УДК 576.3.088

DNA-damaging activity of a new antiviral agent FS-1 in human and rodent tumor cell

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Key words: comet assay, DNA damage, tumor cell

ԴՆԹ-ի վնասվածքներ առաջացնող նոր հակավիրուսային FS-1 գործոնի ակտիվությունը մարդու և մկների ուռուցքային բջիջներում

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Հետազոտվել է հեռանկարային հակավիրուսային և հակամանրէային FS-1գործոնի ԴՆԹ-ի վնասվածքներ առաջցնելու հատկությունը մկների ուռուցքի և մարդու ուռուցքային գծերի բջիջներում: FS-1 գործոնը ստուգվել է մկների լիմֆոմայի L5178Y բջիջներում 500, 1000 ու 2000 մգ/մլ դոզայով և մարդկային Hele ու Caco-2 ուռուցքային գծերի բջիջներում առանց էկզոգեն մետաբոլիկ ակտիվացման 200, 500 ու 1000 մգ/մլ դոզայով: Բոլոր փորձերի արդյունքները բացասական էին: Հիմնվելով այս արդյունքների վրա՝ FS-1 գործոնը հետագայում կարող է հետազոտվել լայն օգտագործման նպատակով որպես անհրաժեշտ հեշտոցային հակավիրուսային գործոն մարդկանց անվտանգության համար HPV ինֆեկցիայի դեմ:

ДНК-повреждающая активность нового антивирусного FS-1 агента в человеческих и мышиных опухолевых клетках

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Изучена способность многообещающего антивирусного и антибактериального FS-1 агента вызывать повреждения ДНК в клетках опухоли мыши и опухолевых линиях клеток человека. FS-1 агент был проверен в дозах 500, 1000 и 2000 мг/мл в клетках лимфомы мыши L5178Y, и в дозах 200, 500 и 1000 мг/мл в человеческих Hele и Caco-2 опухолевых линиях клеток без экзогенной метаболической активации. Результаты всех экспериментов были отрицательны. Основываясь на этих данных, FS-1 агент может быть изучен для его дальнейшего применения против HPV инфекции в качестве перспективного влагалищного антивирусного агента.

Introduction

Cervical cancer causes about 250,000 deaths annually worldwide, with women in developing countries accounting for 80% of these deaths. It is well known that infection of the cervical epithelium with human papillomavirus (HPV) increases the risk of premalignant lesions and progression to cervical cancer [1]. Antiviral agents which kill HPV can be effective in prevention of cervical cancer [1,10].

Recently a very effective drug against many strains of microbes and viruses was developed in Kazakhstan. FS-1 is a complex of iodine with synthesized polysaccharides. It is very potent in veterinary used against many infectious factors of microbial and viral origin. Because of its low toxicity in animals it is possible to use it as topical vaginal microbicide.

This compound has been tested for its mutagenicity in the Salmonella/microsome assay in five strains of microbes (TA 98, TA 100, TA 102, TA 1535, TA 1538) with and without metabolic activation. Completely negative results were obtained (Prof. S. Knasmьller, paper in preparation).

DNA-damaging activity of FS-1 in mammalian cells in vitro systems is unknown. The aim of this work was to evaluate possible DNA-damaging activity of FS-1 in mammalian cells in vitro (mouse lymphoma L5178Y and human HeLa and Caco-2 tumor cell lines).

Materials and Methods

Chemicals. FS-1 was produced in RSOE ***Anti-infectious drugs***, Almaty, Kazakhstan. All chemicals used in experiments were produced by Sigma-Aldrich (St. Louis, USA or Taufkirchen, Germany).

Cells. Mouse lymphoma L5178Y cells were cultured in suspension in RPMI 1640 medium supplemented with antibiotics (95 units/ml penicillin, 95 μg/ml streptomycin), 0.25 mg/ml glutamine/ml, 107 μg sodium pyruvate/ml and 10% heat-inactivated horse serum. Cell cultures were grown in a humidified atmosphere with 5% CO_2 in air at 37° C. To study the DNA-damaging activity first the toxicity of the compound must be evaluated because the tested substances should be used at doses which do not induce death of more than 30% of cells [8]. The experiments were conducted in flasks containing 5 ml of RPMI with 2 x 106 lymphoma cells (incubation time – 18 h) at concentrations of 100, 500, 1000 and 2000 μg/ml. The cell number was evaluated by means of cells counter (Coulter, UK). Since we could not obtain signs of substantial toxicity, the compound was used in the mentioned concentrations (the number of live lymphoma cells was between 98.0% and 81.0% compared with negative control). Two independent experiments with two parallel cultures were carried out.

HeLa (human cervix carcinoma) and Caco-2 (epithelial colorectal adenocarcinoma) cells were obtained from Laboratory U 322 INSERM ***Retrovirus et Maladies Associeftes***, Marseilles, France, and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) heat-inactivated fetal bovine serum and 100 U/ml penicillin/streptomycin (all reagents were from Sigma, USA). Cells were kept at 37° C in humidified 5% CO_2 and 95% air. Cells were allowed to grow in complete medium (containing serum) for 24 h before the start of experiments. The experiments were conducted in flasks containing 5 ml of medium with 2 x 106 HeLa or Caco-2 cells at concentrations of FS-1 100, 200, 500, 1000 and 2000 μg/ml (incubation time – 18 h). The cell number was evaluated by means of cells counter (Coulter, UK). Since 2000 μg/ml and 1000 μg/ml of FS-1 were toxic for both HeLa and Caco-2 cells (ca. 90% and 75% dead cells, respectively for both tumor cell lines), the compounds were tested at doses of 200, 500 and 750 μg/ml.

Comet assay. Comet (single cell gel electrophoresis) assay was carried out as described in our recent papers [6]. Cells were stained with ethidium bromide. A fluorescence microscope at 200 (Komet 5, Kinetic Imaging Ltd., Liverpool, UK) was used for analysis of experiments with mouse and human tumor cells. Fifty cells in total (25 per slide) were analyzed and results were expressed as % DNA in the tail region. Two independent experiments with 2 parallel cultures were carried out.

As a positive control methyl methanesulfonate (MMS) was used in all experiments at concentration 30.0 μg/ml for 3 h incubation (because of high potency of this agent to induce genotoxic effect, [12]). As negative control phosphate buffered saline (PBS) was used.

Statistical analysis. Mann-Whitney U-test was applied to calculate differences between the groups by means of GraphPad Prism (version 3.02) MicrosoftWord.

Results and Discussion

Mouse lymphoma L5178Y cells are a good and sensitive model to study DNA-damaging effects of various agents by means of the comet assay [1]. HeLa and Caco-2 are the most frequently used human cells for various kinds of investigations, including DNA damage studies [4-6].

It seems that FS-1 is more toxic for human tumor cells than for mouse lymphoma cells because in L5178Y cells 2000 μg/ml was not toxic at all, although in human HeLa and Caco-2 cells a dose of 1000 μg/ml induced about 50% cell death.

The results of the experiments on potential DNA-damaging effect of FS-1 on three tumor cell lines are presented in Table 1.

Table 1

DNA-damaging activity of FS-1 in human tumor cell lines HeLa and Caco-2 and in mouse lymphoma L5178Y cells (measured by a computer-aided image analysis system Komet 5, mean of two independent experiments)

Cell line	Compound (concentration)	DNA in tail, % (mean±SE)
HeLa	FS-1 (200)	2.5±0.7
	FS-1 (500)	2.6±0.5
	FS-1 (750)	3.4±1.1
	MMS (30)	44.8±2.2*
	PBS	2.5±0.6
Caco-2	FS-1 (200)	3.2±0.3
	FS-1 (500)	2.7±1.0
	FS-1 (1000)	2.4±0.8
	MMS (30)	37.4±2.3*
	PBS	2.7±1.1
L5178Y	FS-1 (500)	3.5±0.5
	FS-1 (1000)	3.8±0.4
	FS-1 (2000)	3.4±0.4
	MMS (30)	29.4± 3.5*
	PBS	2.6±0.5

* $p<0.001$; Mann-Whitney U-test; MMS – methyl methanesulfonate;
PBS - phosphate buffered physiological saline

It can be seen that no genotoxic effect was found after incubation of cells with FS-1 for 18 h. The positive control induced substantial DNA damage in all experiments. The background DNA damage level was in the range of previous published results [1,5,6].

Hence, FS-1 showed no activity in the comet assay in human and mouse tumor cells.

L5178Y mouse lymphoma and HeLa cells lack ability to metabolize mutagens/carcinogens because of absence of phase I enzymes whereas Caco-2 cells have possibility for metabolic activation of xenobiotics by means of cytochromes CYP1A1, CYP1A2 and CYP1B1 [2, 9]. The absence of activity in Caco-2 cells which possess phase I enzymes, and also in HeLa and mouse lymphoma cells suggest that either the compound has no ability to be transformed into biologically active metabolites, or after the transformation of the compound metabolites have equal genotoxic potential with the parent compound. Our data confirm completely negative results in the Salmonella/microsome assay in five strains of microbes both with and without metabolic activation with S9 mix (Prof. S. Knasmьller, personal communication).

It is noteworthy, that recently Witte et al. [11] showed that the in vitro comet assay can be proposed as an alternative to cytogenetic assays in early genotoxicity screening of drug candidates.

Human papillomavirus (HPV) infection is the second leading cause of cancer-related morbidity and mortality among women due to its very close association with cervical cancer (approximately 24 HPV genotypes specifically infect the genital and oral mucosal system) [3]. The mucosal HPVs are most frequently sexually transmitted, and they are responsible for the most common sexually transmitted diseases throughout the world. HPV infection can lead to cervical cancer, which remains difficult to treat [3]. It is believed that an additional approach to

HPV prevention is the use of microbicidal agents to block or inactivate the virus [7]. Currently, there are not any effective, commercially available, microbicides that can do this. Some such agents are under the trials [3].

Topical vaginal microbicides are practical, female-controlled, and inexpensive agents for prevention of sexually transmitted diseases including HPV. These kinds of agents may be especially attractive in developing countries, where vaccine delivery is economically challenging. And, hence, the results obtained are very important for the next level of the trials with FS-1 which can be used as topical vaginal antiviral agent.

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Acknowledgement. A. Nersesyan carried out a part of the experiments at the Institute of Pharmacology and Toxicology, Wьrzburg, Germany, thanks to DAAD Fellowship.