J. Pharm. Pharmacol. 1990, 42: 655–657
Communicated January 24, 1990

Potent inhibition of prostaglandin inactivation in rabbit gastric antral mucosal slices by selenium ions in-vitro

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Abstract—The effect of selenium ions on prostaglandin (PG) catabolism and synthesis in rabbit gastric antral mucosal slices has been examined. Selenium ions had a potent inhibitory effect on the inactivation process for PGE_2 and $PGF_{2\alpha}$. Simultaneously, the levels of PGE_2 and $PGF_{2\alpha}$ were increased. These results suggest that selenium ions have the potential to increase the levels of biologically active PGs in gastric mucosa by preventing their inactivation and that this effect may represent some pharmacological action of selenium ions.

Important interactions between selenium ions and arachidonic acid metabolism appear to exist and the possibility that the action of selenium ions is linked to the element's effects on prostaglandins (PGs) is suggested by several observations. However, results regarding the influences of selenium ions on PG generation are difficult to interpret, because they have been shown either to increase (Doni et al 1983; Schiavon et al 1984) or to decrease (Fujimoto et al 1985) PG synthesis. On the other hand, much evidence indicates that endogenous PGs are important in gastric mucosal cytoprotection and other gastric functions (Bennett 1976; Miller & Jacobson 1979; Robert 1979). Recently Preclik et al (1987) have reported the predominant synthesis of PGE_2 and $PGF_{2\alpha}$ in the mucosa of rabbit stomach, and suggested their special importance in the regulation of many functions of the gastric mucosa. Since PG-catabolizing enzymes, 15-hydroxy-PG dehydrogenase and PG Δ13-reductase, have been found in the 100 000 *g* supernatant prepared from rabbit stomach (Moore & Hoult 1978), PG release from gastric mucosa depends, not only on the synthesis supported by enzymes conducting PG formation, but also on enzymes associated with PG catabolism. It has also been shown that in the rabbit, antral mucosa contains large amounts of PG-catabolizing enzymes, 15-hydroxy-PG dehydrogenase and PG Δ13-reductase (Spenney 1979a, b). Few reports have explored the action of selenium ions on PG degradation. These findings prompted us to examine the effect of selenium ions on the in-vitro production of PGE_2, $PGF_{2\alpha}$ and their metabolites by rabbit gastric antral mucosal slices.

Materials and methods

Male rabbits (2–2·5 kg) were used. Whole stomachs were removed from anaesthetized (sodium pentobarbitone, 30 mg kg^{-1}) rabbits, rinsed with distilled water and rapidly chilled in ice-cold 0·9% NaCl (saline). Antral mucosa was obtained by gentle stripping from the underlying muscular layer and was cut into approximately equal slices (a 3 × 7 mm piece) with a razor blade on an ice-cold petri dish. In all experiments, slices of gastric antrum mucosa (0·4 g) were preincubated in 4·0 mL of 0·15 M KCl/0·02 M Tris-HCl buffer (pH 7·4) at 4 °C for 15 min. Following preincubation, the medium was discarded, the slices rinsed twice with the Tris-HCl buffer and incubated with various concentrations of sodium selenite at 37 °C for 30 min, as described previously (Fujimoto & Fujita 1982; Fujimoto et al 1983). After incubation, the medium was extracted with 30 mL of light petroleum (b.p. 30–70). The aqueous phase was then acidified (approximately pH 3) with 0·5 M HCl and extracted with 30 mL of ethyl acetate. PGs in the extracted lipid were simultaneously determined by a high-pressure liquid chromatographic (HPLC) method using 9-anthryldiazomethane (ADAM, Funakoshi Pharmaceutical Co., Tokyo) (Nimura & Kinoshita 1980; Fujita et al 1986). The HPLC separation and quantitative determination of PGs esterified with ADAM have been described previously (Kiyomiya et al 1986; Yamaki & Ohishi 1989). Since ADAM contains many impurities which interfere with the HPLC determination, the purification of PGs esterified with ADAM (PGs-ADAM) was attempted by use of a normal-phase silica cartridge (Sep-pak, Waters Associates). The cartridge was prepared by rinsing it with 10 mL of methanol followed by 20 mL of toluene–chloroform (1 : 1 v/v). The sample was passed through the cartridge. The cartridge was washed with toluene–chloroform (1 : 1 v/v, 10 mL) and the PGs-ADAM was then quantitatively eluted with acetonitrile–methanol (4 : 1 v/v, 10 mL). ADAM derivatives of PGs were separated in reverse-phase chromatography and simultaneously quantitated by employing a Shimadzu model RF-535 fluorescence spectrofluorometer (excitation 365 nm, emission 412 nm) with a 16 μL flow cell. The liquid chromatograph system used in this study was a Shimadzu model LC-6A system equipped with a YMC packed column (ODS A-303 type, 4·6 mm i.d. × 25 cm, Yamamura Chemical Lab. Co., Kyoto). Two solvent systems were employed: (a) acetonitrile–water–phosphoric acid (62 : 37·9 : 0·1 v/v) at a flow rate of 1·2 mL min^{-1} at 30 °C, which separates 6-keto $PGF_{1\alpha}$ (the stable hydrolysis product of PGI_2), PGE_2, PGD_2, 15-keto PGE_2, 15-keto $PGF_{2\alpha}$, 13,14-dihydro-15-keto PGE_2 and 13,14-dihydro-15-keto $PGF_{2\alpha}$, but does not allow separation of $PGF_{2\alpha}$ from thromboxane B_2 (TxB_2, the stable hydrolysis product of TxA_2), and (b) methanol–acetonitrile–water (3·8 : 1·0 : 1·2 v/v) at a flow rate of 1·0 mL min^{-1} at 20 °C, which allows separation of $PGF_{2\alpha}$ and TxB_2. A linear relationship between the height of fluorescent peak and the amount of PG standard injected was observed over the range 0–50 ng for each PG or TxB_2. The detection limit was approximately 1 ng under the conditions used. The recovery of 6-keto $PGF_{1\alpha}$, PGE_2, $PGF_{2\alpha}$, PGD_2, TxB_2, 15-keto PGE_2, 15-keto $PGF_{2\alpha}$, 13,14-dihydro-15-keto PGE_2 and 13,14-dihydro-15-keto $PGF_{2\alpha}$ during extraction and purification was 90·1 ± 3·7%, 94·8 ± 2·0%, 97·2 ± 2·2%, 96·8 ± 1·4%, 97·6 ± 2·2%, 90·6 ± 4·7%, 95·5 ± 0·8%, 99·0 ± 2·4% and 98·5 ± 1·3%, respectively (means ± s.e.m., n = 4). The coefficient of variance for the assay of 6-keto $PGF_{1\alpha}$, PGE_2, $PGF_{2\alpha}$, PGD_2, TXB_2, 15-keto PGE_2, 15-keto $PGF_{2\alpha}$, 13,14-dihydro-15-keto PGE_2 and 13,14-dihydro-15-keto $PGF_{2\alpha}$ was 1·5%, 1·8%, 1·0%, 2·7%, 2·5%, 2·0%, 2·8%, 1·2% and 1·9%, respectively (n = 4).

By this method it was demonstrated that the sum of PGE_2, $PGF_{2\alpha}$, 13,14-dihydro-15-keto PGE_2 and 13,14-dihydro-15-keto $PGF_{2\alpha}$ amounted to more than 70% of the total PG release from the antral mucosa in-vitro under the present experimental conditions. The other PG products were 6-keto $PGF_{1\alpha}$ (14%), TxB_2 (11%) and PGD_2 (3%). Negligible amounts of 15-keto PGE_2 or 15-keto $PGF_{2\alpha}$ were found in the incubation medium,

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indicating a very efficient transformation into 13,14-dihydro-15-keto PGE_2 or 13,14-dihydro-15-keto $PGF_{2\alpha}$. Thus, in our experiment, the release of PGE_2, 13,14-dihydro-15-keto PGE_2, $PGF_{2\alpha}$ and 13,14-dihydro-15-keto $PGF_{2\alpha}$ reflects a composite of the activities of both PG-synthesizing and PG-catabolizing enzymes in rabbit gastric antral mucosa.

The values presented herein are the means ± s.e.m. Statistical significance was calculated using Student's paired *t*-test.

Results

As illustrated in Fig. 1, gastric antrum mucosa from rabbits possessed a high capacity for catabolizing PGE_2 and $PGF_{2\alpha}$ to their respective 13,14-dihydro-15-keto derivatives. Antral mucosal slices under basal conditions, without the addition of selenium ions, released about equal amounts of parent PG (PGE_2 or $PGF_{2\alpha}$) and its metabolite. The preparation produced $PGF_{2\alpha}$ plus its metabolite/PGE_2 plus its metabolite in a ratio of 0·47. The rate of PGE_2 synthesis appears to be significantly higher than $PGF_{2\alpha}$.

Selenium ions (0·125–2 mM) reduced the release of basal 13,14-dihydro-15-keto PGE_2 and 13,14-dihydro-15-keto $PGF_{2\alpha}$ in a dose-dependent manner. Selenium ions inhibited the formation of 13,14-dihydro-15-keto $PGF_{2\alpha}$ to a greater extent than that of

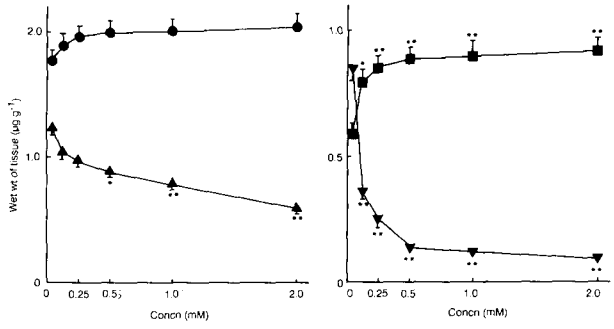

FIG. 1. Effect of selenium ions on the release of prostaglandin E_2 (PGE_2, ●), 13,14-dihydro-15-keto PGE_2 (DKE_2, ▲), $PGF_{2\alpha}$ (■) and $DKF_{2\alpha}$ (▼) from rabbit gastric antral mucosal slices. Slices were incubated for 30 min at 37 °C in 0·15 M KCl/0·02 M Tris-HCl buffer in the presence of different concentrations of selenium ions. Each point indicates the mean of 5 experiments; vertical lines show s.e.m. * $P < 0·02$ compared with the corresponding value in the absence of selenium ions. ** $P < 0·01$ compared with the corresponding value in the absence of selenium ions.

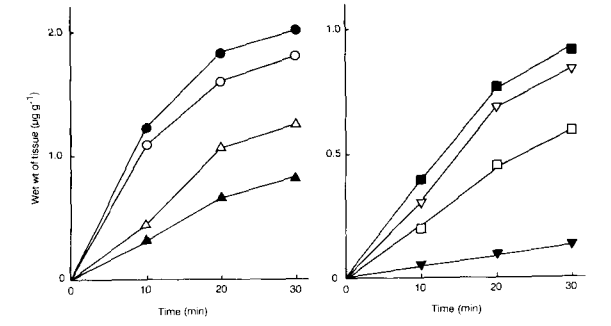

FIG. 2. Time course of prostaglandin E_2 (PGE_2), 13,14-dihydro-15-keto PGE_2 (DKE_2), $PGF_{2\alpha}$ and $DKF_{2\alpha}$ release from rabbit gastric antral mucosal slices. Incubations were for 30 min at 37 °C in 0·15 M KCl/0·02 M Tris-HCl buffer in the absence (○ PGE_2; △ DKE_2; □ $PGF_{2\alpha}$; ▽ $DKF_{2\alpha}$) and the presence of 1 mM selenium ions (● PGE_2; ▲ DKE_2; ■ $PGF_{2\alpha}$; ▼ $DKF_{2\alpha}$). Each point indicates the mean of 5 experiments (s.e.m. values were less than 5%).

13,14-dihydro-15-keto PGE_2 (13,14-dihydro-15-keto PGE_2, 15–51% inhibition; 13,14-dihydro-15-keto $PGF_{2\alpha}$, 56–87% inhibition). On the other hand, the release of PGE_2 was increased slightly by the addition of selenium ions. The release of $PGF_{2\alpha}$ was enhanced more markedly than that of PGE_2.

The effect of selenium ions (1 mM) was apparent within 10 min after addition to the incubation mixture and persisted for 30 min (Fig. 2).

Discussion

The gastric mucosa is capable of synthesizing and inactivating PGs. Thus PGs that emerge from gastric mucosa are the net result of these two opposing properties. The present study showed that selenium ions had a potent inhibitory effect on the inactivation process for PGE_2 and $PGF_{2\alpha}$ in rabbit gastric antral mucosa. Simultaneously, the levels of PGE_2 and $PGF_{2\alpha}$ were increased. It can be conceived that selenium ions increase the levels of biologically active PGs in gastric mucosa by preventing their inactivation.

In the presence of selenium ions the net increased amount of PGE_2 or $PGF_{2\alpha}$ was smaller than the net decreased amount of 13,14-dihydro-15-keto PGE_2 or 13,14-dihydro-15-keto $PGF_{2\alpha}$ (PGE_2, 37–65%; $PGF_{2\alpha}$, 41–44%). It is possible that selenium ions participate in a process which leads to inactivation of PG cyclo-oxygenase in gastric mucosa. Several reports suggest that PG synthesis is altered by selenium ions. Supplementation of a normal diet with selenium ions was reported to increase PGI_2 synthesis by aorta and to increase glutathione peroxidase activity in erythrocytes from rats (Doni et al 1983). When added to the culture medium of porcine aortic endothelial cells, selenium ions increased glutathione peroxidase activity and PGI_2 production (Schiavon et al 1984). On the contrary, we have reported that selenium ions inhibit the generation of PGE_2 in rabbit kidney medulla slices (Fujimoto et al 1985). Selenium is an integral component of glutathione peroxidase (Chance et al 1979). Glutathione peroxidase is an important enzyme in destroying H_2O_2 and organic hydroperoxides such as lipid hydroperoxides (Hoekstra 1975). Very low concentrations of hydroperoxides are required to activate PG cyclo-oxygenase, and in their absence, there is very little enzyme activity (Hemler & Lands 1980). On the other hand, higher concentrations of these substances can inhibit PG cyclo-oxygenase (Nugteren & Hazelhof 1973; Powell & Gravelle 1985). So, it seems possible that PG cyclo-oxygenase activity can be enhanced or inhibited by selenium ions depending on the cell type and the cellular levels of hydroperoxides.

Further studies are needed to clarify the mechanism of action of selenium ions which may affect the activity of PG-catabolizing enzymes in gastric mucosa; however, the present study serves to emphasize that selenium ions have the potential to increase the PG levels in gastric mucosa by inhibiting the inactivation process for PGs. The increased levels of PG may participate in some pharmacological action of selenium ions.

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J. Pharm. Pharmacol. 1990, 42: 657–659
Communicated September 21, 1989

Effect of zinc sulphate on acetic acid-induced gastric ulceration in rats

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Abstract—The effects of zinc sulphate on gastric ulcer healing rate and mucosal mucus content of acetic acid-induced ulceration in rats have been assessed. Daily treatment with zinc sulphate progressively accelerated ulcer healing in a dose-dependent manner with a significant increase observed on day 15 after ulcer induction in rats treated with 44 and 88 mg kg^{-1} zinc sulphate. A significant increase in gastric mucosal adherent mucus was also observed in those animals treated with 88 mg kg^{-1} zinc sulphate. The results suggest that a minimum treatment period of 15 days is needed for the zinc sulphate to be effective, and that zinc ions may promote gastric ulcer healing by enhancing mucus formation to prevent acid back-diffusion into the gastric mucosa.

It has been shown that oral zinc sulphate accelerates the healing of wounds caused by excision of pilonidal sinuses (Pories et al 1967), and several workers have suggested that zinc may accelerate the healing of chronic leg ulcers (Husain 1969; Greaves & Skillen 1970; Sergeant et al 1970). Recent observations have shown the protective effect of zinc in gastric ulceration could be due to its ability to prevent gastric mucosal mast cell degranulation, with a concomitant decrease in histamine levels in the gastric secretion (Ogle & Cho 1977b). Zinc also depresses histamine release from the gastric mucosal mast cells following vagal over-reactivity which occurs under stressful conditions (Cho & Ogle 1977; Ogle & Cho 1977a). Furthermore, zinc pretreatment in normal rats causes an increase in gastric mucus content (Cho & Ogle 1978). Thus, it was decided to investigate whether zinc treatment would be able to hasten the healing of chronic ulcers induced by a topical application of acetic acid to rat stomachs.

Materials and methods

Female Sprague-Dawley rats (220–250 g) were fed a standard laboratory diet (Ralston Purina Co., USA), and kept in a room with controlled humidity (65–70%) and temperature (22 ± 1 °C).

Chronic ulcer induction. Rats were starved for 24 h before use, but were allowed free access to an 8% sucrose in 0·2% NaCl w/v solution, which was removed 1 h before experimentation. Experiments were conducted between 13.00 and 17.00 h because at this time the stomachs were almost empty and a full stomach was found to result in inconsistent ulcer formation. Under a light ether anaesthesia, a midline epigastric incision exposed the stomach and a cylindrical plastic mould (6·5 mm in diameter) was firmly placed upon the anterior serosal surface of the gastric wall. Sixty μL of 100% acetic acid was pipetted into the mould and allowed to remain for 60 s as described by Okabe et al (1970). The acid solution was then removed using a syringe; the mould was rinsed 3 times with 0·9% NaCl w/v (saline) to prevent possible damage to surrounding tissues close to the point of acid