IRSTI 34.23.23 https://doi.org/10.26577/eb.2021.v89.i4.15

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# ASSESSMENT OF FS-1 GENOTOXIC EFFECT ON THE BUCCAL EPITHELIUM OF RABBITS

Antimicrobial activity of the drug FS-1 is due to the content of iodine halogen, which is quite active. Currently, potassium triiodide-based preparations, such as Lugol's solution, Iodopyron, Betadine and others, are already used as antiseptics. Studies have already been conducted confirming the ability of FS-1 to increase the permeability of the bacterial cell membrane, which allows antibiotics to act more efficiently, and in vitro and in vivo studies have been conducted to evaluate the cytotoxicity and genotoxicity of FS-1 in laboratory animals with systemic exposure. In this experiment, effect of FS-1 on the site of primary contact, the buccal epithelium, was studied. Based on the experiment results, we could conclude about the possible use of FS-1 as an oral antiseptic. Three rabbits were used in the experiment, buccal epithelium smears of which were taken before the administration of 4 mg/kg FS-1 (control samples) and after the administration of FS-1 (experimental samples). Total period of FS-1 daily administration was 14 days, which is a period for self-renewal of the epithelium. Obtained smears of the cells were examined under a microscope for the presence of cytogenetic aberration. As a result, there was no significant change after administration of FS-1, since the level of cells with karyolysis, karyorrhexis, micronuclei, and nuclear protrusions remained at about the same level. Therefore, it was concluded that FS-1 does not cause cytogenotoxic effect upon prolonged exposure to rabbit buccal epithelium.

**Key words:** FS-1, iodine, buccal epithelium, cytogenotoxic effect, karyolysis, karyorrhexis, micronuclei, nuclear protrusions.

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## Қояндарды буккал эпителийіне ФС-1 генотектілігін бағалау

ФС-1 препаратының микробқа қарсы белсенділігі йод галогенінің құрамына байланысты, ол өте белсенді. Қазіргі уақытта калий триодидіне негізделген препараттар, мысалы, Луголь ерітіндісі, Иодопирон, Бетадин және басқалары антисептиктер ретінде қолданылады. ФС-1 бактериялық жасуша мембранасының өткізгіштігін жоғарылату қабілеттілігін растайтын зерттеулер жүргізілді, бұл антибиотиктердің тиімдірек әрекет етуіне мүмкіндік береді, ал in vitro және in vivo жүйелерінде зертханалық жануарларда цитотоксикалық және генотоксикалықты бағалау үшін зерттеулер жүргізілді. Бұл экспериментте ФС-1-тің алғашқы байланыс торабына, бұлшықет эпителийіне әсері зерттелді. Экспериментте үш қоян қолданылды, 4 мг/кг ФС-1 (бақылау үлгілері) енгізілгенге дейін және ФС-1 (эксперименттік үлгілер) қабылдағаннан кейін алынған бүршік эпителийінің жағындылары. ФС-1-ті күнделікті қолданудың жалпы кезеңі 14 күнді құрады, бұл эпителийдің өздігінен жаңару кезеңі. Алынған жасушалардың жағындылары цитогенетикалық аберрацияның бар-жоғына микроскоппен қаралды. Нәтижесінде ФС-1 қабылдағаннан кейін айтарлықтай өзгеріс болған жоқ, өйткені кариолиз, кариорексия, микроэлектр және ядролық протездері бар жасушалардың деңгейі шамамен бірдей деңгейде қалды. Сондықтан, FS-1 қоян букальды эпителийіне ұзақ уақыт әсер еткенде цитогенотоксикалық әсер етпейді деген қорытындыға келді.

**Түйін сөздер:** ФС-1, иод, буккальды эпителий, цитогенотоксикалық әсер, кариолиз, кариорексис, микроэлементтер, ядролық шығулар.


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**Оценка генотоксичности ФС-1 на буккальный эпителий кроликов**

Антимикробная активность лекарственного препарата ФС-1 обусловлена содержанием галогена иода, который является довольно активным. В настоящее время в качестве антисептиков уже применяются препараты на основе трииодида калия, такие как раствор Люголя, Иодопирон, Бетадин и другие. Ранее уже были проведены исследования, потверждающие способность ФС-1 увеличивать проницаемость клеточной мембраны бактерии, что позволяет антибиотикам действовать эффективнее, а также были проведены исследования in vitro и in vivo оценки цитотоксичности и генотоксичности ФС-1 на лабораторных животных при системном воздействии. В данном эксперименте было изучено влияние ФС-1 на участок первичного контакта – буккальный эпителий. Исходя из результатов эксперимента, мы могли сделать вывод о возможном применении ФС-1 в качестве орального антисептика. В эксперименте были использованы три кролика, мазки буккального эпителия которых были взяты до введения 4 мг/кг ФС-1 (контрольные образцы) и после введения ФС-1 (экспериментальные образцы). Общий период ежедневного введения ФС-1 составил 14 дней, что является периодом для самообновления эпителия. Полученные мазки клеток были исследованы под микроскопом на наличие цитогенетических аберрации. В результате не было выявлено значительного изменения после введения ФС-1, так как уровень клеток с кариолизисом, кариорексисом, микроядрами и ядерными протрузиями оставался примерно на таком же уровне. Следовательно, был сделан вывод о том, что ФС-1 при длительном воздействии на буккальный эпителий кроликов не оказывает цитогенотоксического влияния.

**Ключевые слова:** ФС-1, иод, буккальный эпителий, цитогенотоксическое влияние, кариолизис, кариорексис, микроядра, ядерные протрузии.

## Introduction

There are many iodine-containing pharmaceuticals, among which are antiseptics based on potassium triiodide – Betadine, Lugol's solution, Iodopyron, iodonate and so on [1,2]. The use of iodine is not limited by medicine; it is also used in industry for the production of liquid crystal displays, high-power gas lasers based on excited iodine atoms, halogen lamps, lithium-ion batteries for cars and etc [3]. Except simple solutions of iodide and potassium iodide, there are also iodophors – complexes of triiodide with polymers, which when applied to the skin do not cause skin irritation, but at the same time they have antimicrobial properties. For example, a study of the irritant effect on the eyes of rabbits with the instillation of 1.5-2% povidone iodine for three days led to damage to the cornea [4]. Nevertheless, the use of a 5% povidone-iodine solution in the fungal keratitis model was more effective than using 5% natamycin [5].

Among halogens – iodine stands out for its distinctive characteristics. The large electron shell and the increased internuclear distance determine the isolation of the nucleus [6]. The addition of strong Lewis acids ($Li^+$, $Na^+$, $K^+$) to the medium increases the solubility. Due to this property, iodine becomes more mobile and can be easily polarized by other elements, such as lithium. Regarding solubility, iodine dissolves in substances that can polarize it, for example, water, possessing oxygen in its composition, which polarizes iodine. Upon dissolution of iodine, the solution becomes brown, and polyiodides blue or brown [7]. Iodine can interact with other organic substances, forming complexes of the type: [(amino acid)] $K^+ \cdot I3^-$, which is the equation of the simplest complex compounds of iodine. For example, in a complex with glycine, potassium, and water, an iodide ion interacts with six glycine molecules and forms an energetically stable structure [8,9]. When interacting, for example, with aromatic molecules, diiodide forms charge-transfer complexes, in which iodine acts as a Lewis acid. Such complexes are studied in the framework of supramolecular chemistry of iodine, which includes other iodine complexes with the properties of forming donor-acceptor, hydrophobic, hydrogen and halogen bonds [10,11].

Iodides also form complex compounds with lithium and potassium halides, polypeptides and α-dextrin, which can affect the stability of the structure. For example, α-dextrin is a component of the drug Armenicum intended for the treatment of HIV infection, and in these complex iodides are located inside the dextrin helix and exhibit acceptor proper-

ties for α-dextrins and donor properties for lithium halides [12]. In combination with phenylalanine, iodides dimerize phenylalanines, which leads to the formation of hydrogen bonds between amino acid molecules and ensures the binding of the entire molecule to a three-dimensional structure [13]. In combination with amylose, an increased or decreased concentration of iodides determines a shift of system equilibrium to the corresponding direction. A key observation is also that with the addition of iodide to the polyiodide chain in the amylose molecule it becomes less stable, which makes it very different from the complex of iodine with xylan. Also, the polypeptide complex of iodine with magnesium and lithium chloride in FS-1 exhibits inhibitory properties for *Mycobacterium tuberculosis* RNA polymerase, which disrupt the process of transcription of bacterial RNA leading to cell lysis. In addition, FS-1 increases the permeability of the bacterial cell membrane for antibiotics, which makes therapy more effective [14].

Despite the fact that iodine, which is part of FS-1, is bound to a complex with bioorganic ligands, its antimicrobial activity persists. Similarly, in relation to the multiresistant strain of *M. tuberculosis* SCAID 187, the minimum inhibitory concentration is 27.7 μg / ml [15]. In this regard, assessment of the damaging effect on the site of primary contact, when taking FS-1 inside, that is, the mucous membrane of the oral cavity is relevant. Previously, cytotoxicity and genotoxicity of FS-1 were evaluated on a battery of tests, which include *in vitro* and *in vivo* studies in laboratory animals within systemic exposure [16]. In the conducted experiment, FS-1 acted directly on the buccal epithelium, consequently the study was aimed to investigate the local effect of FS-1.

## Materials and methods

*Animals*. Healthy male 6-8 month old rabbits weighing 2.3kg±10% were obtained from the Kazakh Scientific Research Veterinary Institute, Almaty, Kazakhstan. Animals were held under barrier conditions in a biosafety level III animal laboratory at 23±2°C, 50±10% relative humidity, 12/12 h light/dark cycle. All the animals received the standard forage (Assortiment Agro Ltd., Russia) and deionized water consumed *ad libitum*. According to recommendations of the OECD guideline 405, 2-3 rabbits are used for similar tests.

*Design of experiment*. Three rabbits orally received aqueous solution of FS-1 at dose of 4 mg/kg (estimated dose for clinical study) for 14 days. Smears of buccal epithelium obtained before administration of solution were considered as control sample smears, then smears of buccal epithelium obtained on 15$^{th}$ day were considered as experimental sample smears. Aqueous solution of FS-1 was administrated by syringe. After experiment rabbits were not euthanized. The study was approved by the Ethics Committee of the Scientific Center for Anti-Infectious Drugs (protocol No. 9, from May 4, 2018).

Microscopic preparations were fixed by 96% ethanol and stained by Giemsa (5% solution), because it is the basic staining method for detection of cytogenetic aberrations.

Cells were analyzed for the presence of cytogenetic lesions, proliferation arrest, apoptotic and necrotic cells. As amount of obtained cells was not very large and not enough for calculating one thousand cells, up to ten cells from one rabbit were taken into account for measuring degree of cytotoxicological and genotoxic impact. According to pictures the most frequent cytogenetic lesions were nuclear protrusions, karyopyknosis and apoptotic bodies presented even before administration of iodine coordination compound. Similar picture was observed after subchronic administration, level of which was not significantly different from those obtained before the introduction of iodine coordination compound aqueous solution.

Cells with aberrations were calculated as ratio between amount of cells with aberration and normal cells. Aberration index (AI) was calculated as the ratio of the total number of cells with aberrations to the total number of analyzed cells.

*Statistical analysis.* The mean value ($\bar{x}$) and standard deviation (SD) were calculated for each variable measured and analyzed statistically by Wilcoxon matched-pairs signed rank test to determine significant differences between groups at $P< 0.05$.

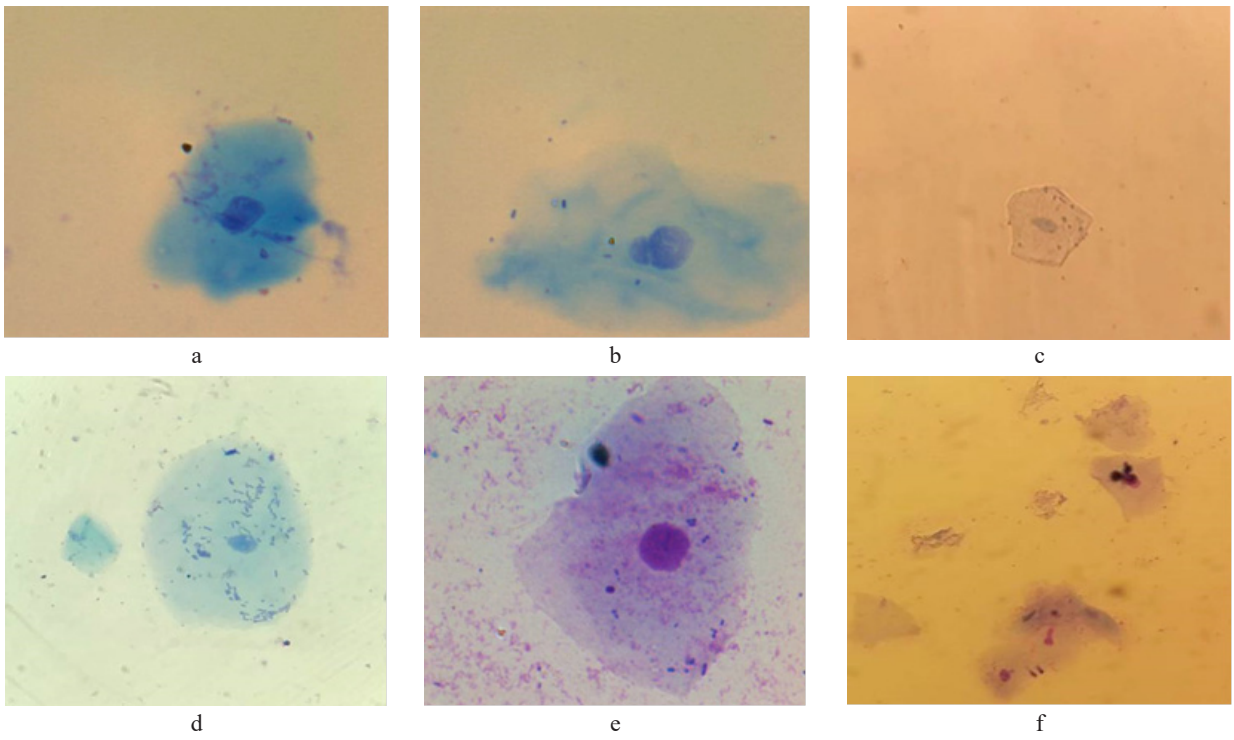
## Results and discussion

During analysis of buccal epithelium cells, we noticed normal cells with unchanged morphological structure as well as cells with micronucleus, nuclear protrusion, karyolysis, and karyorrhexis (Figure 1).

Figure 1 shows microphotography of rabbit buccal epithelium cells and types of cytogenetic aberrations. Normal buccal epithelium cell has full and undamaged structure of nucleus. Nuclear protrusion is a one of the types of cytogenetic aberrations, like micronucleus it can be formed by fragments of chromosomes or by whole chromosomes lagging behind in violation of the spindle of division. Karyorrhexis (disintegration of the cell nucleus into parts) and

karyolysis (dissolution of the cell nucleus particles that has disintegrated due to karyorrhexis) are the final stages of necrobiotic cell death.

Microscopic observation of buccal epithelium cells let us reveal amount of cytogenetic aberrations both in control and experimental sample (Figure 2).




a – normal buccal epithelium cells; b – nuclear protrusion;
c, d – karyolysis; e – micronucleus; f – karyorrhexis

**Figure 1** – Buccal epithelium cells

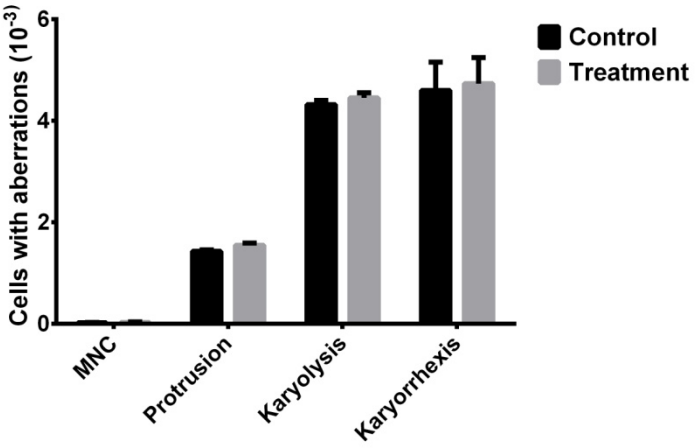



**Figure 2** – Types and amount of aberrations in buccal epithelium

As a result of the microscopic analysis, cells with karyolysis, karyorrhexis, micronuclei, nuclear protrusions that formed during cell division were detected, but their quantity in the experimental group did not exceed the control group ($p = 0.125$). Moreover, the cytogenetic aberration indices in the initial samples of buccal epithelial cells were significantly less than the control indices of the cytogenetic aberration level of human buccal epithelium [17]. Probably, observed cells are associated with spontaneous mutations occurring in the buccal epithelium.

Studies of FS-1 genotoxicity and cytotoxicity *in vitro* and *in vivo* has been conducted repeatedly [18]. In addition, it was found that potassium iodide affects apoptosis of genetically modified lung cancer as a result of increased intracellular levels of iodides, which leads to a significant decrease in tumor size [19]. A study of the influence of molecular iodine on the development of carcinogenesis of the mammary gland revealed that iodine induces apoptotic activity against cancer cells, which manifests itself in a cytotoxic effect [20]. A similar mechanism of action of iodine and its compounds can manifest itself in various directions, namely, by the formation of oxidative stress, apoptosis, necrosis, cell cycle arrest, decrease in the proliferation rate, or in altered cell differentiation. The type of observed mechanism depends on the dose and iodine exposure time. For example, iodine concentration of 20 millimoles contained in povidone-iodine inhibited the proliferation of MCF 7 breast carcinoma, IPS melanoma, A549 and H1299 lung carcinoma, and a Lugol's solution with an iodine content of 20-80 millimoles reduced the growth MCF-7 cells. Significantly high doses of molecular iodine were also studied: 50, 100, 200 millimoles in the composition of povidone-iodine and Lugol's solution, among which the highest concentration of 200 millimoles induced decrease in proliferation rate by about 70%, and in the case of Lugol's solution a significantly lower effect of reduction in 26%. At lower concentrations of 50 and 100 millimoles, cell proliferation decreased by 63% and 56% [21, 22]. These studies allows us to suggest the efficacy of povidone-iodine, Lugol`s solution, and triiodide as antitumor agents. We suppose that high concentration of FS-1 may explain manifested forms of cells, which are consistent stages of necrobiosis. As it is known, karyolysis is the final and irreversible stage, implying the inevitable cell death, while apoptosis manifests itself in a violation of the cell membrane integrity and structure of the cell nucleus, which leads to its fragmentation, as a result of which micronuclei formed in our study [23]. Also, cells that were formed as a result of abnormal course of cell division, namely at the stages of interphase and anaphase mitosis, were noticed [24]. Budding of nucleus in interphase and the lagging of nuclear fragments from chromosomes in anaphase led to the formation of nuclear protrusion [25]. Mitotic errors could be caused at the level of spontaneous mutations, while necrosis could take place due to high concentration of iodine, which caused damage to the cell [26].

## Conclusion

Based on the obtained results, it can be concluded that FS-1 does not cause cytogenotoxic effect on buccal epithelium cells during prolonged exposure to the oral mucosa of rabbits.

## Conflict of interests

The authors declare that there are no competing interests regarding the publication of this paper.

Authors express huge gratitude to Candidate of biological sciences, Head of the Laboratory of Pharmacology and Toxicology of JSC "Scientific Center of Anti-Infectious Drugs" Ibragimova Nailya Akhtamovna for help in the conducted work.

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