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EFFECT OF VAGOTOMY, α-TOCOPHEROL, AND ARACHIDENE ON LIPID PEROXIDATION IN DIFFERENT PARTS OF THE GASTORDUODENAL ZONE IN RATS WITH EXPERIMENTAL PEPTIC ULCER

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Activation of lipid peroxidation (LPO) is one of the leading factors reducing the resistance of the mucous membrane of the gastroduodenal zone [3, 6, 7]. LPO is initiated by stress stimulations [8] and is often the result of a deficiency of antioxidants in the tissues [5]. There is no doubt about the fact that various products of free-radical reactions are the cause of damage to the integrity of the cell membranes and, consequently, of the viability of the cell as a whole [4]. In this connection it can be postulated that disturbance of regulation of LPO may play an essential role in the pathogenesis of peptic ulcer.

The aim of this investigation was to study the role of LPO in experimental peptic ulcer and the effect of vagotomy, antioxidants, and arachidene on this process.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 140-160 g. Duodenal ulcer (DU) was induced by a single subcutaneous injection of cysteamine (Fluka, Switzerland) in a dose of 30 mg/100 g by the method in [14]. There were four groups of animals. Rats of group 1, 24 h after receiving the injection of cysteamine, underwent one of three versions of subdiaphragmatic vagotomy: 1) complete truncal vagotomy (CTV); 2) incomplete — division of one vagus trunk (ITV); and 3) proximal selective division of separate branches of the vagus nerve (PSV) by the method in [11]. The mucous membrane of the fundal and antral portions of the stomach and of the duodenum was removed 2 weeks after the operation and fixed in liquid nitrogen until required for use. Before receiving the injection of cysteamine the rats of group 2 were given daily subcutaneous injections of α-tocopherol (TP) in a dose of 5 mg/100 g for 5 days. For 5 days before the injection of cysteamine, rats of group 3 were given 0.1 ml of arachidene daily. Arachidene is a mixture of polyunsaturated fatty acids, which was synthesized in the Laboratory of the M. I. Lomonosov Moscow Institute of Fine Chemical Technology, directed by Corresponding Member of the Academy of Sciences of the USSR Professor R. P.

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Evstigneeva. The rats of group 4 were subjected to combined treatment with TP and arachidene for 5 days before receiving the injection of cysteamine. The mucous membrane was removed from the rats of groups 2, 3, and 4 24 h after the injection of cysteamine and fixed in liquid nitrogen. In all series the mucous membrane of the gastroduodenal zone was examined visually and microscopically.

LPO was investigated in homogenates of the mucous membrane from different parts of the gastroduodenal zone. Activity of LPO was judged by the accumulation of malonic dialdehyde (MDA) during incubation for 30 min at 37°C [4]. The incubation medium for determination of the intensity of ascorbate-dependent LPO consisted of 40 mM Tris-HCl buffer (pH 7.4) and 0.5 mM ascorbate. The reaction was initiated by the addition of protein of the homogenate in a final concentration of 10 mg/ml. The volume of the incubation sample was 1 ml.

During investigation of the activity of enzymic LPO the composition of the incubate medium was as follows: 40 mM Tris-HCl buffer (pH 7.4), 2 mM NADPH, and 0.1 mM $FeCl_3$. The protein concentration and volume of the sample were the same as for determination of ascorbate-dependent LPO.

The reaction in both versions was stopped by the addition of TCA in a final concentration of 25%. The samples were cooled in an ice bath and centrifuged for 20 min at 2000 g and 4°C. To 1 ml of supernatant was added 0.5 ml of a 0.9% aqueous solution of 2-thiobarbituric acid (TBA) and the mixture was incubated for 20 min at 100°C. After cooling the samples were subjected to spectrophotometry at 532 nm. The measured extinction value was multiplied by the molar coefficient for MDA, namely $1.56 \cdot 10^5$ $M^{-1}cm^{-1}$. The results were expressed in nanomoles DMA/mg protein/30 min. Protein was determined by Lowry's method [12].

EXPERIMENTAL RESULTS

Cysteamine, which causes specific and selective ulcer formation in the duodenal mucosa, activates LPO strongly in the mucous membrane of the fundal part of the stomach only 24 h after its injection. Activity of both ascorbate-dependent and NADPH-dependent LPO was increased almost equally under these circumstances. With the different versions of vagotomy, activity of LPO was most effectively reduced after PSV: activity of ascorbate- and NADPH-dependent LPO was reduced by 1.47 and 1.66 times respectively compared with the control. Preliminary premedication with TP (before injection of cysteamine) led to a sharp decline in LPO activity in the mucous membrane of the fundal portion of the stomach (by 2.78 and 2.5 times compared with the control). These data are of great importance in connection with the view that the presence of bioantioxidants — "traps" for free radicals — is an essential condition for maintaining oxidative reactions of lipids at a level capable of maintaining the integrity of biological membranes, and that the quantity of these very important compounds in the biosystem characterizes its powers of adaptation [1, 2, 5].

Besides the study of the role of the antioxidant TP, the possible cytoprotective action of arachidene also was studied. Preliminary feeding of the animals with arachidene also reduced activation of LPO after subsequent injection of cysteamine, although arachidene was rather less effective than TP.

With the combined use of TP and arachidene the cytoprotective action of these substances on the mucous membrane of the fundal part of the somach was exhibited to the greatest degree, with respect both to ascorbate- and NADPH-dependent LPO.

Analysis of the data on the effect of the various correcting factors on LPO activity in the mucous membrane of the gastric fundus thus shows that TP brought about the greatest effect, especially when combined with arachidene, and in conjunction with vagotomy, when PSV was more effective than CTB and TTV.

The comparative study of the action of the procedures described above on the mucous membrane of other parts of the gastroduodenal zone, namely the antral portion of the stomach and the duodenum, showed that the degree of the pathological changes observed was considerably higher in the mucosa of the antral portion of the stomach and reached a maximum in the duodenal mucosa, i.e., in the zone of the ulcer.

For instance, activity of ascorbate- and NADPH-dependent LPO increased after injection of cysteamine by 7.5 and 5.8 times, respectively, compared with the control. TP, alone and together with arachidene, lowered the intensity of ascorbate- and NADPH-dependent LPO in the

duodenal mucosa by 4.3 and 5.5 times, respectively. Activity of LPO also was considerably depressed by PSV (by 2.18 times in the case of ascorbate-dependent LPO).

It can be concluded from these results that cysteamine causes marked activation of LPO in all parts of the mucous membrane of the gastroduodenal zone and, in particular, in the duodenum. Pharmacological agents (TP, arachidene, and a combination of both) reduce the activity of free-radical reactions by different degrees, and TP acts most effectively in conjunction with arachidene. Preliminary investigations [9] showed that a 5-day course of these preparations prevented ulcer formation in the duodenum and the development of erosions in the antral portion of the stomach after injection of cysteamine in 75% of cases. Of the different versions of vagotomy, PSV was most effective. The impression is gained that this operative technique, with its effect on metabolic processes in the tissues, reduces LPO activity, and so promotes regenerative processes in the duodenal mucosa.

On the basis of the results described above showing the effect of these pharmacological agents on LPO it can be concluded that, besides activation of free-radical reactions, other factors may also be involved in the pathogenesis of ulcer formation. These may include the prostaglandin-synthesizing activity of cells of the mucous membrane, the products of which are known to play an important role in cytoprotection, and in the regulation of secretion and of the circulation in the gastrointestinal trace [13], and also the glutathione system [15] and interaction between the glutathione system and bioantioxidants [10].

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