

PROTECTION BY CALCITONIN IN INDOMETHACIN-INDUCED GASTRIC ULCERATION IN RATS

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Abstract

Calcitonin is a polypeptide with hypocalcemic effect, but also with significant action on the digestive system. After 10 years from its discovery was found that parenteral calcitonin inhibits resting and pentagastrin-induced gastric acid secretion in humans and laboratory animals. Consecutively, anti-ulcer effect of calcitonin was observed on animal models and peptic ulcer patients.

In one acute indomethacin-induced gastric ulcer model, calcitonin is very effective as ulcer treatment. Administered in 2,5; 5; 10 and 20 UI/ kg reduces significantly the occurrence, number and severity of the gastric ulcerations produced by indomethacin. Gastro-protective effect of calcitonin in this case may be explained by the mucosal citoprotection, decrease of acid secretion and inhibition of stomach motility.

Keywords: Calcitonin, Indomethacin, ulcer.

Foreword

Calcitonin is a polypeptide hormone, secreted in the C cells of the thyroid gland [1] and other tissues [2], and its first role is in calcium homeostasis. Calcitonin decreases plasma calcium and phosphor [3,4]. Moreover, calcitonin inhibits gastric secretion in humans [5] and animals [6,7,8,9,10], and also has gastro protective effect in experimental ulcer models [8,11,12]. Hence, we enquired calcitonin effects on indomethacin-induced gastric ulcer in rats.

Material and method

All experiments were done on albino Wistar Bratislava female rats from UMF "Iuliu Hațieganu" Biobase, Cluj-Napoca. Animals were kept in standard lab conditions, normal feeding, water ad libitum and natural dark/light cycle. Five randomized groups were formed of 10 rats each. After 12 hours fasting period, all groups received a single dose of 50 μmoles/kg indomethacin. Animals were sacrificed after 8 hours. Stomachs were taken into study, opened at the greater curvature, and washed with saline solution, examined with a 5x magnifying lens. Mucosal shape and ulcer indexes were considered. The 5 groups were as follows: I- control, only indomethacin; II- 2.5 UI / kg calcitonin with 15 minutes before indomethacin and

repeated 3 and 5 hours later; III, IV and V were treated with 5, 10 respectively 20 UI /kg calcitonin with the same protocol as group II.

Statistical analysis

Parametric and non-parametric ulcer indicators were used. Ulcer occurrence was taken as a percentage and statistical significance were represented as 2x2 tables. Gastric ulcerations were counted differentially: total number of ulcers, number of ulcers larger than 1mm, and extended, confluent ulcers. Antral ulcers were taken into account separately. These indexes were expressed as mean and standard error ($\bar{x} \pm e.s.$) in a bilateral "t" Student test. All indicators were compared among groups and were considered significant at $p < 0.05$ [13]. Severity of ulcers were calculated by ulcer index (U.I.) on a scale of 0-4. Based upon U.I. we calculated the protection rate (P.R.) with the following formula $P.R. \% = (U.I. \text{ control} - U.I. \text{ treated}) / U.I. \text{ control} \times 100$. Percentage of P.R. was considered relevant at absolute values greater than 33% [14].

Drugs:

1. Indomethacin pulvis. A 2% suspension was prepared in methyl-cellulose mucilage according to Romanian Pharmacopoeia X edition. Injected dose was 50 μmoles/kg (17.89 mg/kg) in 1 ml/ kg volume after dilution adjustment.

2. Synthetic eel calcitonin (Calcitonin® Sclavo

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Siena) liofilized powder. After dissolving and diluting accordingly to the above mentioned doses, 2 ml / 100 g was injected.

Results

No differences in behavior were noted between the five groups.

Occurrence of large ulcers (> 1mm) is 100% in the control group. Confluent ulcers have 10% occurrence in the control group. Calcitonin reduces the percentage of gastric lesions for all types of ulcerations. Calcitonin groups do not have confluent ulcers (Table I).

Control group has 11.9 ± 2.44 gastric ulceration and 7.7 ± 1.99 large ulcers. After calcitonin administration a substantial decrease of these two indexes is noted. This is more important at the larger doses of calcitonin (Table II).

Severity of ulcers is 1.65 ± 0.27 in the control group and drops significantly in the calcitonin groups (Table II). PR shows highly significant protection in the calcitonin groups (Table III).

Table I. Ulcer's incidence.

| Groups | | % | | |
|----------------------|----------|-------|-------|-----------|
| | | Total | > 1mm | Confluent |
| I | Control | 100 | 100 | 10 |
| II | C 2.5 UI | 40 | 10 | 0 |
| III | C 5 UI | 80 | 40 | 0 |
| IV | C 10 UI | 20 | 0 | 0 |
| V | C 20 UI | 10 | 0 | 0 |
| p (tables 2x2) | I vs. | II | 0.296 | 0.047 |
| | | III | 0.757 | 0.296 |
| | | IV | 0.075 | 0.011 |
| | | V | 0.047 | 0.011 |

Legend: C - calcitonin.

Discussion

The first to notice is the high significance of gastroprotection of calcitonin in 8 hours period. All ulcer indicators are affected. Our laboratory already demonstrated this effect on restraint and reserpine-induced ulcer models. We could try to explain this effect by calcitonin mucosal cytoprotection. This indirect effect is probably

by prostaglandin release, observed on ex vivo mucosa models [15], and in the same extent may be the cause of inhibition of gastrics ecretion, observed in experimental [16] and clinical conditions [17,18]. An extra interest in the indomethacin model is the inhibition of gastric motility induced by calcitonin [19,20]. Indomethacin was found to accelerate gastric evacuation, fact that was vaguely related to its ability to damage gastric mucosa [15]. As much as its hypocalcemic effect, this seems not to be involved in the protective action of calcitonin against ulcers [21]. A central nervous effect also is possible [22,23]. Central calcitonin receptors exist [24] and intraventricular injection of calcitonin reduces gastric secretion in rats [25]. Extensive literature data exists regarding restraint [6,26,27,28,29], anti-inflammatory drugs [30,31], reserpine [32], acetic acid [33] and corticosteroid [34] experimental ulcer models, on calcitonin gastroprotection. Clinical data also shows symptom relief and antisecretory effect of short-term calcitonin administration in peptic ulcer patients [35,36]. Clinical use is unfortunately limited by the requirement of frequent parenteral administration.

Conclusions

1. Indomethacin induces in 100% of cases ulceration in rat stomach corpus.
2. Calcitonin in the used doses decreases significantly occurrence, number and severity of gastric mucosal lesions induced by indomethacin.
3. Gastroprotective effect can be explained mainly by indirect citoprotection and inhibition of gastric acid secretion.

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Table II. Number of ulcers ($\bar{x} \pm e.s.$)

| Groups | | Body weight | Total | > 1 mm | U.I. |
|--------|----------|------------------|-----------------|----------------|-----------------|
| I | Control | 144.8 ± 6.07 | 11.9 ± 2.44 | 7.7 ± 1.99 | 1.65 ± 0.27 |
| II | C 2.5 UI | 143.6 ± 5.76 | 4.1 ± 2.9 | 1.3 ± 1.3 | 0.35 ± 0.19 |
| III | C 5 UI | 146.1 ± 6.7 | 3.2 ± 0.82 | 0.8 ± 0.35 | 0.5 ± 0.1 |
| IV | C 10 UI | 143.7 ± 6.28 | 0.2 ± 0.13 | 0 | 0.1 ± 0.07 |
| V | C 20 UI | 144.2 ± 5.67 | 0.2 ± 0.2 | 0 | 0.03 ± 0.03 |
| p | I vs. | II | 0.8877 | 0.0548 | 0.0013 |
| | | III | 0.8874 | 0.0034 | 0.0012 |
| | | IV | 0.9013 | 0.0002 | 3.97E-05 |
| | | V | 0.9433 | 0.0002 | 3.5E-07 |

Legend: C - calcitonin.

Table III. Protection ratio.

| Groups | R p % | | | |
|-------------|---------------------|-------------------|--------------------|--------------------|
| | Calcitonin 2.5 u.i. | Calcitonin 5 u.i. | Calcitonin 10 u.i. | Calcitonin 20 u.i. |
| Control vs. | 78.79 | 69.67 | 93.94 | 98 |

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