Tr. J. of Medical Sciences
29 (1999) 551–554

Cihat GÜZEL[1]
Cemil SERT[2]
Zeki KANAY[3]
Doğan KURT[3]

The Protective Effect of Vitamins E and C on The Gastric Mucosal Barrier in Rats Irradiated With X-Rays

Received: December 10, 1998

Departments of [1]Physiology and [2]Biophysics, Faculty of Medicine, [3]Department of Physiology, Faculty Veterinary Science, Dicle University, Diyarbakır-Turkey

Abstract: The protective effect of vitamins E and C on the gastric mucosal barrier in rats irradiated with X-rays was investigated. Thirty-two male Wistar Albino rats were used. The animals were divided into four groups and arranged of follows: the first group (n=8) was the sham group; the second group (n=8) was the control group which was irradiated with X-rays, the third group (n=8) to which vitamin C was administered and the fourth group (n=8) to which vitamin E was given. The animals in the groups except the sham group wereirradiated with 8.9 Gy X-rays. Twenty-four hours later, the animals were sacrificed by cervical dislocation. The stomach was removed and opened along the greater curvature. The amounts of mucus and phospholipid, which are the components of gastric mucosal barrier, were determined by Corne and Baur's method. The amounts in irriadiated rats were found to be lower relative to those in the rats which were not irradiated ($p<0.001$, $p<0.001$, respectively). The amounts were observed to be higher in the vitamin C administered group compared with the irradiated control group ($p<0.001$, $0<0.05$, respectively). In the group to which vitamin E was given, the amounts were also found to be higher relative to those in the control group ($p<0.001$, $p<0.05$ respectively).
The results indicate that vitamins E and C protect the gastric mucosal barrier against X-rays.

Key Words: X-rays, Gastric mucosal barrier, Vitamin E, Vitamin C.

Introduction

The gastric wall is continuously exposed to various exogen and endogen damaging agents. The gastric mucosal barrier is an important structure protecting the gastric wall against damaging agents (1, 2). X-rays possibly slow down the reproduction and regeneration capacity of the vessels in muscle and submucosa in the gastrointestinal tract and of the cells in connective tissues (3, 4). Free oxygen radicals entirely mediate the cytotoxic effects in gastrointestinal epithelium (5). Prostaglandins play a significant role in protecting the gastric mucosal barrier by increasing stimulation of mucus and bicarbonate secretion, mucosal blood flow and phospholipids (6). In addition, in the present study, it is shown that prostaglandin E_2 protects the mucosal cells againts the fatal effect of radiation (7). Vitamins E and C defend the integrity of the cell membrane against oxidant agents as antioxidants (8, 9). In the studies carried out, it was determined that the vitamin E and C stimulated the prostaglandin synthesis (10, 11).

The purpose of the present study was to investigate whether Vitamins E and C, when their effects are taken into account, protect the gastric mucosal barrier in X-ray irradiated rats.

Materials and Method

Thirty-two Wistar Albino Rats two months old, weighing 200±20 gr were used. They were divided into four groups. The groups were arranged as follows : the first group (n=8) was the sham group; the second group (n=8) was the control group, which was irradiated with 8.9 Gy X-rays; the third group (n=8), to which Vitamin C was administered, and the gourth group (n=8), to which vitamin E was given. Vitamin C and E were intragastrically given to the third and fourth groups, respectively, dissolved in oil (Vit E) and in water (Vit C) at a dose of 400 mg/kg/day for 30 days. For 30 days, physiological serum was loaded at the same volume to the sham group and the control group irradiated with X-rays.

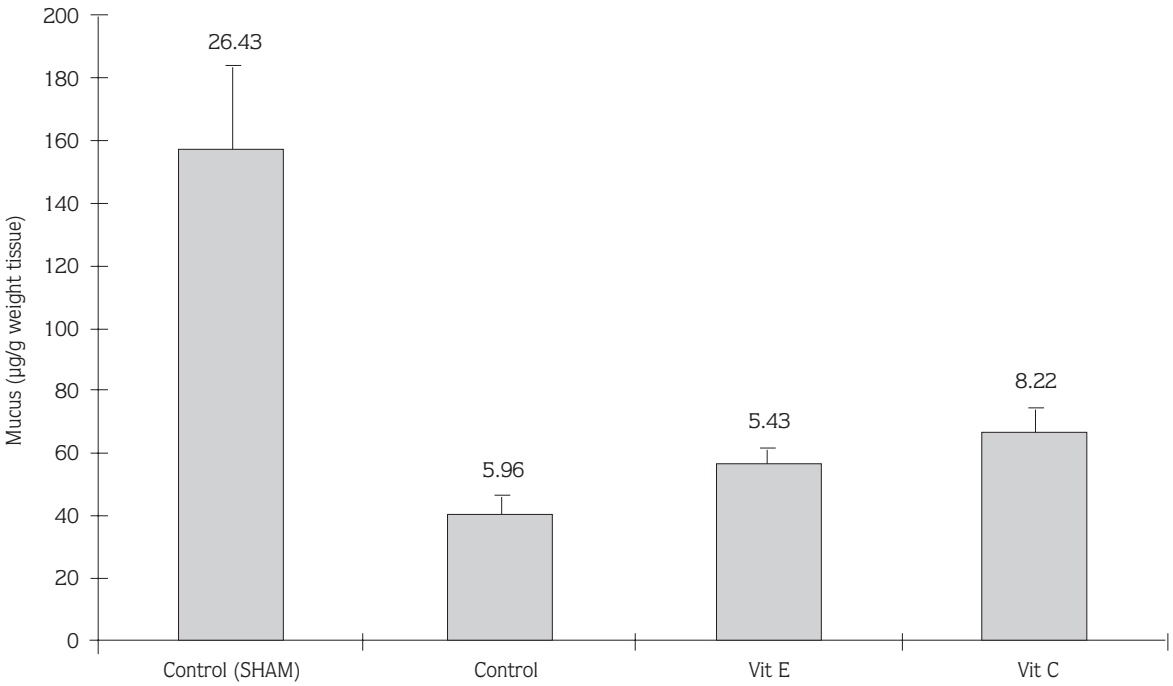

Figure 1. Effect of vitamin E and C on x-ray irradiated rats on mucus component of gastric mucosal barrier (Values are mean±SEM).

At the end of 30 days, the rats in the second, third, and fourth groups were irradiated with 8.9 Gy X-rays with a Toshiba DRX 1603 B (0.7nm Al, 125kVp, 200m AS, 10 cm distance) X-ray machine. They were separately placed (ventro-dorsal position) in a Plexiglas box and were kept under controlled temperature (22˚C) and a photo period of 12 h light and 12 h dark. The animals were kept hungry for 24 h (but allowed water). The animals were sacrificed by cervical dislocation. The abdomen was opened all along the median line. The stomach was taken out and separated into two pieces along the minor and major curvatures. Amounts of mucus were then determined in one piece according to the method of Corne et al. (12). One piece of stomach, taken out for determination of the amount of mucus was kept for two hours in Alcian Blue solution. After this proçedure, the stomachs taken from solution were put into $MgCl_2$ solution at 37˚C and incubated for two hours. The absorbance of $MgCl_2$ was measured at 605 nm in a spectrophotometer, and the amounts of mucus were obtained through analysis. In the other piece of the stomach, the amount of phospholipid was measured by the method of Baur (13). In order to determine the concentration of phospholipid, 0.2 ml mucosal extract was placed in the tubes. One millitre of nitric acid was added to each tube and heated by flame until no more nitric acid fumes were observed and then cooled. To each tube was added 1 ml of ascorbic acid –TCA, 0.5 ml of ammonium molybdate (1%) and 1 ml of arsenit cirtare and mixed. After 15 minutes, absorbtion of the mixture was measured at 700 nm againsta blank. The amount of phospholipid was calculated by the equation below:

Amount of phospholipide (mg/dl)= absorption of sample/Absorption of standard

= Concentration of standard x dilution coefficient

The mucosa of the other piece of stomach mucosa was scraped according to this method. The absorbance of supernatant obtained from this scraping, which underwent various extraction procedures, was measured at 700 nm in a spectrophotometer, and the amounts of phospholipid were substracted. The data were analysed for significance by using the Mann Whitney U test.

Results

As seen in Figures 1 and 2, X-ray decreased amounts mucus and phospholipid, which are the components of the gastric mucosal barrier : ($p<0.001$, $p<0.001$, respectively). The effects of vitamins E and C on mucus levels in the gastric mucosal barrier in X-ray irradiated rats are seen in Fig.1. The effects of vitamins C and E on phospholipid levels in the gastric mucosal barrier in X-ray

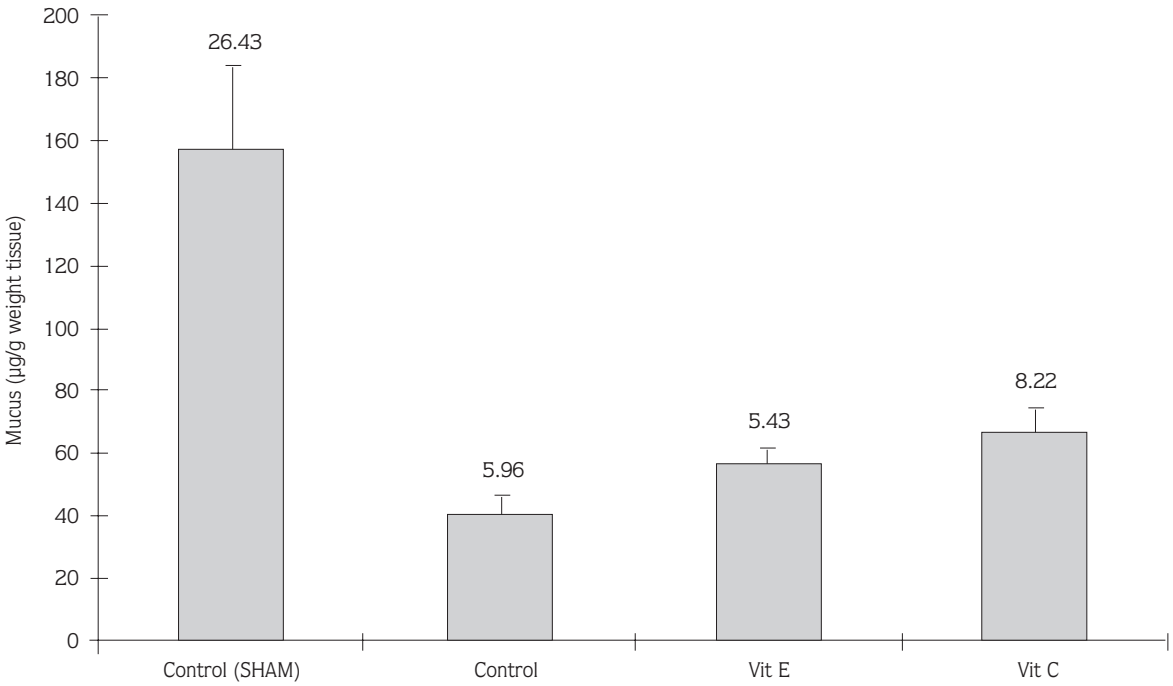

Figure 2. Effect of vitamin E and C on x-ray irradiated rats on phospholipid component of gastric mucosal barrier.

irradiated rats are seen in Fig.2. It was observed that the amounts of mucus and phospholipid were higher in rats given vitamin E relative to those of the control group, which were irradiated with X-rays ($p<0.001$, $p<0.05$, respectively). In the rats irradiated with X-rays, vitamin C significantly prevented a decrease in the levels of mucus and phospholipid, which are the components of the gastric mucosal barrier ($p<0.001$, $p<0.05$, respectively).

Discussion

According to the results of our study, vitamins C and E significantly reduced the decrease in the levels of mucus and phospholipid, which arethe components of the gastric mucosal barrier, in the X-ray irradiated rats. Radiation possibly slows down the reproduction and regeneration of the vessels in muscle and submucosa in the gastrointestinal tract and the cells in the connective tissue (3, 4). In the studies carried out, it was determined that the radiation used experimentally caused ulceration in the small intestines of the rats (14). Acute radiation damage is the result of the death of rapidly renewed epithelium cells in the mucosa (15). Water radiolizes with ionizing radiation, and radicals such O_2, OH, H_2O_2 occur. There is not an intestinal enzyme system which clears OH, the most dangerous radical that is formed. Therefore, free radicals formed in this way deteriorate the integrity and renewal rate of cell membrane, thus leading to ulceration in GIS (16). Prostaglandins play an important role in the strengthening of the gastric mucosal barrier (6). In addition, it has been shown that prostaglandin E_2 protects mucosal cells against the fatal effects of radiation (7). Vitamin E, inhibiting the lipid peroxidation, as a result of free radicals, functions as an antioxidant scavenging free radicals (8). Furthermore, it was found that vitamin E protects the integrity of the cell membrane against oxidant agents (17). In the studies carried out by Lonnier et al. and Soheir et al. it was observed that vitamin E had a protective effect as an antioxidant against the injury in gastrointestinal mucosa resulting from radiation (16, 18). Vitamin E also stimulates synthesis of prostaglandins by activating phospholipase A_2 enzyme (10). However, Behrens et al. (19), in their study in which they aimed to explain whether the effect of vitain E protecting the gastric mucosa against ulcerogenic agents exhibits its influence by was of prostaglandins or other mechanisms, have shown that vitamin E did not inhibit the occurrence of gastric ulcer caused by indomethacin which is an cyclooxygenase enyzme inhibitor. In the studies carried out, it was determined that vitamin C has a radioprotective effect (18, 20). Clara (11) has found that vitamin C increases the synthesis and secretion of prostaglandins. Charole et al. (21) have suggested that the mucus covering the upper surface of epithelial cells

functions as an antioxidant against free radicals resulting from radiation.

According to our findings, vitamins C and E indicate the effect protecting the gastric mucosal barrier in X-ray irradiated rats. These vitamins are thought to show their effects in two ways; first, they function as an antioxidant against the free radicals as a result of radiation. Second, it may be said that vitamins E and C may indirectly strengthen the gastric mucosal barrier by increasing the synthesis and secretion of prostaglandins, which increase the amounts of mucus and phospholipid, which are important components of the gastric mucosal barrier. However, we are of the opinion that further studies are needed to explain whether other mechanisms play any role in these effects of vitamins E and C or not. As a result, we can say that vitamins E and C protect the gastric mucosal barrier against radiation damage.

References

1. Aase S. Disturbances in the balance between aggressive and protective factors in the gastric and duodenal mucosa. Scand J Gastroenteral 24: 17-23, 1989.
2. Allen A, Leonarn JA. The mucos barrier: Its role in gastroduodenal mucus protection. J Clin Gastroenterol 10 (suppl.1): 593-98, 1980.
3. Black, W.C., Gomez, L.S., Yuhas, J.M., Kligermen, M.M. Quantitation of the late effects of x radiation on the large intestine. Cancer 45: 1980.
4. Hamilton, E., Franks, LM. Cell proliferation and aging in mouse colon. 11. late effects of repeated x-irradiation in young and old mice. Europ J Cancer 16: 663-69, 1980.
5. Parks DA, Bulkley GB, Granger DN. Role of oxygen derived free radicals indigestive tract diseases. Surgery 94: 415-22, 1983.
6. Reinhart WH, Muller O, Halter F. Influence of long term 16, 16 dimethyl prostaglandin E2 treatment on the rat gastrointestinal mucosa. Gastroenterology 85: 1003-10, 1983.
7. Hanson WR, Thomas C.16, 16, dimethyl prostaglandin E2 increases survivial of murine intestinal stem cells when given before phone radiation. Radiat Res 96: 393-98, 1983.
8. Morenkova ST, Tabutsadze TV, Federova LM, et al. Effect of vagotomy, a-Tocopherol, and arachidene on lipid peroxidation in different parts of the gastroduodenal zone in rats with experimental ulcer. Bulletin of Experimental Biology and Medicine. 5: 532-34, 1987.
9. Sobala GM. Ascorbic acid in the human stomach. Gastroenterology 97: 357-63, 1989.
10. Hirata F. The regulation of lipomodulin a phopholipase inhibitory protein in rabbit neutrophillis by phosphorilation. J Biol Chem 256: 7730-33, 1981.
11. Clara FP. Ascorbic acid, PGE2 and acetylcholine interaction: The effect on isolated smooth muscle. Acta physiol Pol: 37(1): 18-24, 1986.
12. Corne SJ, Morriessay SM and Woods RJ. A method for quantitive estimation of gastric barrier mucus. J Physiol 242: 1168-79, 1974.
13. Baur JD, Ackerman PG. Phospholipids clinical laboratory methods. C.O. Mosby Conp. St. Louis: 450-51, 1974.
14. Weiss SJ. Oxygen ischemia and inflamation. Acta Physiol Scand. 126: 9-37, 1986.
15. Ito Hissao, Meistrich Marvin L, Barkley H. Thomas, Thames Howard D. and Milas Luka. Protection of acute and late radiation damage of the gastrointestinal tract by WR-2721. Radiation Oncology Biol. Phys. Vol.12. 211-19, 1986.
16. Lonnier RE, Jamie D, Lawrence D, et al. Mucosal protective effects of vitamin E and Misoprostol during acute radiation-ınduced enteritis in rats. Dig Dis Sci. 37: 205-14, 1992.
17. Toshkazu Y, Yasuda M, Meada S, et al. Vitamin E in Gastric mucosal injury induced by ischemia-reperfision. Am J Clin Nutr 53: 21, 1991.
18. Soheir M, El Nahas S, Fathy E, Mattar and Amal A. Mohamed. Radiopretictive effect of vitamins C and E mutation Resarch. 301: 143, 1993.
19. Behrens GZ, Mueller R, Gresellin E, Behrens WA. Lack of vitamin E cytoprotective effects on Indomethacin-induced gastric lesions Res Comm Chem Pathol. and Pharmachol. 72(3): 327-335, 1981.
20. Linda HC, Gilbert A, et al. Vitamin C, Vitamin E and Cancer. Anticancer Research 8: 739-48, 1988.
21. Charole E Cross, Barry H, Adrien A, Antioxidant protection A function of tracheobronchial and gastrointestinal mucus. Lancet. 1328-29, 1984.