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MORPHOLOGICAL STUDIES UPON THE ULCEROGENIC EFFECT OF SINGLE AND MULTIPLE APPLICATIONS OF ACETYLSALICYLIC ACID ON GASTRIC MUCOSA IN ALBINO RATS

M. TZANEVA[1] , D. PRANGOVA[1] AND A. VODENICHAROV[2]

[1]Department of Pathoanatomy, Forensic Medicine, Deontology and Foreign Languages, Faculty of Medicine; [2]Department of Anatomy, Faculty of Veterinary Medicine, Thracian Unievrsity, Stara Zagora, Bulgaria

Summary

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The changes in gastric mucosa, occurring after experimentally induced acute and chronic acetylsalicylic acid (ASA) damage were studied. The experiments were performed on 40 male albino Wistar rats. At hour 24 after the application of a single ASA dose of 250 mg/kg b.w., deep erosions in the corpus mucosa in a process of epithelization were found out. Around the fibroblasts, located at the bottom and on the edges of those erosions, single collagen fibrils were observed. After a 10- and 20-day application of ASA, deep chronic erosions were seen 24 hours following the last application. Both the light microscopic and the electron microscopic studies revealed an inflammation infiltrate consisting of lymphocytes, plasmatic cells, fibroblasts and single macrophages. Along the edges and at the bottom of deep chronic erosions, single collagen fibrils were discovered by Van Gieson staining and by elecron microscopy. Few blood vessels were present at the bottom of erosions. Those results evidenced that ASA decreased collagen synthesis and slowed down the angiogenesis at the bottom of deep chronic gastric erosions.

Key words: acetylsalicylic acid, collagen, deep erosions, fibroblasts

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely used medicines (Vainio and Morgan, 1998). They have an ulcerogenic effect in humans and experimental animals depending on the applied doses (Konturek et al., 1993; Manevska, 1994, Tzaneva et al., 1994; Tolekova et al., 1995; Tzaneva and Julianov, 1997; Hull et al., 1997; Vainio and Morgan, 1998; Stolte et al., 1999; Tzaneva, 1999; Tolekova, 2000, Vodenicharov and Tzaneva, 2000).

In patients with chronic gastric ulcer, the NSAID adminsitration delayed the process of tissue regeneration (Levi et al., 1990). The precise mechanisms, by which the acetylsalicylic acid (ASA) and other drugs from this group perform their ulcerative effect is not elucidated. After an acute damage, tissue regeneration depends on extracellular matrix proteins secretion (Potehin and Paukov, 1997). The latter help the fixation, differentiation and proliferation of different cell types that fill the tissue defect (Lanas et al., 1998). Collagen is one of extracellular matrix pro-

teins with a high concentration in granulation tissue (Gillessen et al., 1993). The major amount of collagen in the granulation tissue is formed by fibroblasts and myofibroblasts (Schurch et al., 1998; Serini et al., 1999). The number of fibroblasts increases during the healing of acute gastric lesions and afterwards they are transformed in myofibroblasts. The latter appear in granulation tissue by the 8^{th} day after the damage, reach a peak by the 15^{th} day and then their amount gradually decreases. After the 30^{th} day they are no longer observed (Schurch et al., 1998).

The aim of the present study was to study the changes and processes of tissue damage, that occurred after experimentally induced acute and chronic lesions of gastric mucosa with ASA.

MATERIALS AND METHODS

The studies were performed on 40 male white Wistar rats weighing 180-220 g. ASA was applied orally singly or repeatedly (once per 24 h) at a dose of 250 mg/kg b.w., preliminary dissolved in 1 ml polyethylene-glycol 400 (PEG 400). The animals were divided into four groups:

Group I (n =10). Five rats were intact and another 5 treated orally only with 1 ml PEG 400 (control group). The solvent was administered once daily for 3 days.

Group II (n=10). The rats were treated with a single ASA dose.

Group III (n=10). The animals received ASA for 10 days.

Group IV (n=10). The animals received ASA for 20 days.

ASA or PEG 400 were administered prior to the morning feeding via a stomach tube according to Yeomans (1976). The rats were euthanized using an ether anaesthesia, 24 hours after the last treatment. Immediately after the euthanasia, the stomach was excised along the *curvatura minor*. Peaces with a size of 2x5 mm were taken from the wall of the corpus and the anthrum and fixed in 10% neutral formaldehyde. The further processing included routine techniques for dehydratation in an ascending ethanol series, clearing in xylene and embedding in paraffine. The prepared sections (5-8 μm) were stained with hematoxillin/eosin, PAS reaction, alciane blue and Van Gieson.

Specimens from the same places were taken for electron microscopic investigation in all groups as well. Pieces of 1 mm^3 were fixed in 2% glutaraldehyde solution in 0.1% cacodilate buffer (pH 7.4) for 24 hours, washed in cacodilate buffer and postfixed in 0.1% osmium tetroxide (OsO_4). Afterwards the samples were washed in cacodilate buffer, dehydrated in an ascending ethanol series, processed in propylene oxide-epon and embedded in epon (Merck, Darmstadt, Germany). Semithin sections were prepared, stained according to Hunphrey & Pittman (1974) and submitted to a light microscopic study in order to determine areas suitable for transmission electron microscopic study. They served to prepare ultrathin sections, contrasted with uranyl acetate and lead citrate.

RESULTS

Light microscopic study

Control group. The morphology of the gastric mucosa in control animals - untreated and PEG 400 treated, was normal. After a Van Gieson staining, a moderate amount of collagen around the basal parts of the glands was found out.

ASA-treated rats. At hour 24 after the single ASA treatment, deep acute erosions, located in the corpus mucosa were found out. The edges of those erosions were inclined and lined by mucus-

secreting epithelial cells. The Van Gieson staining revealed a lack of collagen along the edges and at the bottom of erosions. After 10-days and 20-days ASA applications, along the edges of deep chronic erosions, a foveolar hyperplasia was observed 24 hours after the last application (Fig. 1).

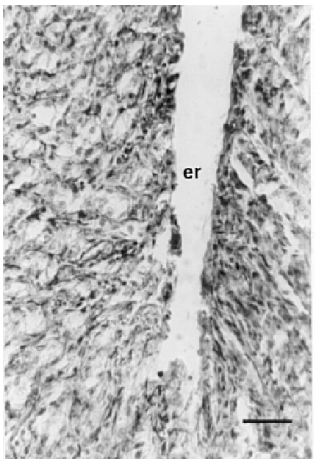

Fig. 1. Deep chronic erosion (er) in the corpus gastric mucosa after a 10-days treatment with ASA. Van Gison staining. Bar = 45 μm.

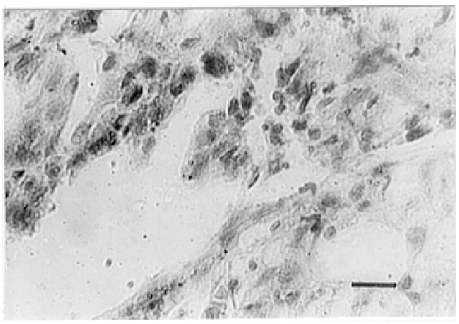

Fig. 2. Inflammation infiltrate at the bottom of a deep chronic gastric erosion after a 10-days treatment with ASA, consisting of lymphocytes, plasmatic cells, macrophages and fibroblast-like cells. Van Gison staining. Bar = 200 μm.

On the edges and at the bottom of erosions, a round-cell infliltrate was present, consisting of lymphocytes, plasmatic cells, fibroblast-like cells and single macrophages (Fig. 2). In separate cases, collagen was seen following a Van Gieson staining. At the bottom of deep chronic erosions, few blood vessels were found out.

Transmission electrone microscopic study

Control group. Around the corpus glands, large fusiform cells with thin processes were observed. The nucleus was oval or elongated and contained euchromatin and heterochromatin. The latter was located primary in the periphery of the nucleus that often appeared indented (Fig. 3).

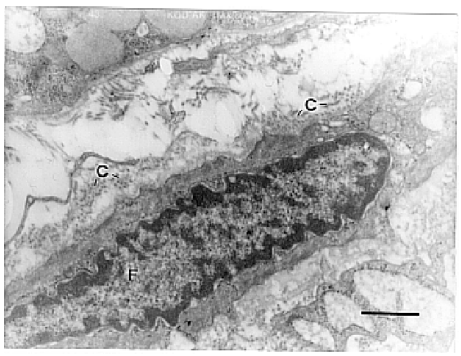

Fig. 3. Fusiform cell similar to fibroblast (F) with an elongated indented nucleus and a moderate amount of pericellular collagen fibrils (C) in the control group. Bar = 0.5 μm.

Mitochondria were located perinuclear. The Golgi apparatus (GA) was similarly located. Within cell cytoplasm, a well developed granulated endoplasmic reticulum (GEPR) and multiple free ribosomes (Fig. 4) were observed. Pericellular, multiple dispersed or grouped collagen fibrils were discovered. The morphology of those cells was similar to that of fibroblasts.

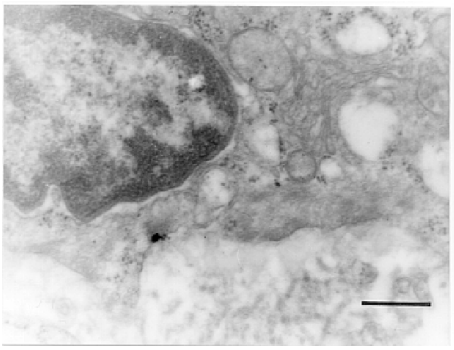

Fig. 4. Ribosomes and polyribosomes, a well developmed Golgi apparatus and mitochondria in the cytoplasms of a fusiform cell similar to fibroblast from the control group. Nearby, there is a moderate amount of collagen fibrils. Bar = 0.26 μm.

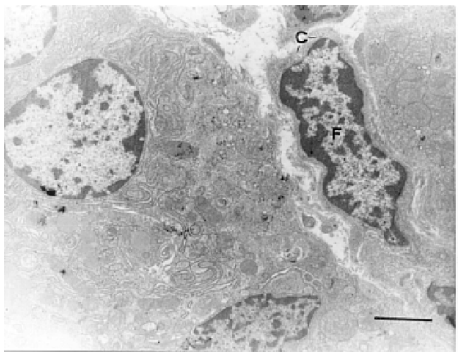

Fig. 5. Fusiform cell similar to fibroblast at the bottom of a deep chronic gastric erosion following a 20-days ASA treatement with single collagen fibrils (C). Bar = 1.5 μm.

ASA-treated rats. By the 24th hour after receiving a single ASA dose, single collagen fibrils were found out around the fibroblasts at the bottom and along the edges of deep acute erosions. The inflammation infiltrate was not different among the ASA-treated groups. It consisted of lymphocytes, plasmatic cells, fibroblasts and single macrophages. Fibroblasts were with an elongated nucleus, sometimes with indented edges and heterochromatin, located mainly peripherally. GEPR was moderately developed. A significant number of free ribosomes was observed in cell cytoplasm. The GA and mitochondria were perinuclear positioned and moderately developed. Around the fibroblasts, single collagen fibrils appeared (Fig. 5).

DISCUSSION

The present results, obtained after the reproduction of the model of acute and chronic gastric damage are similar to those in previous studies of ours (Vodenicharov and Tsaneva, 2000). At the same time, they correlate well with the reports of other authors, having applied ASA at dose rates of 120 or 250 mg/kg b.w. (Yeomans et al., 1973; Yeomans, 1976). In our experiments, 24 hours after the applcation of a single 250 mg/kg b.w. dose of ASA, the deep acute erosions of the corpus mucosa were almost entirely epithelized.

In the vicinity of deep gastric erosions, relatively few collagen fibrils were observed. This fact corresponds to the finding of others (Lanas et al., 1998) that collagen content in gastric fibroblast cell lines decreases following ASA application.

In our studies, the synthesis of collagen at the bottom as well as along the edges of deep chronic gastric erosions remains reduced after 10-days and 20-days ASA applications .

The precise mechanisms acting in ASA-impaired collagen synthesis, are not clear enough. It is known that several substances as prostaglandins, growth factors, cytokines etc. regulate the processes of collagen synthesis and secretion (Polk et

al., 1992; Hull et al., 1997; Asakura et al., 1998; Lanas et al., 1998; Piazuelo et al., 1998; Vainio and Morgan, 1998). It is also known that prostaglandins decrease collagen secretion and inhibit the migration and proliferation of fibroblasts (Lanas et al., 1994, 1998; Oiazuelo et al., 1995, 1998). Recent studies evidence that following an ASA application, tissue prostaglandin level decreases (Hull et al., 1997; Vainio and Morgan, 1998). Thus we consider that most probably, prostaglandins are not responsible for the reduced collagen synthesis. On the other side, the inhibited prostaglandin synthesis is accompanied by increase in leukotrienes - arachidonic acid derivatives. An increased leukotriene LTB4 content is found out in the gastric mucosa of patients, receiving NSAIDs for a long time (Hudson et al., 1991). Recently it is proved that the leukotrienes LTC4, LTB4, LTD4 modulate and enhance the collagen secretion from skin fibroblast cell lines (Lanas et al., 1998). It could be therefore concluded that leukotrienes are not in direct relationship with reduced collagen synthesis either.

After ASA administration, several growth factors are expressed in the damaged gastric mucosa: epidermal growth factor (EGF), transforming growth factor α (TGFα) and transforming growth factor β1 (TGFβ1) etc. (Wright et al., 1990; Polk et al., 1992). EGF is expressed in epithelial cells, located adjacent to gastric lesions (Wright et al., 1990). This growth factor stimulate the collagen synthesis when applied upon gastric fibroblast cell cultures (Lanas et al., 1998). TGFα appears in cell destruction foci and it is demonstrated that this has a stimulating effect upon the synthesis of extracellular matrix proteins (Polk et al., 1992). According to Asakura et al. (1998) and Serini et al. (1999), the most potent stimulator of extracellular matrix synthesis is TGFβ1. Apart stimulating collagen synthesis, this growth factor exerts a similar effect to fibronectin, proteoglycans and the other extracellular matrix proteins, acting indirectly via the platelet-derived growth factor (PDGF-BB). It is evidenced that ASA administration inhibits the release of PDGF-BB in serum and thus, TGFβ1 is not able to explicit its stimulating effect upon the collagen synthesis (Vissinger et al., 1993). Moreover, it is recently reported that the preliminary treatment with PDGF-BB prevents the unfavourable effect of ASA and indomethacin on the healing of acetate-induced gastric ulcers in rats (Arcuz et al., 1997). Therefore, we suppose that the little amount of collagen observed in our experiments, is most probably due to reduced PDGF-BB formation.

After a 10-days and 20-days administration of ASA, we did not observe any transformation of fibroblasts into myofibroblasts at the bottom and along the borders of deep chronic gastric erosions. According to data reported by others (Schurch et al., 1998), this transformation is most expressed in the granulation tissue 15 days following the initial damage and is obligatory for tissue regeneration. The decreased collagen synthesis, in our opinion could be due to insufficient fibroblastic stimulation on one part and to inhibition of their myofibroblastic transformation on the other, although the clear reason for this is hard to be formulated.

As to the few blood vessels at the bottom of chronic gastric erosions, our opinion is similar to that of Hull et al. (1997) and Ukawa et al. (1998) that reduced angiogenesis could be due to inhibited prostaglandin or collagen synthesis. It have to be pointed out however that the exoge-

nous collagen application stimulates the formation of young blood vessels that appeared as early as five days after the damage while in untreated controls this occurred not before the 15th day (Redlich et al., 1998).

Our results evidenced that ASA reduced collagen synthesis and slowed down angiogenesis at the bottom of deep chronic gastric erosions.

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Correspondence: Dr. Maria Tzaneva
Department of Pathoanatomy, Forensic Medicine, Deontology and Foreign Languages,
Faculty of Medicine,
Thracian University,
11 Armeiska str.,
6003, Stara Zagora,
Bulgaria