for 90 min and the sugar concentration was measured before and after incubation. Student's *t*-test with $p < 0.05$ was used to compare the mean ± standard error of the mean values for intestinal absorption rates of various sugars in different groups of rats.

Results – The optimum effective dose of most sugars for intestinal absorption was 100 mg/dL and the best inhibitory dose of acarbose was 1 mg/mL. The rate of intestinal absorption of glucose and galactose in the presence of acarbose was significantly reduced in both normal and diabetic rats, while fructose and sucrose absorption was not affected significantly by acarbose in diabetic rats. Mannose absorption was not affected significantly by acarbose.

Conclusion – Acarbose directly arrested the intestinal absorption of most hexoses at different rates, probably due to different mechanisms involved in the intestinal absorption of monosaccharides.

Keywords acarbose diabetic rats intestinal absorption streptozocin

Introduction

There are a number of reports on the blood glucose lowering effect of acarbose.[1–4] Acarbose is a microbial pseudotetrasaccharide widely used in the treatment of both insulin-dependent and non-insulin-dependent diabetes mellitus.[5–7] The therapeutic action of this hypoglycemic drug is generally attributed to its á-glucosidase inhibitory effects on carbohydrate digestion in both man and rats.[8–11] This reduces the digestion of oligosaccharides in the proximal half of the small intestine, delaying, by prolonging the absorption of monosaccharides after the meal. Thus, in patients with diabetes, acarbose decreases postprandial hyperglycemia. Acarbose is also reported to cause a reduction in hexose absorption in animals.[12]

The mechanism and rate of intestinal absorption are not the same for all monosaccharides. Glucose and galactose are believed to be primarily absorbed by an active transport system,[8] while facilitated transport is known to be involved in fructose absorption.[13] The present study was undertaken to further elucidate the normal intestinal absorption of hexoses and the effect of acarbose on the rate of intestinal absorption of monosaccharides in normal and streptozocin-induced diabetic rats. A range of increasing concentrations of sugars and acarbose was used to determine the best dose for sugar absorption and acarbose inhibition.

Materials and Methods

Male Sprague-Dawley rats weighing 250 to 300

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g were selected and maintained on a stock pellet diet. They were allowed free access to food and water at room temperature with a light/dark phase of 12/12 hours. The rats were housed in the animal house of the Medical School, Shiraz University of Medical Sciences, Shiraz, Iran. Animals were entered in one of two groups, a normal (control) group consisting of 40 rats and a normal or streptozocin-induced diabetic group of 120 rats receiving acarbose (Table). Rats were made diabetic by injecting one dose of streptozocin solution (40 mg/kg body weight) in the tail vein.[14] After one week, animals with blood glucose of 300 mg/dL or more were considered diabetic.

Everted sac technique

Rats were deprived of food, but not water, for 24 hours before being sacrificed by stunning and decapitation between 9 and 10 AM. The procedure described by Wilson and Wiseman[15] for everted sac technique was followed. Pieces of intestine 15 to 20 cm long, were excised from the small intestine distal to the duodenojejunal flexure. The procedure was carried out on a piece of glass placed on a 37ºC water bath immediately after the animals were sacrificed. One end of the intestine was ligated to a glass rod end with a piece of sewing thread and the rod was pushed in, to evert the intestine. Both ends of the everted intestine were ligated with thread before they were placed in an incubation flask. Incubation solution was 50 mL of Kreb's bicarbonate containing the sugar under study. Acarbose concentration was 1 mg/mL. Incubation was performed for 90 min in a shaking water bath at 37ºC with carbagen (95% O_2 + 5% CO_2) gas flow through the incubation flasks. The hexose concentration was measured before and after incubation. After incubation, everted sacs were dried in a Petri dish at 80ºC in an oven and weighed.

Intestinal absorption and sugar concentration

Sugar concentrations of 0.25, 0.50, 1.00, 1.50, 2.00 and 2.50 g/L were used in the incubation mixture. Gray and Olefsky used sugar concentrations of up to 0.2 g/L.[16] Glucose concentrations were measured using an enzymatic method with Zist Shimi kits (Zist Shimi Company, Iran). Galactose, fructose and mannose concentrations were measured using the method of Somogyi.[17] Sucrose was acid hydrolyzed and quantitated using the Somogyi method.

Best effective dose of acarbose

Acarbose concentrations of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/mL in normal saline incubation buffer, containing a sugar and the everted sac of intestinal segment, were used to determine the acarbose concentration that caused the greatest reduction in intestinal absorption of sugars during the incubation period. An acarbose concentration of 1 mg/mL was used by Hirsh et al.[18] The sugar concentration of the incubation mixture was measured before and after 90 min of incubation. The sugars used were glucose, galactose, fructose, mannose and sucrose.

Student's *t*-test with $p < 0.05$ was used to compare the mean ± standard error of the mean (SEM) values for the rates of intestinal absorption of various sugars.

This research project carried out at Shiraz University of Medical Sciences was approved by the Ethics Committee.

Results

Figure 1 shows that in normal rats, the rate of intestinal absorption depended on the sugar concentration. Glucose, galactose and mannose absorption increased with concentration up to 100 mg/dL, with slower increases at higher concentrations, reaching a plateau at 150 mg/dL. However, the rate of fructose absorption increased up to 2.50 mg/dL. The effect of acarbose

Table. Rate of sugar absorption in the presence of acarbose (1 mg/mL) in normal and diabetic rats compared with corresponding control group.

	Sucrose (n = 8)	Fructose (n = 8)	Mannose (n = 8)	Galactose (n = 8)	Glucose (n = 8)
Control (normal)	10.6 ± 0.9	12.0 ±1.3	16.8 ± 2.1	32.6 ± 0.9	37.1 ± 0.7
†Normal + acarbose	9.0 ± 0.5	10.8 ± 1.1	15.3 ± 1.4	24.0 ± 0.78	24.2 ± 2.2*
Control (diabetic)	21.1 ± 0.8	17.6 ± 3.5	12.5 ± 1.4	18.1 ± 2.3	20.0 ± 0.5
‡Diabetic + acarbose	14.1 ± 1.4*	12.1 ± 0.8*	11.1 ± 1.0	10.9 ± 1.5*	13.5 ± 2.0*

*$p < 0.05$ using Student's *t*-test. † = intestinal sac from control rats incubated with 50 mL of sugar (100 mg/dL) and acarbose (1 mg/dL) at 37 C for 90 min. ‡ = intestinal sac from diabetic rats incubated with 50 mL of sugar (100 mg/dL) at 37 C for 90 min. Values for intestinal absorption for each group are mean ± SEM.

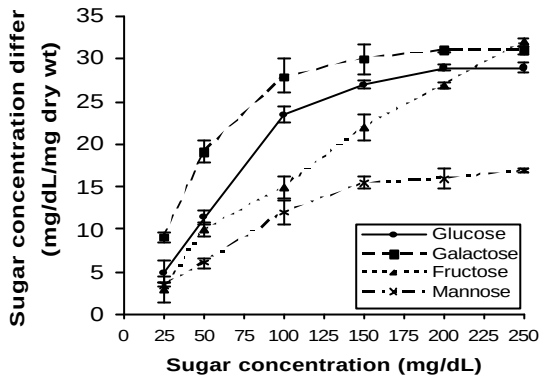

Figure 1. Effect of concentration of monosaccharides on *in vitro* intestinal absorption in normal rats. Values are mean ± SEM of five experiments at each point.

concentration on monosaccharide absorption by intestinal segments from normal rats after 90 min incubation at 37ºC is illustrated in Figure 2. Similar data are shown for diabetic rats in Figure 3. Although the patterns of reduction in intestinal absorption by acarbose for normal and diabetic rats were not similar, an acarbose concentration of 1 mg/mL caused the greatest reduction in the intestinal absorption of all four sugars.

Effect of acarbose on intestinal absorption of sugars in normal and diabetic rats

Table 1 demonstrates the effect of acarbose on the rate of intestinal absorption of glucose, galactose, fructose, mannose and sucrose in the intestinal segments of normal and diabetic rats. Each sugar was used at a concentration of 100 mg/dL and acarbose at a concentration of 1 mg/mL. Acarbose caused a significant reduction in intestinal absorption of glucose and galactose in both normal and diabetic rats. However, intestinal absorption of fructose was only reduced in diabetic rats in the presence of acarbose. Intestinal absorption of mannose was not significantly reduced by acarbose in either normal or diabetic rats. Intestinal absorption of sucrose in the presence of acarbose was significantly decreased in the intestinal segments of diabetic rats but not of normal rats.

Discussion

In the present study, intestinal absorption was investigated with a wide range of hexose concentrations. A range of acarbose concentrations was also used and the dose that caused the maximum inhibition in intestinal absorption of monosaccharides was adopted. The difference in the shapes of the curves for the intestinal absorption of hexoses (Figure 1) is probably explained by the presence of different absorption mechanisms for monosaccharides. The decrease in intestinal absorption of glucose brought about by acarbose in normal and diabetic rats, and of fructose in diabetic rats, found in the present study is in line with the finding of other workers.[10] In our study, acarbose did not cause a reduction in the intestinal absorption of mannose in normal or diabetic rats, which is again probably related to the different mechanisms of absorption. However, no published data are available on mannose intestinal absorption. A previous report shows that intestinal absorption of sucrose is hampered in the presence of acarbose,[16] but we only found this inhibition in diabetic rats, and not in normal rats. This contrast may be due to the different sucrose concentrations used in the two studies.

Although acarbose is an intestinal á-glucosidase inhibitor and affects carbohydrate intestinal absorption indirectly,[8–11] our study and others have shown that it can also have a direct effect on the intestinal absorption of hexoses.[5, 19] Acarbose inhibits the entrance of glucose and galactose into intestinal mucosal cells. This function of acarbose resembles the effect of compounds such as phloridzin,[13] as acarbose inactivates sodium-

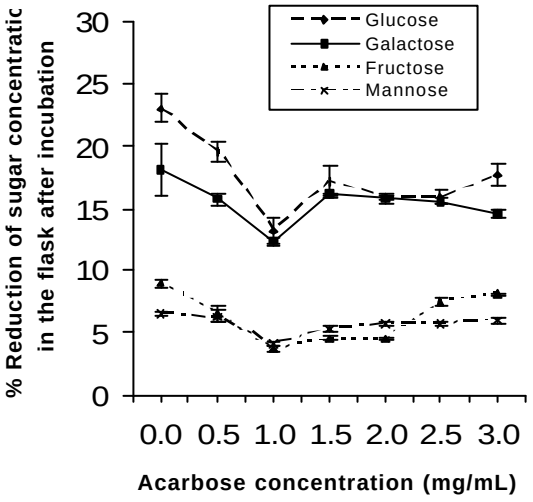

Figure 2. Effect of acarbose concentration on the rate of *in vitro* intestinal absorption of monosaccharides (100 mg/dL) after 90 min incubation in normal rats. Values are mean ± SEM of five experiments at each point.

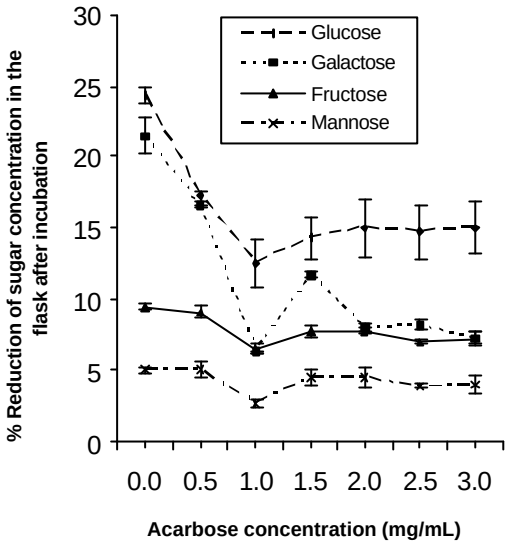

Figure 3. Effect of acarbose concentration on the rate of *in vitro* intestinal absorption of monosaccharides (100 mg/dL) after 90 min incubation in diabetic rats. Values are mean ± SEM of five experiments at each point.

glucose cotransporter (SGLT-1), which transports glucose and galactose into the intestinal mucosal cells. Acarbose activates a secondary messenger in the intestine (RS1), which inactivates SGLT-1 by phosphorylation, leading to the inhibition of absorption of hexoses such as glucose and galactose.[13]

Acarbose also inhibits intestinal absorption of fructose. Intestinal absorption of fructose is dependent, to some extent, on a transport protein called GLUT-5. [8, 13] Acarbose inhibits GLUT-5 protein, but since fructose is also transported into intestinal mucosal cells by the GLUT-2 system,[13] which is resistant to the acarbose inhibitory effect, the overall inhibitory effect of acarbose on intestinal absorption of fructose is not severe.[8] The hypoglycemic effect of acarbose along with the lowering effect on blood lipids has allowed acarbose to be considered as an important oral drug for diabetic patients.

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