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Original Article

Acute high blood glucose level attenuates the formation of gastric ulcers in male Wistar rats

Taofeek Olumayowa Usman*[1], Lawrence Aderemi Olatunji[1,2] and Abdul Rasak Akinola Alada[3]

[1]Department of Physiology, College of Health Sciences, University of Ilorin, Ilorin, Nigeria. [2] Department of Pharmacology, Kyungpook National University, School of Medicine, Daegu, Republic of Korea. [3] Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria

*Corresponding author: Taofeek O. Usman. E-mail: mrtaophyc@yahoo.ca; Tel.: +2348068449324

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ABSTRACT: Studies have shown that hyperglycemic state induced by intravenous glucagon or glucose infusion may lead to inhibition of gastric acid secretion through the inhibition of gastric vagal activity. Conversely, hypoglycemic state has been reported to cause an increase in gastric acid secretion by increasing vagal stimulation. The gastrointestinal side effects of non-steroidal anti-inflammatory drugs (NSAIDs), especially on the stomach, are one of the more serious complications in individuals taking these drugs. The aim of this study was therefore to study the effect of a high or low blood glucose level on gastric ulcers formation induced by NSAID, indomethacin. Thirty male Wistar rats weighing between 200 and 250g were randomly divided into three groups of 10 rats each, receiving intravenous infusion of normal saline (control; group 1), glucose (hyperglycemic; group 2) or insulin (hypoglycemic; group 3) followed by oral administration of indomethacin (25mg/kg). The animals were sacrificed 4 hours after indomethacin administration and the stomach was removed to estimate the ulcer score (US). The ulcer score was significantly reduced in the hyperglycemic group compared to the control group, while no significant change was observed in the hypoglycemic group. In conclusion, these results indicate that high blood glucose levels attenuate the formation of NSAID-induced gastric ulcers while insulin-induced hypoglycemic state may not affect the formation of NSAID-induced gastric ulcers.

KEYWORDS: Hyperglycemia, hypoglycemia, gastric ulcer, NSAID, indomethacin, rats.

INTRODUCTION

The blood glucose level is the amount of glucose present in the blood of a human or animal. The human body naturally tightly regulates blood glucose levels as a part of metabolic homeostasis and it is usually kept between a range of 64.8 and 104.4 mg/dl (Henry, 2001). Hyperglycemia, or high blood glucose, is a condition in which an excessive amount of glucose circulates in the blood plasma. This is generally a glucose level higher than 180 mg/dl. Temporary hyperglycemia is often benign and asymptomatic. Blood glucose levels can rise well above normal for significant periods without producing any permanent effects or symptoms (Guigliano *et al*., 1997). Hypoglycemia is the medical term for a state produced by a lower than normal level of blood glucose (Cryer, 1999). It can produce a variety of symptoms and effects but the principal problems arise from an inadequate supply of glucose to the brain, resulting in impairment of function (neuroglycopenia). Effects can range from mild dysphoria to more serious issues such as seizures, unconsciousness, and (rarely) permanent brain damage or death (Cryer *et al*., 2009).

Gastric ulcer, also known as peptic ulcer, is a localized area of erosion in the stomach lining, resulting in abdominal pain, possible bleeding, and other gastrointestinal symptoms (Ramakrishnan and Salinas, 2007). It is defined as mucosal erosions equal to or greater than 0.5 cm (Malagelada *et al*., 2007). As many as 70–90% of ulcers are associated with *Helicobacter pylori*, a spiral-shaped bacterium that lives in the acidic environment of the stomach (Chey and Wong, 2007). There is normally a balance between the amount of

acid that you make and the mucus defense barrier. An ulcer may develop if there is an alteration in this balance allowing the acid to damage the lining of the stomach or duodenum.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are drugs with analgesic and anti-pyretic effects which have anti-inflammatory effects at high doses (Stuart and Warden, 2010). The most prominent members of this group of drugs are aspirin, ibuprofen and indomethacin and they are all available over the counter (Stuart and Warden, 2010). It has been demonstrated that indomethacin and aspirin inhibit prostaglandin (PG) biosynthesis (Vane; 1971). A reduction in the biosynthesis of PG through inhibition of cyclooxygenase (COX) is the pharmacological background to both the anti-inflammatory action (Vane; 1971) and the harmful side effects of indomethacin, as well as other NSAIDs (Hawkey; 2000). Since indomethacin inhibits both COX-1 and COX-2, it inhibits the production of prostaglandins in the stomach and intestines, which maintain the mucous lining of the gastrointestinal tract (Hawkey; 2000; Lanza *et al*., 2009). Indomethacin, therefore, like other non-selective COX inhibitors can cause peptic ulcers. The gastrointestinal side effects of NSAIDs, especially in the stomach, are one of the more serious complications in persons taking these drugs (Hawkey, 2000; Lanza *et al*., 2009).

Studies have shown that hyperglycemia, when induced by intravenous glucagon or glucose infusion leads to inhibition of gastric acid secretion. Other studies reported that intravenous glucose infusion inhibits basal, pentagastrin-, sham feeding-, and meal-stimulated gastric acid output (Lam *et al*; 1993; Lam *et al*; 1994; Lam *et al*; 1995). It was also reported that an acute elevation in blood glucose inhibits intravenous amino acid induced gastric acid secretion. It has been discovered that a high blood glucose level, acting on a glucoreceptor area in the ventromedial hypothalamus, results in vagal inhibition and subsequently, inhibition of gastric acid secretion (Doong and Yang, 2003).

Several studies have investigated the effect of reduced blood glucose level, most especially insulin-induced, on gastric acid secretion and it was first discovered as far back as 1927 that hypoglycemia causes an increase in gastric acid secretion. It was discovered that intravenous insulin was a hypoglycemic stimulus of gastric acid secretion (Qvigstad *et al*; 1999). It is mediated by the vagi and can be ended by the administration of glucose. The effect of the vagus nerve has been shown to be through direct stimulation of the parietal cells of the stomach and this response is abolished following vagotomy.

It appears that studies on the specific effect of a hyperglycemic or hypoglycemic blood glucose level on gastric ulcer is lacking. The aim of this study was therefore to determine the effect a high or lowered blood glucose level would have on gastric ulcers induced by a known NSAID (Indomethacin).

MATERIALS AND METHODS

Animals and Grouping

The study was carried out in the animal house of the College of Health Sciences, university of Ilorin. Thirty male Wistar rats weighing between 200 and 250g were used in this study. The animals were fed on standard rat chow and allowed free access to drinking water except on days when they had to be fasted before a procedure. They were kept in wire mesh cages for one week to allow for acclimatization. The animals were randomly divided into 3 groups with 10 rats in each group. The first group was the control group that received only indomethacin. The second group was the hyperglycemic group that received 2g/kg body weight of glucose plus indomethacin while the third group was the hypoglycemic group that received 10I.U./kg body weight of insulin plus indomethacin.

Methods

Simulation of elevated or lowered blood glucose level was done by intravenous infusion, via the femoral vein, of glucose or insulin load, as appropriate. The blood glucose was estimated using a glucometer (OneTouch^{TM} Ultra). Ulcer was induced in the animals by giving indomethacin of 25mg/kg body weight orally as was described by Morsy and Fouad (2008). For the determination of the ulcer score, the animal would first be sacrificed and then their stomach would be removed. The stomach is then inflated by injecting 10 ml of 2% formalin, immersed in 2% formalin for 10 min to fix the gastric tissue wall, and opened along the greater curvature. Then, the regions of hemorrhagic lesions developed along the stomach wall were studied under a microscope.

The ulcer scores (Adami *et al*., 1964) can be placed on a scale, with 0=no lesions; 1=haemorrhagic suffusion; 2=from one to five small ulcers; 3=many ulcers, more than five, or one ulcer of marked size; 4=many ulcers of marked size; 5=perforated ulcers.

Table 1: Average body weight, initial and final blood glucose levels. (*p<0.05)

	Group 1	Group 2	Group 3
Average body weight (g)	217.6±1.27	220.5±2.00	219.3±1.68
Initial blood glucose (mg/dl)	67.7±2.20	59.3±8.61	59.8±7.23
Final blood glucose (mg/dl)	66.5±3.95	79.0±6.25	32.3±6.13*

Study Protocol

The animals were fasted for 12-18 hours and their blood glucose level was first measured before commencement of the experiment. Those in the control group were given indomethacin immediately after estimation of blood glucose and were left for four hours before being sacrificed for the

estimation of the ulcer score as earlier described. Animals in the treated groups were anaesthetized after their blood glucose was determined. They were then infused with glucose or insulin as earlier described. Ten minutes after infusion, the blood glucose level of the animal was checked to confirm onset of hyperglycemia or hypoglycemia as the case may be and this served as the post-infusion blood glucose level. Animals with a blood glucose of 180mg/dl or higher were taken as hyperglycemic while those with a blood glucose level of 40mg/dl were taken as hypoglycemic. Once this has been achieved, the animal is given indomethacin orally and left for four hours (Filaretova *et al*., 2002; Morsy and Fouad, 2008) before being sacrificed. Prior to the animals being sacrificed, the blood glucose of the animal was again estimated to serve as the final blood glucose.

Statistical Analysis

All results are presented as means ± S.E.M. All data were analyzed using the statistical package for social sciences software (SPSS Inc, U.S.A). ANOVA and Post hoc analysis were used to check for significance between groups and paired t-test was used to test for significance within groups. A p value less than 0.05 was taken as being significant.

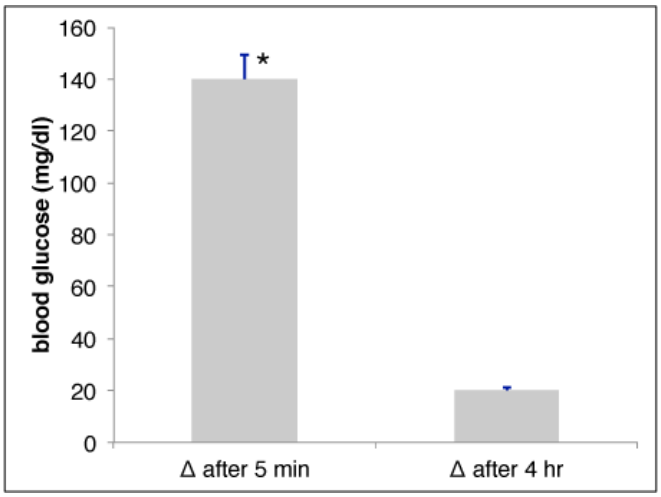

Figure 1: Change in blood glucose 5 mins and 4hours after infusion of high glucose load. *p<0.05

RESULTS AND DISCUSSION

Following the bolus infusion of glucose in rats in group 2 (hyperglycemic group), there was a significant increase (p<0.05) in blood glucose levels. This increase however, had returned to control level four hours after glucose infusion (Table 1, Figure 1).

When insulin was administered to the rats in group 3 (hypoglycemic), there was a significant fall (p<0.05) in the blood glucose to hypoglycemic levels. Four hours later, the blood glucose level of the rats had only increased slightly but was still significantly lower than the control level (Table 1, Figure 2).

Effect of glucose or insulin infusion on ulcer score

There was a very significant (P<0.05) decrease in the ulcer score in the hyperglycemic group (group 2) compared to the control group (group 1) whereas, the hypoglycemic group (group 3) did not present any significant changes in the ulcer score when compared to the control group (Figure 3).

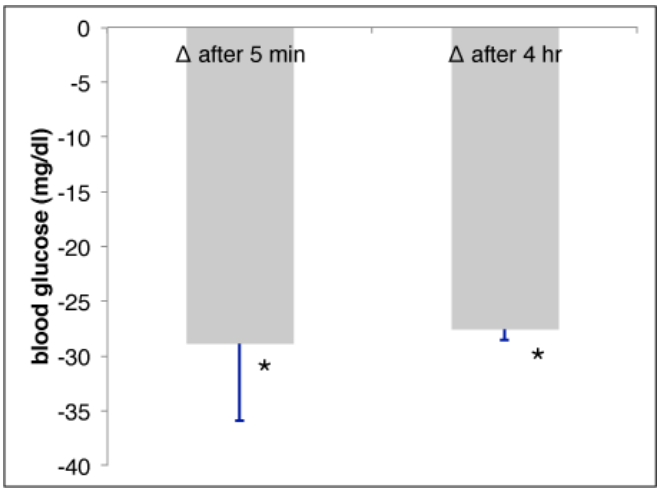

Figure 2: Change in blood glucose 5mins and 4hours after infusion of insulin. *p<0.05

The results of the present study show that infusion of a high glucose load leads to a significant reduction in the ulcer score (P< 0.01). On the other hand, simulation of hypoglycemic state through the infusion of insulin did not cause any significant change in the ulcer score of the stomach following indomethacin administration.

It is very tempting to conclude that the reduced ulcer score discovered in the hyperglycemic group was due to the inhibition of acid secretion that accompanies an hyperglycemic state but the final glucose reading we got from the rats after four hours show that the blood glucose level had dropped back to control levels, and considering the fact that the dosage of indomethacin administered requires four to six hours to achieve maximum action, the inhibition of acid secretion by hyperglycemia cannot be justified as a reason for the reduced ulcer score discovered. The increased insulin that would have been secreted following glucose infusion which enabled the blood glucose to return to normal levels might have been suggested as a possible reason but considering the fact that the group that received insulin administration didn't show a significant change in ulcer score compared to the control group makes this line of reasoning improbable.

The probable explanation for the reduced ulcer score would be that it is due to increased endogenous production of prostaglandins which has been discovered to occur following acute hyperglycemia as reported by Lash *et al* (1999) and Gonlachanvit *et al* (2003). Endogenous prostaglandins like prostaglandin E_2 have also been indicated in the healing of indomethacin-induced ulcers both in the stomach and in the small intestine through the endogenous prostaglandin receptors (Jones, 1999). This explanation is very probable because the increased endogenous production of prostaglandins would help to counteract the inhibitory effect of indomethacin on prostaglandin synthesis and subsequently protect the gastric mucosa from being eroded.

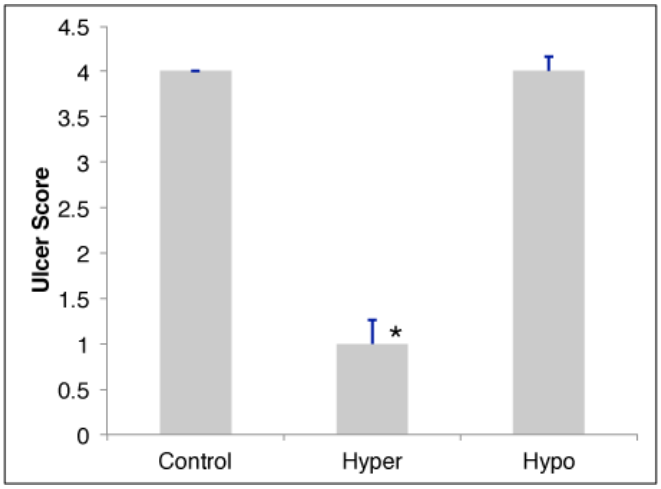

Figure 3: Ulcer score for the control, hyperglycemic and hypoglycemic groups. *p<0.05

The reduced ulcer score noticed in this group however does not imply that inducing hyperglycemia can be used to cure gastric ulcers. This is because long-term administration would be required, and the possibility that chronic administration of hyperglycemic doses of glucose could induce a diabetic state in man cannot be completely excluded. However, increasing the blood glucose level before using NSAIDs can go a long way in protecting against its ulcerative effects.

The insignificant change in ulcer score noticed in the hypoglycemic group does not corroborate with the previous finding that hypoglycemia increases acid secretion. Further studies are being undertaken in our laboratory to provide a possible explanation for this.

In conclusion, these results indicate that an elevated glucose level in a non-diabetic state could attenuate the formation of NSAID-induced gastric ulcers while insulin-induced hypoglycemic state may not affect the formation of NSAID-induced gastric ulcers.

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