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Ameliorative effects of telmisartan in diabetic rats with indomethacin-induced gastric ulceration

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ABSTRACT

The protective effects of telmisartan, the angiotensin II-receptor antagonist, were investigated in rats with type 2 diabetes mellitus exposed to acute gastric ulceration. Following successful induction of diabetes, telmisartan treatment (1 mg/kg/day, orally) was started and continued for 8 weeks, after which acute gastric ulceration was induced by indomethacin. Telmisartan significantly attenuated the hyperglycemia and hypoinsulinemia in diabetic rats. Also, telmisartan significantly reduced the elevations of total gastric acid output, pepsin activity, gastric ulcer index and gastric mucosal tumor necrosis factor-α, nitric oxide, malondialdehyde and caspase-3 activity, and restored the depleted antioxidant defenses (reduced glutathione level, and superoxide dismutase and catalase activities) caused by indomethacin administration in diabetic rats. Histopathological gastric tissue damage induced by indomethacin in diabetic rats was ameliorated by telmisartan treatment. Immunohistochemical analysis revealed that telmisartan markedly attenuated the reduction in insulin content of pancreatic islet β-cells, and prevented the indomethacin-induced overexpression of inducible nitric oxide synthase and nuclear factor-κB in gastric mucosa of diabetic rats. It was concluded that telmisartan represents a potential therapeutic option to reduce the risk of gastric ulceration induced by nonsteroidal anti-inflammatory drugs in type 2 diabetic patients.

1. Introduction

Acute gastric inflammation and ulcer disease occur with high prevalence in patients with type 2 diabetes mellitus, and are strongly correlated with the duration of diabetes. Also, peptic ulcers related to diabetes mellitus are more severe with slow healing rate and often associated with complications such as gastrointestinal bleeding (Pietzsch et al., 2002; Boehme et al., 2007). Previous studies demonstrated that streptozotocin-diabetic animals have increased vulnerability of the gastric mucosa to various ulcerogens such as ischemia/reperfusion, stress, ethanol and nonsteroidal anti-inflammatory drugs (Goldin et al., 1997; Korolkiewicz et al., 1999; Tashima et al., 2000; Brzozowska et al., 2004). The mechanism underlying the increased susceptibility of gastric mucosa in diabetic animals to damage is multifactorial and includes alteration of gastric motility (Perdichizzi et al., 1996), impairment of duodenal bicarbonate secretion (Takehara et al., 1997), attenuation of angiogenesis and dysfunction of capsaicin-sensitive neurons involved in the protection of gastric mucosa (Tashima et al., 1998). However, increased production of reactive oxygen species and proinflammatory cytokines seems to play a major role (Brzozowska et al., 2004; Iwai et al., 2004; Hung, 2005; Mohan Kumar et al., 2006).

Nonsteroidal anti-inflammatory drugs are one of the most commonly used medicines throughout the world. However, gastric ulceration remains a major problem limiting their clinical usefulness (Hawkins and Hanks, 2000). Indomethacin, a representative of this group of drugs, is frequently used and clinically relevant experimental model for induction of acute gastric ulcer. Indomethacin causes gastric ulcers through various processes, including generation of reactive oxygen species, initiation of lipid peroxidation, infiltration of leukocytes, induction of apoptosis, and inhibition of prostaglandin synthesis (Bech et al., 2000). Decreased prostaglandin level impairs almost all aspects of gastroprotection and increases acid secretion which in turn, aggravates the ulcer (Miller, 1983).

Angiotensin II, the central product of the renin–angiotensin system, induces oxidative stress and inflammation (Welch, 2008), and constricts the gastric vasculature (Heinemann et al., 1999) by activating the angiotensin II type 1 (AT1) receptor. Telmisartan is a highly selective AT1-receptor antagonist approved for treatment of hypertension. On the other hand, telmisartan acts as a partial agonist on the nuclear peroxisome proliferator-activated receptor-γ that has been reported to exert anti-oxidative and anti-inflammatory effects

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(Benson et al., 2004). Recent studies demonstrated that telmisartan provided a significant antidiabetic effect and ameliorated hyperglycemia and hypoinsulinemia in streptozotocin- and spontaneously diabetic rats (Goyal et al., 2008; Hasegawa et al., 2009). Also, candisartan and telmisartan significantly attenuated gastric mucosal lesions induced by cold-restraint stress and indomethacin in non-diabetic rats (Pavel et al., 2008; Morsy et al., 2009). Therefore, telmisartan has the potential to protect against gastric ulcer associated with diabetes mellitus, and to the best of our knowledge, this is the first study to investigate the gastroprotective effect of AT1-receptor blockers in diabetic animals.

This was encouraging to conduct the present study in order to evaluate the protective effects of the AT1-receptor antagonist, telmisartan in rats with type 2 diabetes mellitus exposed to indomethacin-induced gastric injury. Also, the possible mechanisms underlying these protective effects were investigated.

2. Materials and methods

2.1. Drugs

Telmisartan, indomethacin, streptozotocin and nicotinamide were obtained from Sigma Chemical Company, USA. Telmisartan was prepared in 0.5% aqueous solution of carboxymethyl cellulose sodium, indomethacin was suspended in 1% aqueous solution of Tween 80, streptozotocin was dissolved in 0.1 M citrate buffer (pH 4.5), and nicotinamide was dissolved in normal saline. All the used drugs were always freshly prepared.

2.2. Animals

Male Sprague–Dawley rats, weighing 180–200 g were obtained from the Animal House, College of Medicine, Al-Ahsa, King Faisal University. The animals were kept at standard housing facilities (24 ± 1 °C, 45 ± 5% humidity and 12 h light/dark cycle). They were supplied with standard laboratory chow and water ad libitum, and left to acclimatize for 1 week before the experiments. The experimental protocol was approved by the Local Animal Care Committee and the experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals.

2.3. Induction of type 2 diabetes mellitus

Type 2 diabetes was induced in overnight fasted rats by a single i.p. injection of streptozotocin (60 mg/kg), 15 min following an i.p. administration of nicotinamide at a dose of 110 mg/kg (Masiello et al., 1998). The induction of diabetes was checked 72 h and on the seventh day after streptozotocin administration by measuring the random blood glucose concentration using blood glucose meter (Accu-Chek, Roche Diagnostics, Mannheim, Germany). Rats displaying blood glucose above 250 mg/dl were considered diabetics and were included in the study. The control rats were injected with the vehicle of streptozotocin (0.1 M citrate buffer, pH 4.5).

2.4. Experimental design

The rats were randomly divided into five equal groups ($n = 6$, each). The first group was the non-diabetic rats, without induction of gastric ulceration, and served as control. The second group was the non-diabetic rats with gastric ulcer. The animals of the third and fourth groups were rendered diabetics and received oral 0.5% aqueous solution of carboxymethyl cellulose sodium (vehicle of telmisartan) or telmisartan at a dose of 1 mg/kg/day (Kobayashi et al., 2008), respectively, for 8 weeks following successful induction of diabetes, and then subjected to gastric ulcer induction. The fifth group was non-diabetic non-ulcer rats and received telmisartan orally for 8 weeks.

Table 1
Effects of telmisartan (TEL) treatment (1 mg/kg/day, orally for 8 weeks) on fasting blood glucose and serum insulin levels of diabetic rats before induction of gastric ulceration.

	Control	Diabetic	Diabetic + TEL	TEL
Blood glucose (mg/dl)	99.50 ± 8.91	350.73 ± 29.42[a]	228.31 ± 21.90[a, b]	87.92 ± 6.94
Serum insulin (ng/ml)	2.14 ± 0.18	0.41 ± 0.04[a]	1.25 ± 0.10[a, b]	1.96 ± 0.14

All the values are expressed as mean ± S.E.M., $n = 6$ in each group.
[a] $P<0.05$ versus control group.
[b] $P<0.05$ versus diabetic group.

2.5. Measurement of fasting blood glucose and serum insulin levels

At the end of 8 weeks and before induction of gastric ulceration, blood samples were collected from the retro-orbital plexus of each animal after 12 h-fasting. A drop of blood was used to measure fasting blood glucose by the blood glucose meter. The blood samples were then centrifuged for 10 min at 2430 *g* to obtain clear sera which were stored at −80 °C. Subsequently, serum insulin level was determined by enzyme-linked immunosorbent assay (ELISA) using rat insulin immunoassay kit according to the recommendations of the manufacturer (Cayman Chemical Company, USA).

2.6. Induction of gastric ulceration and assessment of gastric mucosal lesions

After 8 weeks of telmisartan treatment, gastric ulceration was induced in the respective animals. The rats were fasted for 24 h prior to the experiment in mesh-bottomed cages to minimize coprophagia. They allowed free access to water except for the last hour before the experiment. Acute gastric injury was induced by indomethacin in a single oral dose of 40 mg/kg (Rainsford and Whitehouse, 1980). The control rats received the vehicle of indomethacin (1% aqueous solution of Tween 80), orally. All experiments were performed during the same time of the day to avoid diurnal variations of the putative regulators of gastric functions.

The rats were sacrificed 4 h following indomethacin administration by an overdose of ether. Each stomach was removed and opened along the greater curvature. The stomachs were washed with ice-cold

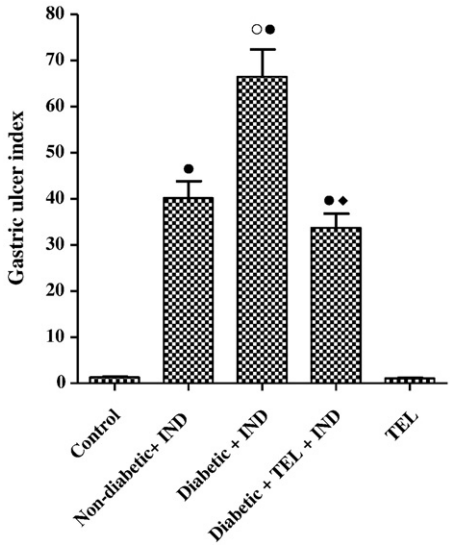

Fig. 1. Effect of telmisartan (TEL) treatment (1 mg/kg/day, orally for 8 weeks) on gastric ulcer index of diabetic rats exposed to indomethacin (IND)-induced gastric ulceration. All values are expressed as mean ± S.E.M., ●$P<0.05$ versus control group, ○$P<0.05$ versus non-diabetic + IND group, ♦$P<0.05$ versus diabetic + IND group.