The influence of the acetylsalicylic acid and its complex compounds with metals on the morphometric characteristics of the stomach mucous membrane

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Abstract. The paper researches the effect of the acetylsalicylic acid (ASA) and its complex compounds with the metals of cobalt (Co²⁺), zinc (Zn²⁺), nickel (Ni²⁺) and manganese (Mn²⁺) at the introduction during 20 days in the dose of 10 mg/kg on the morphometric characteristics of the rats' stomach mucous membrane. It is shown that a long-term introduction of ASA and salicylates of cobalt, zinc, manganese and nickel influences the morphometric characteristics of the animals' stomach mucous membrane.

1. Introduction
Acetylsalicylic acid (ASA, aspirin) as a medicine has been used for more than 100 years; it belongs to the group of nonsteroid anti-inflammatory preparations (NAIP) and has a wide spectrum of therapeutic effects. However, despite its long and wide-spread usage, in the end of XX – the beginning of XXI centuries ASA and its derivatives have drawn much attention both of the researchers and of the medical practice; this has been presented in modern scientific native and foreign literature. One of the most important tendencies of ASA researching is the expansion and amplification of the known therapeutic effect (antipyretic, analgetic, anti-inflammatory, antiaggregant, antioxidant, antidepressant, antimigraine, anxiolytic, etc.) [1, 2]. The use of ASA at the cardiovascular pathology is of great interest, it can widely be used in the treatment and the prophylaxis of coronary heart disease, cardiac insufficiency, hypertension, etc.; this allows estimating the amount of favourable effects, produced by ASA, and ascertain the absence of other medicines, equivalent to it, within the next few years [2].

It is necessary to mention that along with a great number of positive therapeutic effects, ASA has a leading position among all the NAIP not only in the amount of usage, but also in the total amount of side effects, among which there are negative effects on the digestive and immune systems, as well as on the skin and hypodermic tissues [3, 4]. The most frequent undesirable reaction, connected with ASA taking, is gastro toxicity – NAIP- gastropathy. In particular, it is mentioned that a prolonged intake of aspirin causes irritation and damage of the stomach mucous membrane (SMM), heartburn, sickness, and vomiting, erosive gastritis, the increase in the number of bleeding by 1.6 times [5].

Thus, taking into account the clinical efficiency of ASA, not only the expansion and amplification of its therapeutic potential is extremely important, but as well as the reduction, and if possible, the liquidation of the undesirable side effects, limiting the intake of the preparation, including the reduction of the risk of SMM damaging. According to many researchers, the perspective way for completing this task is the creation of complex compounds on the basis of ASA, in particular, with
bivalent metals [3, 6], and rare earth metals [7, 8].

In connection with this it is topical to study the influence of ASA and its complex compounds with the metals of cobalt (Co²⁺), zinc (Zn²⁺), nickel (Ni²⁺) and manganese (Mn²⁺), which, as our research has shown, have a pronounced cardiotropic effect [9], on the morphometric characteristics of SMM; this is the aim of our research.

2. Methods
The animals were taken out of the experiment by decapitation under ether anaesthetic, following the rules of euthanasia, and then we carried out taking the samples of the researched material. In all the groups (control and experimental) decapitation of the animals and taking the stomach fragments were of the same kind. The material for morphological research was taken from the esophageal part (cardiaca) and the body of the stomach (corpus). The choice of these stomach parts is conditioned by the fact that these segments contain different types of integumentary epithelium cells. The material was fixed in 10% buffered formalin (pH 7.2) during 24 hours. After that we carried out hydratation and impregnation with paraffin in the microwave histological processor LOGOS (Miestone, Italy) according to the programme, recommended by the manufacturer. The organs’ parts, impregnated with paraffin, were embedded in the blocks, from which semithin sections with the width 4 μm were made. The sections were coloured with hemtoxylin and eosin [10].

To research the structure of SMM the method of light microscopy was applied: the microscope DM2000 (Leica, Germany) with the objective lenses Plan 10x and Plan 40x; the camera DFC295; the scanner for histopreparations Aperio CS2 (Leica, the USA). The morphometric characteristics of the tissue structures of the histological preparations were obtained by using the system of visual analysis, as well as with the help of the programme ImageJ, calibrating the measuring by the scale of TS-M1 0.01 mm/100 div stage micrometer [10].

The morphometric analysis at the light optical level included measuring the thickness of the mucous membrane of the stomach esophageal part and the plane epithelium, the mucous membrane of the stomach body and the integumentary epithelium, the relative area, occupied with capillaries, the calculation of the central cells (CC) and the accessory cells (AC) per unit of the fundus glands’ mucous membrane.

Non-parametric statistical methods were used as the distribution of the value of variables differed from the regular. The credibility of the statistical differences between the control and the experimental groups were determined with the help of Mann-Whitney test.

3. Results of the research
At the visual assessment the stomach and its mucous membrane had an ordinary appearance, without any pathological changes. The rats’ stomach wall consists of the mucous membrane, submucous base, muscular and serous membranes. The histological research showed that the animals’ SMM structure in the control and experimental groups corresponded to the normal organ’s morphology [11, 12].

The mucous membrane of the esophageal part (without glands) of the animals in the control group is formed by the multilayer keratinizing epithelium, in which from 3 to 6 layers of epithelial cells are visualized, the layers include granular, acanthaceous, basal ones without the signs of cornification; the membrane also formed by its own connective tissue plate and muscle plate of the mucous membrane (lam. muscularis mucosae) (see Figure 1-a). The values of the morphometric characteristics of the rats under control were: the thickness of the SMM esophageal part is 86.00±7.40 μm; the thickness of the plane epithelium of SMM is 19.78±2.79 μm (Figure 2).

After twenty-fold introduction of ASA, ASCo²⁺ and ASZn²⁺ into the animals a statistically significant thickness of the SMM esophageal part by 21.18 % (p≤0.05), 46.00% (p≤0.05) and 44.44% (p≤0.05) happened correspondingly relative to the values of this characteristic in the rats’ control group (Figure 2-a), this apparently was the consequence of the SMM plane epithelium thickness. Thus, after the introduction of ASA into the animals the plane epithelium thickness increased by 50% (p≤0.05), after the introduction of ASCo²⁺ and ASZn²⁺ a more pronounced growth of this characteristic
in average by 78.05% (p≤0.05) was registered relative to the values of the control group (see Figure 2-b). The mentioned changes took place mainly due to the hyperkeratosis of the mucous membrane, which is connected with the keratinizing of the SMM esophageal part epithelium of the animals in the experimental groups during a long period; this is confirmed by the microphotographs, in which the hyperkeratosis development is clearly seen, it is accompanied by the thickening of the granular layer of the SMM esophageal part (Figure 1-b, c, d).

The opposite changes of morphometric characteristics of the SMM esophageal part were observed after the introduction of ASNi^{2+} into the animals: the thickness of SMM and the esophageal part plane epithelium decreased by 35.50% (p≤0.05) and 7.51% (p≤0.05) correspondingly relative to the values of the animals got ASA (Figure 2). These changes of the SMM morphometric characteristics of animals on the background of prolonged ASNi^{2+} introduction can be the result of the desquamation of the integumentary epithelium; this is registered on the histopreparations (Figure 1-e). It is known that the desquamation (detachment) is the result of hyperkeratosis, during which intercellular contacts weaken and the integration of the epithelial layer is damaged [13]. After the multiple introduction of ASMn^{2+} into the animals only the tendency towards the growth of SMM and the esophageal part plane epithelium thickness was noticed, however, statistically significant differences with the control were not observed (p≥0.05) (see Figure 1-f; 2).

Therefore, the multiple introduction of ASA and cobalt and zinc salicylates into the animals leads to the thickening of SMM esophageal part due to hyperkeratosis, the introduction of nickel salicylate, on the contrary, leads to the weakening of SMM as a result of the intensive desquamation of the keratinized epithelium from the mucous membrane, this may be due to the development of the pathological process; and the introduction of manganese does not cause reliable changes of the morphology of this SMM part.

![Figure 1. Mucous membrane of the esophageal part of the stomach: in rats of the control group (a) and rats receiving injections of acetylsalicylic acid (b) salicylates of cobalt (c), zinc (d), nickel (e), manganese (f). Note: 1 - squamous epithelium, 2 - granular layer, 3 - prickly layer, 4 - basal layer. Solid arrow - hyperkeratosis, dotted arrow - desquamation. Hematoxylin-eosin. Lens 40x.](image-url)
Figure 2. Changes in the thickness of the mucous membrane (a) and squamous epithelium of the esophageal part of the stomach (b) in rats of the control group and rats receiving 20-fold injections of acetylsalicylic acid (ASA) salicylates of cobalt (ASCo2+), zinc (ASZn2+), nickel (ASNi2+), manganese (ASMn2+)

Notes:
* – significance level of differences vs. control group of animals (Mann–Whitney U test);
# – significance level of differences vs. ASA group of animals (Mann–Whitney U test).

Mucous membrane of the stomach body consists of a single-layer uniserial cylindrical epithelium, covering gastric fossae, into the base of which the stomach glands open. Diffusely located leukocytes can be observed in the rat’s stomach mucous membrane own plate; lymphocytes and polymorphous nuclear neutrophiles prevail among them. The muscular plate and muscular stomach membrane are formed by the smooth muscular cells. The cells of the muscular plate are located in one layer. The submucous base, presented by the connecting tissue, does not contain glands. There are vascular plexuses and nerve submucous Meissner’s plexus. The muscular cover is well-developed and consists of three layers. From the outside the stomach is covered with a serous membrane, consisting of a single-layer plane epithelium – mesothelium – and a layer of the connecting tissue. In the connecting tissue there are capillary nets among the glands, the nets are formed by the branches of the intramural vessels, penetrating the submucous base. The relative area, occupied by the capillaries, of the rats in the control group was 3.40±0.60%. The capillary nets in the mucous membrane of the stomach body of the animals in all the experimental groups had a few differences, both among the groups and in comparison with the control group, occupying in average 2.8-3.1% of the whole SMM area. There are single eosinophile leukocytes in the SMM stomach body.

CC and parietal AC were well-determined in the glands, in the structure of which the dystrophic changes were not registered. The relative area, occupied by CC and AC, in the rats of the control group was 29.80±4.83% and 40.40±3.36% correspondingly. The analysis of the area, occupied by CC and AC in the mucous membrane of the rats’ stomach bodies of all the experimental groups, showed the absence of statistically significant differences relative to the values of this characteristic of the control group animals; this evidences the absence of the changes in the gastric juice acidity [11].
The thickness of the mucous membrane of the stomach body of the control group animals was 628.40±11.85 μm, and the thickness of the stomach body integumentary epithelium was 19.78±2.79 μm (Figure 4-a, b). The described picture corresponds to the histological and morphometric characteristics of the mucous membrane of the rats’ stomach bodies in the norm [11, 12].
The results of the histological research of the experimental groups rats’ SMM showed that at the introduction of ASA and especially АSNi²⁺ into the animals the apical part of the mucous membrane folds was edematous and loosen, due to the dystrophic-necrotic changes the integumentary epithelium was swollen, most of the cells were desquamated, gastric cells were widened and filled with the mucous mass (see Figure 3-b, e). The morphometric analysis of the mucous membrane of the stomach body thickness confirmed that. Thus, in the animals, that were injected with АSNi²⁺, the statistically significant weakening of the SMM thickness by 9.82% (p≤0.05) was registered both relative to the values of these characteristics in the control group and in the animals, injected with ASA correspondingly (see Figure 4-a). In the rest experimental groups this characteristic did not differ from the same in the control group.

To a greater extent the introduction of ASA and salicylates into the animals led to the change of the integumentary epithelium thickness of the mucous membrane of the stomach body. In the animals in all the experimental groups the decrease of this characteristic was registered, but pronounced to a different degree (Figure 4-b). The most significant decrease in the SMM integumentary epithelium thickness relative to the values in the control animal group was revealed in the rats that were injected with ASA (by 53.45%; p≤0.05), this is, evidently, is the result of the SMM damage during a prolonged intake of this preparation, registered in many researches [3-5]. At the multiple introduction of ASZn²⁺ a reliable decrease of the characteristic by 25.74% (p≤0.05), ASNi²⁺ - by 18.11% (p≤0.05) and ASMn²⁺ - by 21.14% (p≤0.05) was observed relative to the values in the animals’ control group, however, the values of this characteristic were by 13.96%, 21.02% (p≤0.05) and 25.65% (p≤0.05) higher than of the rats in Group 2, that were injected with ASA. At the multiple introduction of ASCo²⁺ into the animals the values of the characteristic approached the same in the control group of rats; this is proved by the absence of statistically significant differences (see Figure 4-b).
Thus, the multiple introduction of ASA into the animals leads to a significant decrease in the thickness of the SMM integumentary epithelium, the introduction of the tested salicylates also causes unidirectional change of this characteristic, however, pronounced to a less degree. Along with this, under the influence of the nickel salicylate, unlike the rats of other experimental groups, there is a considerable weakening of the animals’ mucous membrane of the stomach body.

The issue of prophylaxis of the mucous membrane of gastrointestinal tract damage at a prolonged ASA intake is not completely studied and remains urgent for the clinical practice. Nowadays, several mechanisms of NAIP-gastropathy development are described, besides the key point is given to the systemic action of NAIP, manifested in the inhibition of cyclooxygenase-1 (COG-1) by the mucous membrane of the stomach with the further blockade of the endogenous biosynthesis of prostaglandins E2 and prostacyclins I2, which are the main protective SMM reserves. However, recently the information on the great significance of ASA local effect on the SMM has been accumulated. It is supposed that ASA directly or through anti-inflammatory cytokines can cause the apoptosis of the epithelial cells [14].

It is necessary to mention that while taking ASA per os the absorption of the preparation approaches to 100%, however, the ASA bioavailability (the amount of the preparation reaching the systemic blood flow) makes up 50-68%. During the absorption in the system of the portal vein and in the liver ASA undergoes the presystemic metabolism – hydrolysis [15]. A part of ASA, got into the systemic blood flow, is quickly hydrolyzed by the blood plasma esterase, that is why the period of ASA semiejection is short – not more than 15-20 minutes, and the period of the salicylic acid semiejection and, consequently, of the salicylates is longer, and at taking little doses is 2-3 hours [16].

Since during our research ASA and the tested salicylates were introduced intraperitoneally and got into the blood flow without getting into the gastrointestinal tract, we may assume that all the SMM changes, exposed after their twenty-fold introduction are connected, first, with the mechanism of the systemic, but not of the local action of these compounds, and second, with a longer period of the salicylates’ action in comparison with ASA.

4. Conclusions
1. During the research the influence of ASA and the tested salicylates on the histological and morphometric characteristics of SMM of the animals was found out.
2. At the multiple acetylsalicylic acid introductions the increase of the thickness of the mucous membrane and plane epithelium of the esophageal stomach part and the decrease of the integumentary epithelium of the rats’ stomach body thickness happen.
3. At the multiple nickel salicylate introductions the weakening of the mucous membrane and plane epithelium of the esophageal part of the stomach, mucous membrane and integumentary epithelium of the rats’ stomach body takes place due to the intensive desquamation of the keratinized epithelium.
4. At the multiple introductions of the cobalt and zinc salicylates a pronounced increase of the thickness of the mucous membrane and plane epithelium of the esophageal part of the stomach occurs due to the hyperkeratosis on the background of the weakening of the stomach body integumentary epithelium.
5. At the multiple manganese salicylate introductions the decrease of the integumentary epithelium thickness of the stomach body mucous membrane happens on the background of the invariable thickness of the mucous membrane of the esophageal part and the stomach body.

5. References
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