THE EFFECTS OF ACETAMINOPHENE ON THE ULTRASTRUCTURE OF RAT GASTRIC MUCOSA*

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ABSTRACT

Objective: Investigations revealed the toxic effects of high doses of acetaminophene on liver, kidney and bone marrow. Gastrointestinal disorders such as gastric ulcer were described in patients with chronic liver disease and liver cirrhosis. We aim to investigate the ultrastructural effects of high doses of acetaminophene on gasric fundic mucosa as there was a limited area of investigation on this subject.

Methods: A single dose of acetaminophene (600 mg/kg) was administered to the rats and then they were sacrificied at 3rd(Group I), 6th(Group II) and 24th (Group III) hour following the acetaminophene administration.

Results: In group I, fundic glands were severely degenerated. The most affected cells were the parietal cells with restricted intracellular secretory canaliculi and mitochondrial cristae degeneration. Endoplasmic reticulum of the chief cells were dilated and secretory granules were scanty. ECL and AL cells among the endocrine cells were also affected. Although the degeneration still existed in group II chief cells presented some signs of recovery. ECL and AL cells were active reflecting both the synthesis and secretory phases. Mitotic activity was increased in group III and gasric glands seemed normal, ECL cells were in secretory phase and AL cells were active in synthesis and storage phase.

Conclusion: It was concluded that high doses of acetaminophene resulted in reversible degenerative changes in the gastric glands and the time-related regenerative process was also revealed.

Key Words: Gastric mucosa, acetaminophene, morphology, electronmicroscopy.

INTRODUCTION

Toxic effects of long-term and high dose of acetaminophene usage on the liver, kidney and gastrointestinal tract were revealed in many studies (1-9). As an antipyretic and analgesic, acetaminophene is still being used although its effects on the gastrointestinal tract are under investigation.

The present study aims to describe the ultrastructural changes on the rat gastric mucosa following high doses of acetaminophene.

MATERIAL AND METHODS

Wistar albino female rats (200-220 gr.) used in this study were fed on a standard diet and water ad libitum. One control and 3 experimental groups (Group I, II and III) were established. Control group rats (n=5) were given 1 ml tap water by gavage. Other groups (n=5 for each group) were administered 600 mg/kg acetaminophene (Minoset-Roche) dissolved in 1 ml tap water by gavage. Experimental groups were sacrificied at 3rd (Group I), 6th (Group II) and 24th hour (Group III) following gavage.

For transmission electronmicroscopic examination, stomach fundic mucosa fragments of about 1 mm^3 were fixed in 2.5% phoshate-buffered glutaraldehyde (pH 7.4) and postfixed in 1% O_SO_4 solution for 1 hour. Following Vestopal W embedding, ultrathin sections (400-600 A°) were stained with uranyl acetate and lead citrate and examined in a JEOL 100 C transmission electronmicroscope.

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RESULTS

TEM investigations for all groups were especially focused on parietal cells, chief cells, ECL cells and AL cells population of the gastric fundic mucosa. The following results for each group were observed in 100% of the experimental animals belonging to the experimental group.

Parietal cells of the control group were normal with their intracellular secretory canaliculi and numerous mitochondria (figs.1,2). Chief cells had usually supranuclearly located zymogenic secretory granules and abundant granular endoplasmic reticulum (figs. 1,2). Enterochromaffin- like cells (ECL) and A- like cells (AL) were also noticed among the gastric mucosa (figs. 1,2). ECL cells located at the basal portions of the fundic glands had nucleus with homogeneous chromatin material which showed peripheral aggregations. Membrane-bounded secretory granules at ECL cells described a characteristic eccentric localization of the secretory material (fig.1). AL cells were basally located within the fundic glandular epithelium. They were spherical cells with central nucleus. They possessed membrane-bounded spherical homogeneous secretory granules of equal size (fig.2).

The most affected cell type within the group I was the parietal cells. They were observed with dilated microvilli and restricted lumen of the intrasecretory canaliculi. Aggregated dense material and cristae degeneration were noticed in mitochondria (fig.3). Zymogenic cells described an almost affected ultrastructure reflecting synthesis phase. Dilated endoplasmic reticulum cisterns were widely distributed within the cytoplasm. Scanty, small zymogenic granules were noticed. Golgi complex with dilated saccules were apparent in numerous cells (fig.4). EL and AL cells' ultrastructure reflected both an extreme secretion and degeneration. ECL cells' nuclei were pyknotic with dense chromatin material. Nuclear membranes were dilated. Abundant empty secretory vacuoles and swollen mitochondria with crista degeneration were observed (fig.4). A low degree of ultrastructural change was noticed in AL cells. Secretory material synthesis was obvious with numerous secretory granules of various sizes dispersed in cellular cytoplasm. Lipofuchsin granules were increased in number (fig.3).

In group II, although parietal cells exhibited some structural degeneration, chief cells looked similar to those of the control group. Intracellular secretory canaliculi of the parietal cells were narrowed together with a prominent increase in the tubulovesicular structures. Mitochondria with cristae degeneration were shrunk (fig.5). Chief cells presented numerous GER membranes and abundant zymogenic granules of various sizes (fig.6). The AL cells were the most affected cell type in group II. Nuclei of those cells were invaginated and acquired an ovoid form whereas their chromatin material was clustered on the nuclear membrane. Diffuse secretory granules and vacuoles were increased in number (fig.5) The ECL cells were similar to those of the control group. Empty secretory granules reflected the secretory phase (fig.6).

In group III, mitotic activity was increased in gastric glands. Synthesis and secretory phases in parietal cells and chief cells were noticed. Lumen of the intracellular secretory canaliculi of the parietal cells were dilated (fig.7). Zymogenic cells had numerous GER membranes and the zymogenic granules. ECL and AL cells appeared normal. The AL cells were in the synthesis phase with numerous secretory granules (fig.7) and the ECL cells were in the secretory phase (fig.8) which described an active cell state.

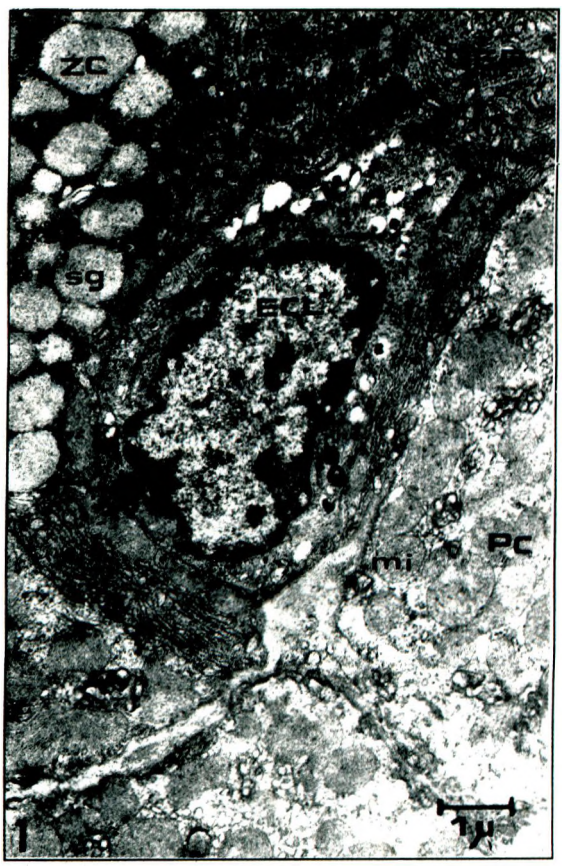

Fig. 1: An ECL cell located between a parietal cell (PC) and a chief cell (ZC) is seen at the control group. mi: Mitochondrion; sg: secretory granule GER: Granular endoplasmic reticulum.

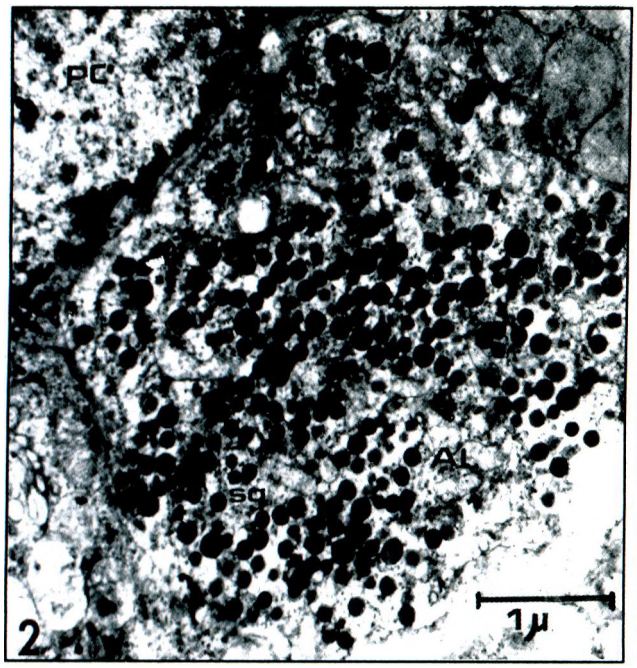

Fig. 2: An AL cell rich in secretory granule (sg) is seen at the basal part of a parietal cell (PC) within the control group.

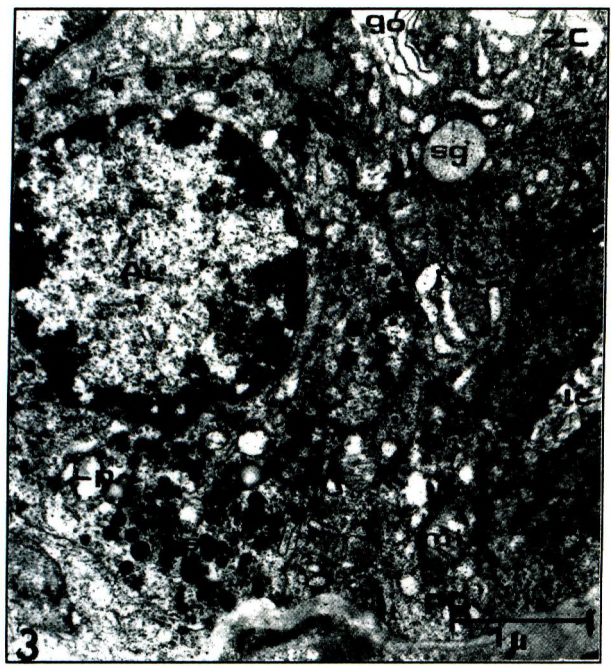

Fig. 3: Group I shows an AL cell with numerous secretory granules (sg) adjacent to a chief cell (ZC) and a parietal cell (PC). Lp: Lipofuchsin granule; Ic: Intracellullar secretory canaliculi; go: Golgi complex; mi: Mitochondrion.

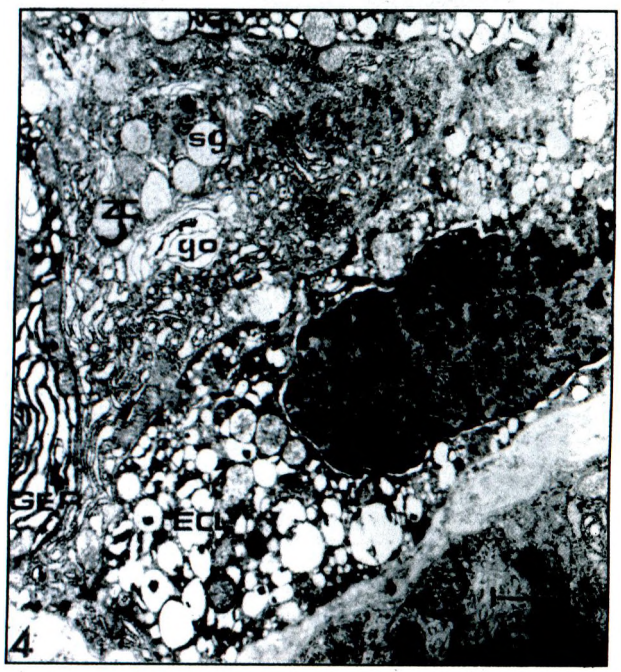

Fig. 4: An ECL cell with numerous secretory granules (sg) is observed at the basal part of a chief cell (ZC). GER: Granular endoplasmic reticulum. go: Golgi complex.

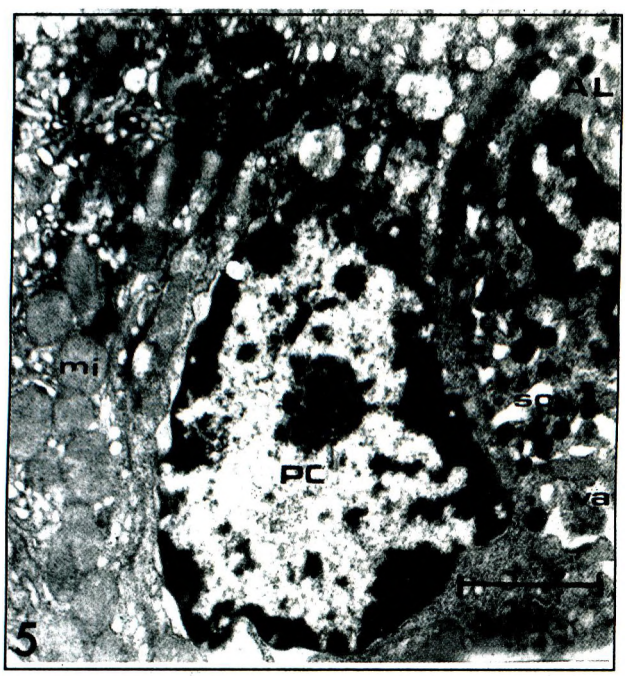

Fig. 5: A parietal cell (PC) with many tubulovesicular structures and an AL cell are shown in group II. va: Vacuole; sg: secretory granule;mi: Mitochondrion

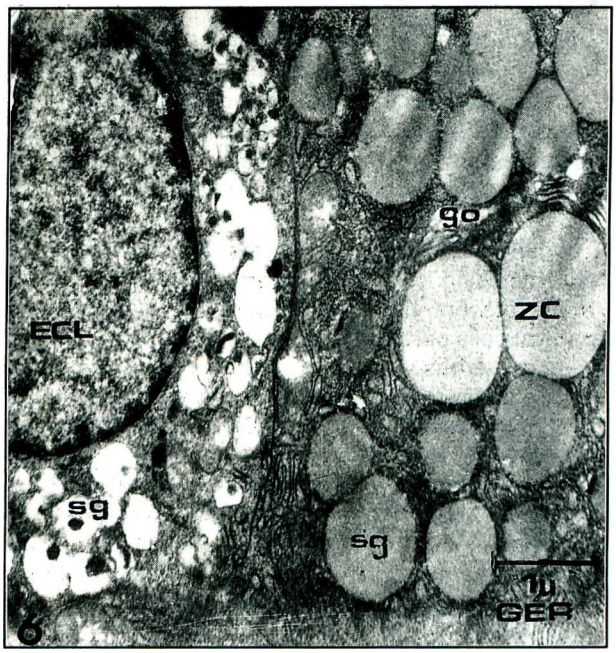

Fig. 6: In group II, a chief cell (ZC) with numerous secretory granules (sg) and a vacuolated ECL cell are seen. GER: Granular endoplasmic reticulum: go: Golgi complex.

Fig. 7: Dilated intracellular secretory canaliculi (lc) of the parietal cell (PC) and an AL cell rich in secretory granules are noticed in group III.

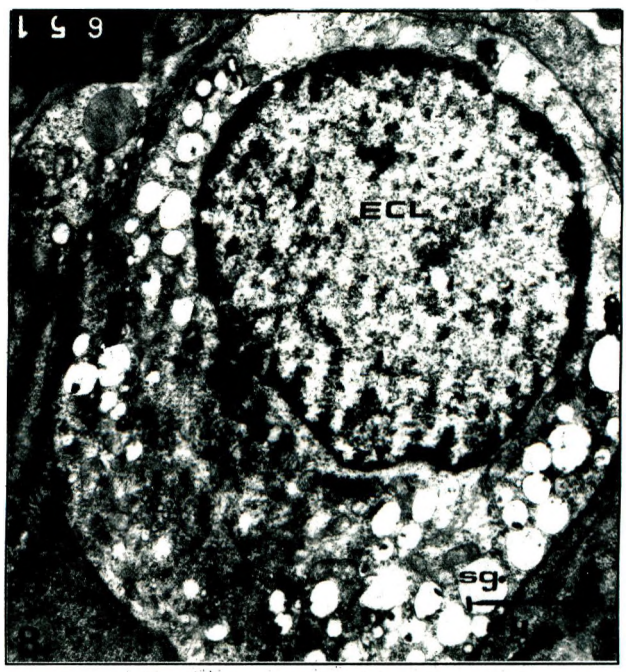

Fig. 8: An ECL cell in the secretory phase is seen in group III. sg: Secretory granule.

DISCUSSION

Acetaminophene is a drug used since the late 19th century. Although adverse effects were demonstrated, it is still widely consumed as an analgesic and antipyretic agent. High doses of acetaminophene and its continuous usage resulted in pathological changes in many tissues and organs such as liver, kidney and bone marrow. Investigations revealed the hepatic necrosis and nephrotoxicity in cases of high dosage and continous usage of acetaminophene. Genotoxic effects on the bone marrow cells were revealed by

Giri et al. (10) in an experimental study. Gastrointestinal system was also affected by various doses and usage time of acetaminophene. As there were limited investigations concerning relationships of gastrointestinal complaints parallel to the acetaminophene usage, the present study was planned to investigate the ultrastructural effects of acetaminophene on the rat fundic mucosa. Nakae et al. (2) revealed hepatic necrosis when 500 mg/kg paracetamol was administered to Sprague- Dawley rats. Oxidative enzymes were decreased in the mentioned study. Many investigators postulated a decrease in some enzymes, especially cytochrome oxidase which is an important enzyme in drug detoxification. Their absence results in cellular degeneration and death (3,4,11). Nazareth et al. (3) pointed out the relationship between mitochondrial membrane potential and cytochrome P450 activity on rat liver slices. The direct effect was on mitochondria followed by other cellular organelle degenerations. Our present study revealed the most prominent adverse effects on gastric glands especially in group I. Mitochondria and GER membranes were the most affected organelles. Fundic glandular parietal cells appeared with many lysosomes and mitochondria with dense matrices and crista degeneration. Intracellular secretory canaliculi were swollen.

Exogenous administration of aspirin and HCl in an experimental study by Guth et al. (7) resulted both in gastric mucosal degeneration and an increase in pepsin secretion. Parallel to the mentioned results, zymogenic cells in group I possessed swollen endoplasmic reticulum cisterns and active Golgi complex. We concluded that those cells were affected in synthesis and secretion phases.

Several studies defined Amine Precursor Uptake and Decorboxylation cells (APUD) or endocrine cells which are important in both coordination and stimulation of the functional activity of the gastrointestinal organs. The relationship among the endocrine cells in different gastric fluid activity range is important to clarify their roles in the regulation of the acid secretion. Many studies emphasized the important effects of enterochromaffine-like cells (ECL), gastrin cells (G), somatostatin cells (D) and A-like cells (AL) on acid secretion (12-19). Hyperacidity caused several effects on ECL, G and AL cells such as an increase both in number and functional activity. Thus, G cells have a trophic effect on parietal cells by means of gastrin secretion. Meanwhile they stimulate ECL cells to secrete histamine (12,16,18). Histamine is known to increase acid secretion in parietal cells (12,15,19). We observed the morphology reflecting a hyperacidity state in ECL cells of group I, with many empty vacuolated granules.

Many studies pointed out the importance of enteroglucagon secreted by AL cells on the acid balance equilibrium by means of acid secretion (13,14). We noticed the active state of AL cells of group I in synthesis and secretion phases and concluded that acetaminophene caused an increase in the acid content of the gastric mucosa.

Toghill et al. (4) reported the hypergylcemia in patients using long-term acetaminophene (35 g/day). In the present study, we observed active phase of parietal cells parallel to the storage phase of AL cells with numerous secretory granules. While parietal cells were in resting state, AL cells were vacuolated with few secretory granules. Degenerative changes observed at 3 hour group seemed to decrease in groups II and III. They looked similar to the control group. Degenerations in parietal cells were still observed in 6 hour group whereas in 24 hour group few degenerative changes were revealed. In group III, gastric glandular cells were normal. A rapid regeneration in group III was evident with numerous mitotic figures in gastric mucosa cells.

We conclude that high doses of acetaminophene may cause serious degenerations on the gastric glands which might be recovered by time.

REFERENCES

1. *Bonkowsky HL, Mudge CH, Murtry RJ. Chronic hepatic inflammation and fibrosis due to low doses of paracetamol. Lancet 1978;36:1016-1018.*
2. *Nakae D, Yamamoto K, Yoshiji H, et al. Liposome-encapsulated superoxide dismutase prevents liver necrosis induced by acetaminophene. J Am Path 1990;136:787-795.*
3. *Nazareth WM, Sethi JK, McLean AE. Effect of paracetamol on mitochondrial membrane function in rat liver slices. Biochem Pharmacol 1991;42:931-936.*
4. *Toghill PJ, Williams R, Stephens JD, Carroll JD. Acute hepatic necrosis following and overdose of paracetamol. Gastroenterology 1969;56:773-776.*
5. *Trumper L, Girardi G, Elias MM. Acetaminophene nephrotoxicity in male Wistar rats. Arch Toxicol 1992;66:107-111.*
6. *Brodie DA, Chase BJ. Role of gasric acid in aspirin-induced irritation in the rat. Gastroenterohepatol 1967;63:604-610.*
7. *Guth PH, Aueres D, Paulsen G. Topical aspirin plus HCl gastric lesions in the rat. Gastroenterology 1979;76:88-93.*
8. *Kauffman GL, Grossman MI. Prostaglandin and cimetidine inhibit antral ulcers produced by parenteral salicylates. Gastroenterology 1978;74:1049-1053.*

9. Shea-Donohue T, Steel L, Montcalm E, Dubois A. Aspirin-induced changes in gasric function: role of endogeneous prostaglandins and mucosal damage. Gastroenterology 1990;98:284-286.
10. Giri AK, Sivam SS, Khan KA. Sister-chromatid exchange and chromosome abberations induced by paracetamol in vivo in bone marrow cells of mice. Mutat Res 1992;278:253-258.
11. Walker RM, Racz WJ, McElligott TF. Scanning electronmicroscopic examination of acetaminophene-induced hepatotoxicity and congestion in mice. Am J Pathol 1983;113:321-330.
12. Andersson K, Mattsson H, Larsson H. The role of gastric mucosal histamine in acid secretion and experimentally induced lesions in rats. Digestion 1990;46:1-9.
13. Beatens D, Rufener C, Srikant B, Dobbs RE, Unger RH, Orci L. Identification of glucagon producing A-cells in dog gastric mucosa. Cell Biol 1976;69:455-464.
14. Chiba T, Taminato T, Kadowaki S, et al. Effects of various gastrointestinal peptides on gastric somatostatin release. Endocrinology 1980;106:145-149.
15. Eisselle R, Rosskopf B, Koop H, Adler G, Arnold R. Proliferation of endocrine cells in the rat stomach caused by drug-induced achlorhydria. Gastroenterology 1991;101:70-76.
16. Hakanson R, Owman C, Sporrong B, Sundler F. Electronmicroscopic identification of the histamine-storing argyrophil cells in the rat stomach. Z Zellforsch 1975;122:460-466.
17. Hakanson R, Tielemans Y, Chen D, Anderson K, Mattson H, Sundler F. Time-dependent changes in enterochromaffine-like cell kinetics in stomach of hypergastrinemic rats. Gastroenterology 1993;105:15-21.
18. Prinz C, Kajimura M, Scott DR, Mercier F, Helander HF, Sachs G. Histamine secretion from rat enterochromaffine -like cells. Gastroenterology 1990;98:284-286.
19. Wallmark B, Skaenberg I, Mattson H, et al. Effects of 20 weeks ranitidine treatment on plasma gastrin levels and gastric enterochromaffine-like cell density in the rat. Digestion 1990;45:181-188.