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CELLULAR MECHANISMS OF β-CAROTENE-INDUCED GASTRIC CYTOPROTECTION IN INDOMETHACIN-TREATED RATS

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ABSTRACT

Mózsik Gy, Bódis B, Karádi O, Király Á, Nagy L, Rumi Gy, Sütő G, Szabó I, Vincze Á. Cellular mechanisms of β-carotene-induced gastric cytoprotection in indomethacin-treated rats. Inflammopharmacology. 1998;6:27–40.

Indomethacin (IND) is a non-steroidal anti-inflammatory agent which is widely used in the treatment of various inflammatory disorders. The drug causes gastrointestinal injury in humans and experimental animals. The aim of these studies was to examine the time course correlation between the macroscopic appearance of mucosal damage, tissue level of PGE_2 and adenosine nucleotide metabolism during the development of indomethacin (IND)-induced mucosal damage and its prevention by β-carotene.

The observations were carried out on both sexes of CFY-strain rats, weighing 180–200 g. Gastric mucosal damage was produced by subcutaneous administration of IND (20 mg/kg). β-Carotene (Hoffman-La Roche, Switzerland) was given intragastrically at the time of IND administration at doses of 0.01, 0.1, 1 and 10 mg/kg. The animals were sacrificed at 0, 1, 2, 3 and 4 h after IND administration when the number and severity of mucosal lesions were noted and the tissue levels of ATP, ADP, AMP, cAMP, lactate and PGE_2 were measured from the total homogenate of gastric mucosa. The ratio of ADP/ATP, the values of the adenylate pool (ATP+ADP+AMP), and 'energy charge' [(ATP+0.5ADP)/(ATP+ADP+AMP)] were calculated.

It was found that: (a) gastric mucosal lesions appear macroscopically 2 h after IND administration; (b) the tissue level of ATP decreased, while ADP was increased 1 h after administration; (c) the most significant decrease in cAMP was found 1 h after IND administration, and thereafter its level returned to baseline; (d) β-carotene dose-dependently prevented the IND-induced mucosal damage and elevated the cAMP level, but it did not alter the mucosal PGE_2 level 3 or 4 h after IND administration; (e) β-carotene produced an elevation in ATP and a decrease in ADP level; (f) no significant changes were found in 'energy charge' of the gastric mucosa in IND-treated animals.

The development of gastric mucosal damage due to IND was associated with increased energy liberation, i.e. transformation of ATP into ADP, and decreased ATP–cAMP transformation. The significant decrease in cAMP preceded the macroscopic appearance of mucosal damage. The increase in ATP–cAMP transformation is involved in the development of β-carotene-induced gastric cytoprotection.

Keywords: indomethacin, β-carotene, gastric mucosal damage and prevention, adenosine nucleotides, cAMP

INTRODUCTION

The multifactorial origin of peptic ulcer disease (PUD) has been emphasized (including increased vascular permeability, altered mucosal biochemistry, decreased sythesis of prostaglandins, increased synthesis of leukotrienes, increase in oxygen free radicals, etc.).

Clinically the PUD can be divided into 'essential' or 'genuine' disease (when no aetiological factor is known) and 'secondary' disease (combined with primary pulmonary, liver endocrine and other diseases) [1].

A significant part of gastrointestinal mucosal damage may be associated with the clinical application of non-steroidal anti-inflammatory compounds, including indomethacin (IND). This compound is frequently used in everyday medical practice in Hungary.

Various animal observations have been carried out with IND in order to evaluate the changes in gastric mucosal biochemistry (cellular ATP, ADP, cAMP), adenylate pool (ATP+ADP+AMP), 'energy charge' [(ATP+0.5ADP)/(ATP+ADP+AMP)] and ratio of ATP/ADP during the development of IND-induced gastric mucosal damage and its prevention by vitamin A cytoprotection [2] and antisecretory doses of atropine and cimetidine [3].

The biochemical measurements were carried out simultaneously in order to study the 'cross-section' of gastric mucosal biochemistry (and to prove or exclude the 'hypoxaemic damage' [3]. Biochemically, the significant decrease in tissue ATP together with the increase in lactate level (which results in impaired oxidative phosphorylation) indicate the presence of tissue hypoxia [4]. However, the increased ATP breakdown in association with the increased level of tissue ADP (without any increase in tissue level of lactate) excludes the presence of tissue hypoxia.

The term 'gastric cytoprotection' was introduced into the international literature by Chaudhury and Jacobson [5] and was adopted by Robert et al. [6,7]. The main point of gastric cytoprotection is that prostaglandins (and later many other chemically different compounds) were found to prevent chemically induced (e.g. 96% ethanol, 0.1 mol/L NaOH, 25% NaCl, 0.6 mol/L HCl and various drugs) gastric mucosal damage without decreasing gastric acid secretion [6,7]. The gastroprotective ('cytoprotective') effects of non-sulphydryl retinoids were proved and documented by our work-team [2,8–11].

The details of gastric mucosal damage caused by chemical, physical and other stress are not well known. Changes in vascular permeability, gastric mucosal biochemistry of the membrane-dependent ATP-dependent energy systems, oxygen-free radical reactions, and tissue hypoxia are thought to occur prior to the macroscopic appearance of gastric mucosal damage; however, we have limited information about the correlation between them (Figure 1). Prostacyclin can modify the vascular events in the gastric mucosa in association with prevention of chemically induced gastric mucosal damage. β-Carotene as a scavenger also prevents the gastric mucosal damage; however, its mucosal protecting effect differs with time in the extent of vascular permeability changes and gastric mucosal biochemistry. Furthermore, β-carotene is a micronutrient provitamin for vitamin A with scavenger properties (Figure 2).

The use of ethanol (as a non-acid-dependent) and HCl (as an acid-dependent) models to produce gastric mucosal damage do not represent the best models to evaluate the time-sequence analysis of biochemical events in the gastric mucosa because about 50% of the total macroscopic mucosal damage is already detectable 5 min after administration of the necrotizing agents [12].

IND produces gastric and intestinal [2,4] mucosal damage which develops over a longer time period. For this reason, the IND model was used to evaluate the mucosal

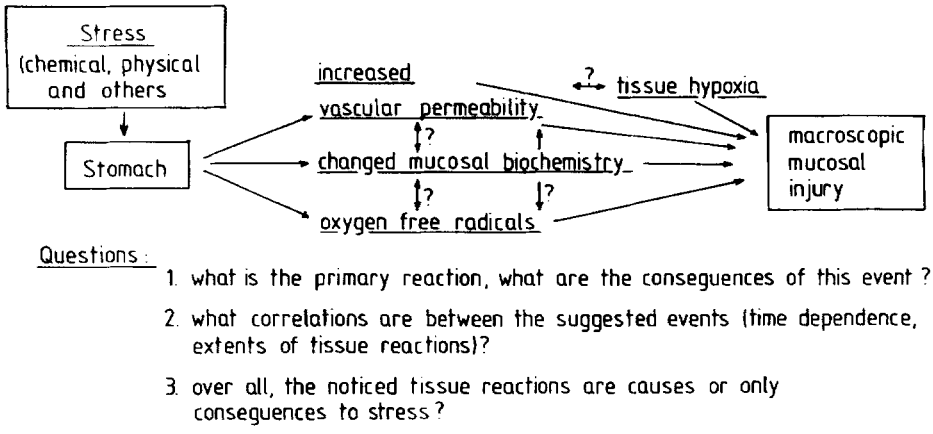

Figure 1. A schematic presentation of our suggested hypothesis for the development of chemically induced gastric mucosal injury

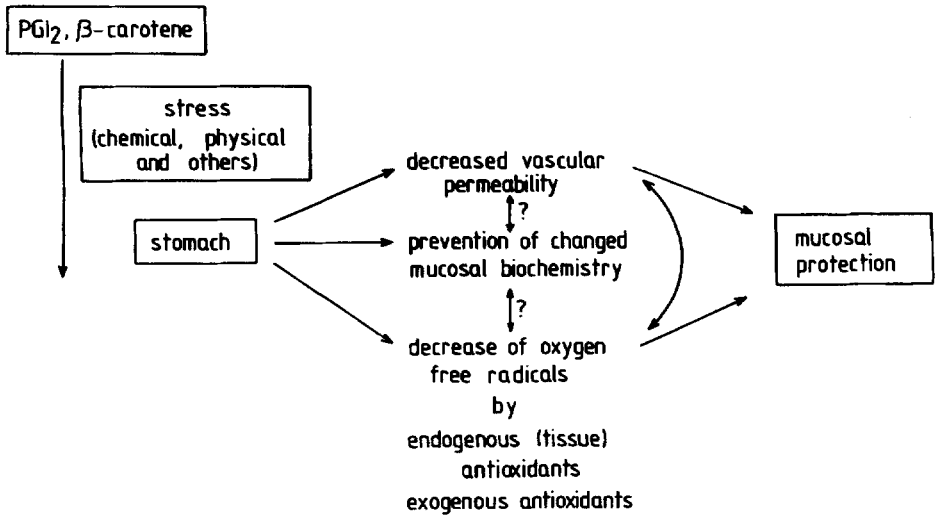

Figure 2. A schematic presentation of our suggested hypothesis for prostaglandin and β-carotene-induced gastric mucosal defence against indomethacin-produced gastric mucosal lesions

biochemical changes responsible for the development of acute IND-induced gastric mucosal damage.

The aims of this study were: (a) to evaluate the IND-induced gastric mucosal damage dependence with time; (b) to test the β-carotene-induced time- and dose-dependent changes in gastric mucosal biochemistry (ATP, ADP, AMP, cAMP, adenylate pool, 'energy charge', ratio of ATP/AD) after IND administration; (c) to study the possible correlations between the changes of PGE_2 and cAMP in the gastric mucosa and the development of IND-induced gastric mucosal damage and its prevention by β-carotene.

MATERIALS AND METHODS

The observations were carried out on both sexes of CFY-strain rats, weighing 180–210 g. The animals were fasted for 24 h before the experiments but they received water *ad libitum*.

The gastric mucosal lesions were produced by subcutaneous administration of IND at a dose of 20 mg/kg. The β-carotene was dissolved in sunflower oil and was given intragastrically to animals at the time of IND administration.

The observations were carried out in different series:

Group A: The animals were treated with IND and were sacrificed 4 h after IND administration, when the number and severity of gastric mucosal lesions were noted.

Group B: The same observations were carried out as in group A; however, the animals received different doses of β-carotene ig at the time of IND administration. The animals were sacrificed 4 h after IND administration, when the number and severity of gastric mucosal lesions was noted, and the biochemical measurements were carried out on the total homogenate of gastric mucosa (Figure 3).

Group C: The animals were treated with IND and sacrificed at 0, 1, 2, 3 and 4 h after IND administration, when the number and severity of gastric mucosal lesions, gastric mucosal PGE_2 and cAMP were measured (Figure 4).

Group D: The same observations were carried out as in group C but the animals received different doses of β-carotene (ig) at the time of IND administration. The number and severity of gastric mucosal lesions were noted. The severity of gastric mucosal lesions was estimated by a semiquantitative scale system published previously [13].

The gastric fundic mucosa was removed immediately after the sacrifice of animals and was placed in liquid nitrogen. The tissue levels of ATP, ADP, AMP and lactate were enzymatically measured (Boehringer Ingelheim, Germany), while the tissue level of cAMP by RIA (Beckton-Dickinson, Orangeburg, SC, USA) from the total

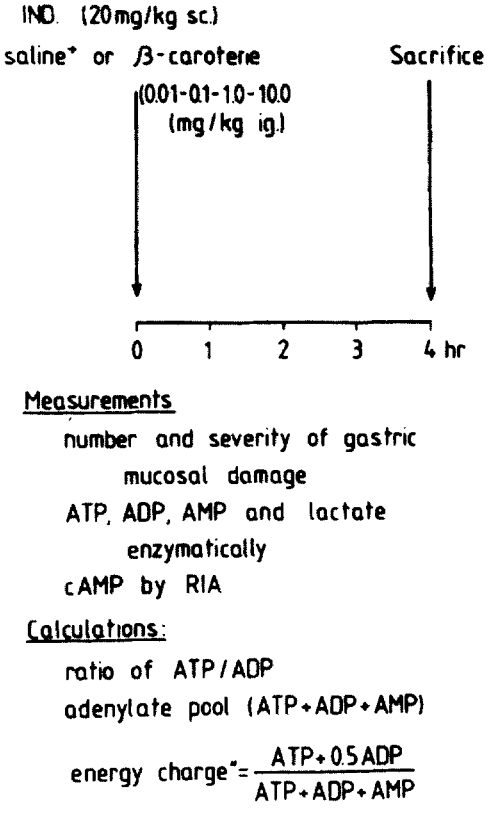

Figure 3. Experimental protocols for the study of biochemical backgrounds of indomethacin-induced gastric mucosal damage and its prevention by β-carotene

homogenate of the gastric mucosa was carried out in ice-cold 0.5 mol/L perchloric acid. The protein content was assayed by the method of Lowry et al. [14]. The ratio of ATP/ADP, the values of the adenylate pool (ATP+ADP+AMP) and the 'energy charge' [(ATP+0.5ADP)/(ATP+ADP+AMP)] were calculated [15]. The biochemical results were expressed to 1 mg mucosal protein (mean ± SEM).

The unpaired Student's *t*-test and the Mann–Whitney test were used for statistical analysis of the results.

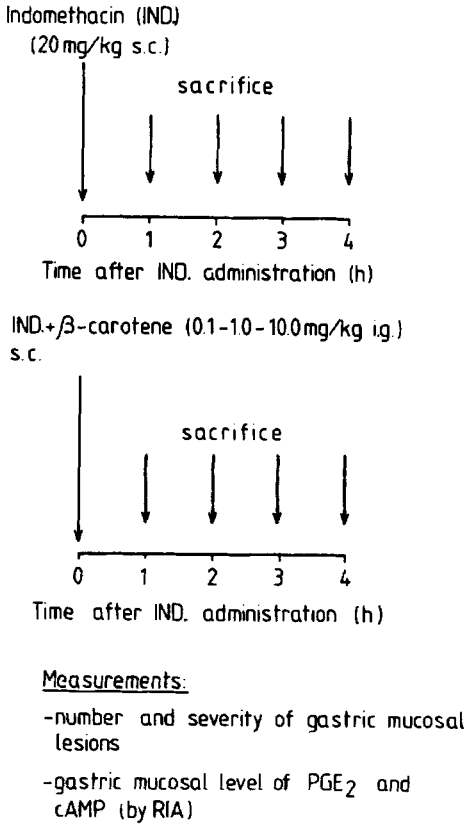

Figure 4. Protocols for the study of gastric mucosal levels of PGE_2 and cAMP in IND-treated rats with or without β-carotene treatment (upper part and lower part, respectively). The development of gastric mucosal damage (number and severity) and actual levels of PGE_2 and cAMP were analysed 0, 1, 2, 3 and 4 h after IND (20 mg, sc) administration

RESULTS

Changes in the gastric mucosal cellular energy systems during the development of indomethacin-induced gastric mucosal damage (4 h observation)

An increased transformation of ATP into ADP, and a decrease in ATP–cAMP conversion was found during the development of IND-induced gastric mucosal damage (see Figures 5 and 6).

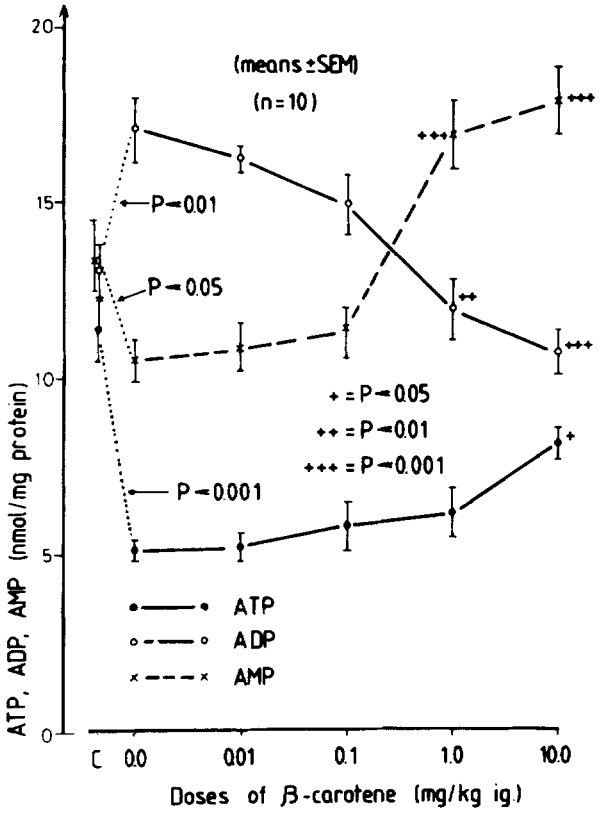

Figure 5. Dose-dependent changes in gastric mucosal levels of ATP, ADP and AMP in 4-h IND-treated rats with and without β-carotene (C = untreated control rats)

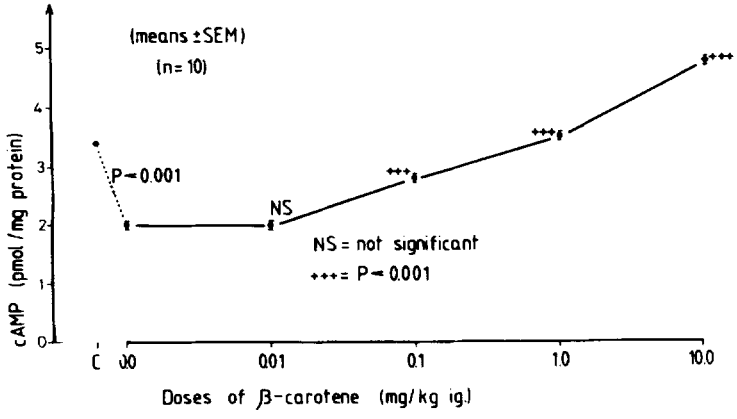

Figure 6. Dose-dependent elevation of gastric mucosal cAMP in 4-h IND-treated rats with β-carotene treatment

β-Carotene-induced changes in the gastric mucosal cellular energy systems in 4-h IND-treated rats

Intragastrically applied β-carotene dose-dependently reduced the number and severity of IND-induced gastric mucosal lesions (Figure 7).

The transformation of ATP into ADP was decreased, while the transformation of ATP into cAMP increased dose-dependently with β-carotene in 4-h rats (Figures 5 and 6).

The ratio of ATP/ADP decreased significantly during the development of IND-induced gastric mucosal damage, and its value dose-dependently increased with β-carotene (Figure 8). However, surprisingly, no significant change was obtained in the values of 'energy charge' under these circumstances. No significant changes were obtained in the tissue levels of adenylate pool and lactate of the rat gastric mucosa under the above-mentioned experimental conditions.

Figures 5, 6 and 8 summarize and indicate clearly that β-carotene-induced gastric mucosal protection is dose-dependently associated with:

(a) The decreased extent of ATP–ADP transformation; and

(b) The increased extent of ATP–cAMP transformation in 4-h IND-treated rats.

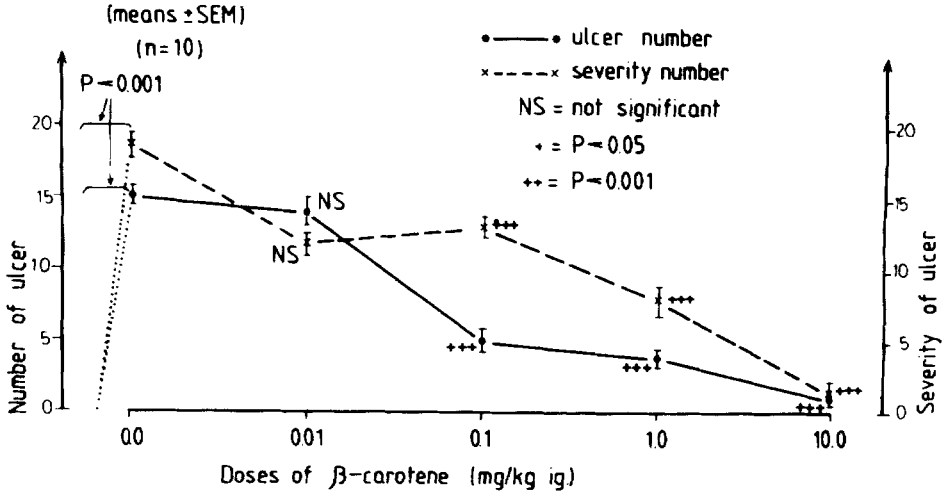

Figure 7. Dose-dependent preventive action of β-carotene on the severity and number of gastric mucosal lesions in 4-h indomethacin-treated rats

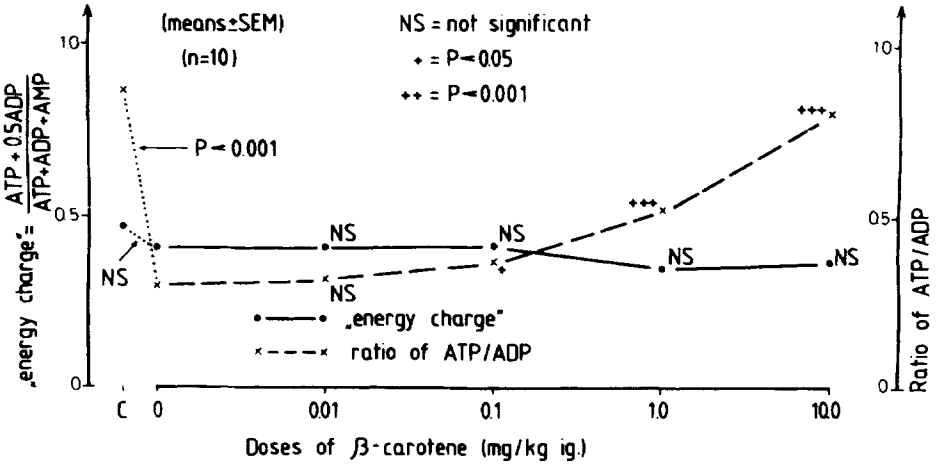

Figure 8. Changes in the ratio of ATP/ADP in 4-h IND-treated rats with and without β-carotene. No change was obtained in the 'energy charge' [(ATP+0.5ADP)/(ATP+ADP+AMP)]

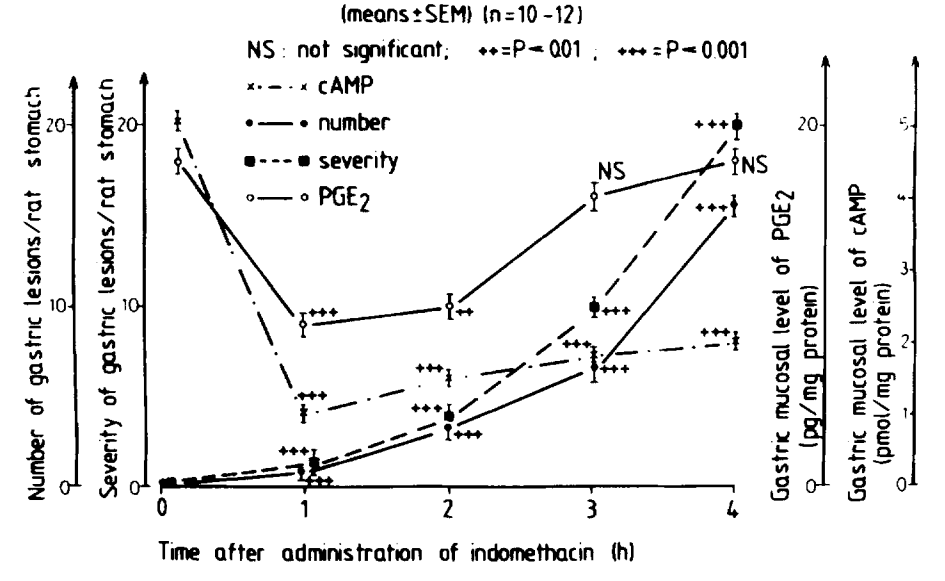

Figure 9. Comparison of the changes of gastric mucosa of 4-h indomethacin-treated rats with and without β-carotene treatment

Correlation between the development of gastric mucosal damage and changes of gastric mucosal PGE_2 and cAMP levels

The gastric mucosal cAMP and PGE_2 levels were significantly decreased at 1 and 2 h, while gastric mucosal damage was increased 4 h after IND administration (Figure 9). The most impressive correlation (Figure 10) exists between the development of gastric mucosal damage (number) and the decrease in gastric mucosal cAMP. The decrease in cAMP was maximal 1 h after IND treatment; no similar extent of decrease was obtained for gastric mucosal PGE_2 (Figure 9). A decreased gastric mucosal cAMP level was observed after 4 h in IND-treated animals when gastric mucosal damage was at its peak.

To clarify the importance of the decrease in gastric mucosal cAMP (the Δ values for changes in gastric mucosal cAMP over the time period of 0 to 1 h) after IND treatment, the correlation between decrease in gastric mucosal cAMP (between 0 and 1 h after IND administration) and number ($y = -49.6x + 117.6$; $r = -0.89$; $p < 0.001$) or severity ($y = -52.2x + 135$; $r = -0.97$; $p < 0.001$) was analysed in IND-treated animals (Figure 10). These calculations showed statistically significant negative correlations.

β-Carotene-induced gastric mucosal protection in IND-treated rats

The number of IND-induced gastric mucosal lesions was dose-dependently reduced by intragastric β-carotene administration (Figure 11).

The gastric mucosal PGE_2 level decreased 1 and 2 h after IND treatment ($p < 0.05$). However, there were no dose-dependent correlations between the changes in PGE_2 level and β-carotene-induced gastroprotection (Figure 12).

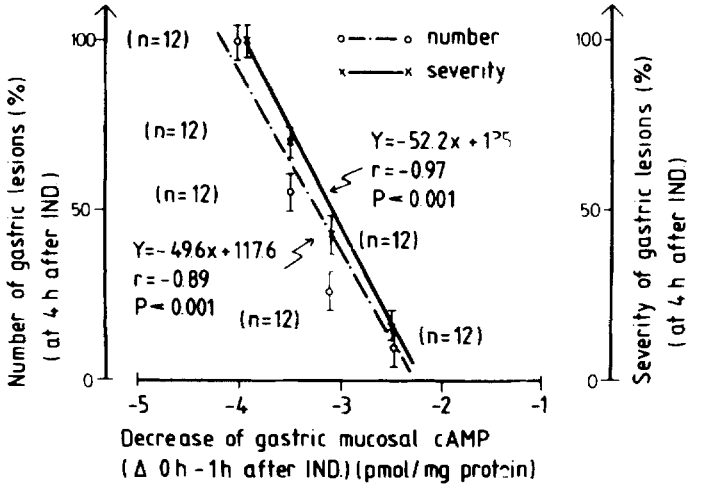

Figure 10. Time-dependent changes in the development of gastric mucosal lesions (number and severity), and gastric mucosal PGE_2 and cAMP levels after administration of IND (20 mg, sc)

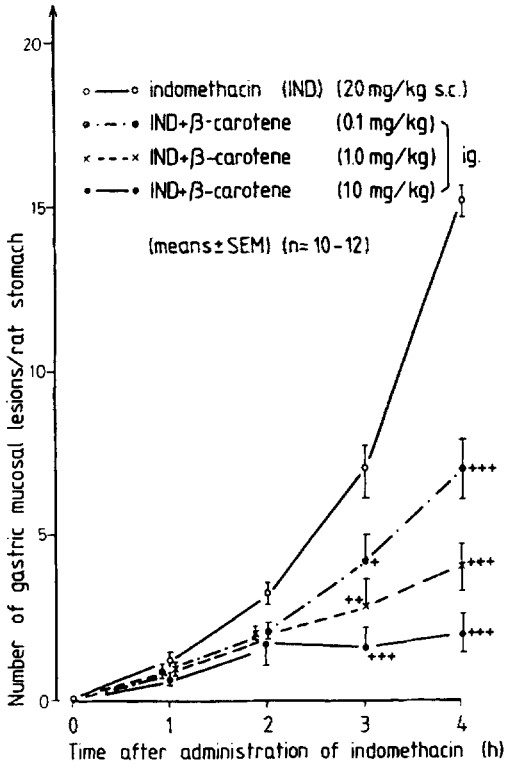

Figure 11. β-Carotene-induced changes in the number of gastric mucosal lesions against time after administration of IND

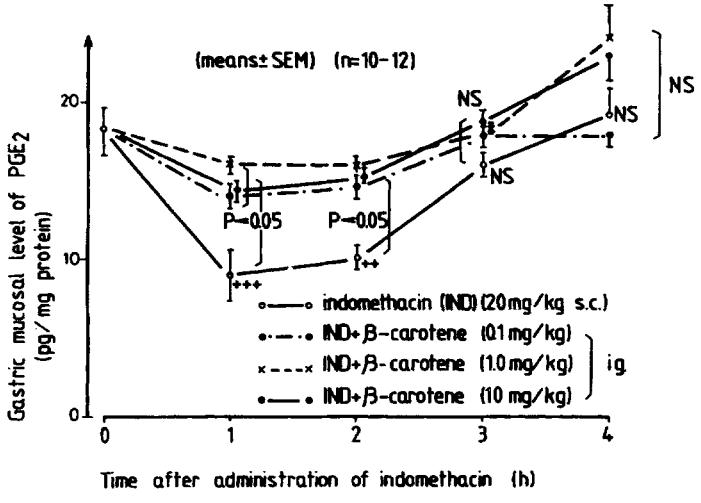

Figure 12. Time-sequence changes in the gastric mucosal levels of cAMP and PGE_2 induced by β-carotene in IND-treated rats

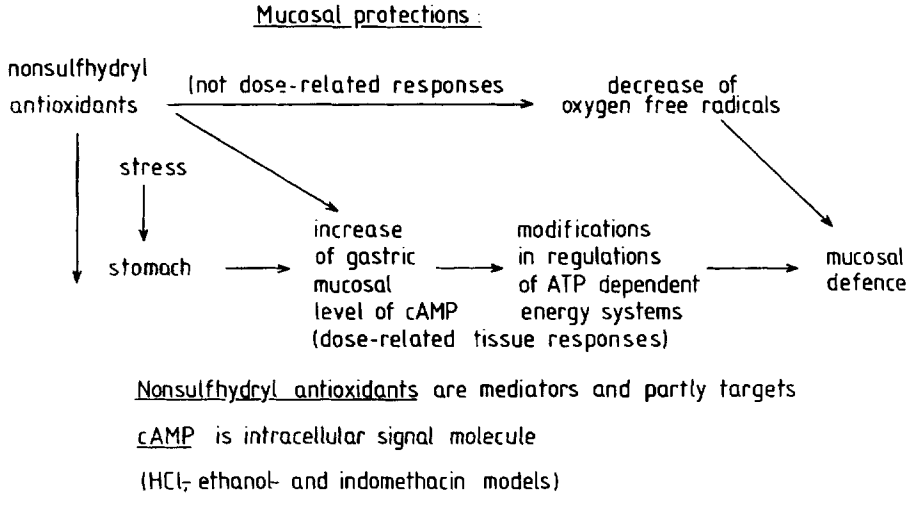

Figure 13. A brief schematic summary of our hypothesis for β-carotene-induced protection of gastric mucosa after IND administration

The gastric mucosal cAMP increased dose and time dependently with β-carotene in IND-treated animals.

Significant correlations were found between the β-carotene-induced decrease in the number of gastric mucosal lesions and the increase in gastric mucosal cAMP level at 1 h ($y = -29.9x + 43.9$; $r = 0.98$; $p < 0.001$) and 4 h ($y = 24.5x + 12.3$; $r = 0.99$; $p < 0.001$) after IND treatment.

DISCUSSION

Unspecific stresses (including chemical-induced stress) produce some specific reactions in target organs (like increase or decrease of gastric secretory responses, etc.).

The plasma membrane plays a key role in the regulation of cells. Interestingly, the responses of cells are also specific; however, the energy supply systems are unspecific in the background to these stresses. Furthermore, the number of receptors for neuropeptides, hormones and mediators is about 130–140, which modify specifically the cells. At the same time, the number of chemicals which damage the gastric mucosa is enormously large; indomethacin is only one of these.

In this study, the development of gastric mucosal damage was associated with a significantly increased degree of ATP–ADP transformation and decreased ATP–cAMP transformation. The IND treatment also inhibited PG synthesis.

It was also proved that the decrease in gastric mucosal cAMP precedes the macroscopic appearance of gastric mucosal damage in IND-treated animals.

Of course, the decrease in gastric mucosal cAMP may be a primary or secondary event due to IND-induced gastric mucosal damage. We believe that the significant decrease in gastric mucosal cAMP is a consequence of increased ATP–ADP transformation in the gastric mucosa produced by IND. Surprisingly, the gastric mucosal damage in this model was associated with active metabolic responses of gastric mucosa.

β-Carotene is a micronutrient with antioxidant properties. The scavenging effect of β-carotene was also suggested in its gastric mucosal protective effect. β-Carotene (like vitamin A) produced contraregulatory changes in membrane-bound ATP-dependent energy systems during the development of IND-induced gastric mucosal damage [2].

It is true that the PGE_2 level increased in the gastric mucosa without any dose-dependent changes, after application of β-carotene in IND-treated animals. The β-carotene produced dose- and time-dependent changes in the gastric mucosal cAMP in IND-treated rats, demonstrating a close correlation with its gastric mucosal protection.

These results clearly indicate that β-carotene-induced gastric mucosal protection does not depend only on its scavenging property. This conclusion is also supported by other observations, i.e. when the gastric mucosal protective effect of β-carotene disappeared after surgical vagotomy and adrenalectomy [16,17]. β-Carotene (as a non-sulphydryl antioxidant) is part mediator and part target, while the cAMP is an intracellular signal molecule (Figure 13).

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