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Short communication

Study of Urotropin for Virucidal Action toward HSV-1 and HSV-2

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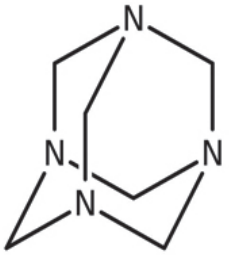
Abstract

Urotropin, a compound used in a wide spectrum of technologies and industries, manifested a marked virucidal effect toward herpes simplex virus (HSV) type 1 *in vitro* contact test.

Keywords: urotropin, virucidal effect, herpes simplex virus



Резюме

Уротропин, съединение използвано широко в редица технологии и в промишлеността, показва отчетлив пряк вирусоциден ефект спрямо вирус херпес симплекс тип 1 в комтактен тест *in vitro*.

Urotropin (hexamethylene-tetramine, hexamine) causes allergy in some humans, related with its capacity to be absorbed through the skin. The rash is the usual symptom of this effect. The compound is used in the rubber and textile gums, in the production of phenol-formaldehyde resins, in the medicine (in urinary system inflammation, flu, meningitis, etc.), in photographic industry, in the production of explosives, for corrosion defense, as protein modifier, as chemical analysis reactive, in deodorants, etc.

Evidently, urotropin is present in large spectrum of technologies where the human skin could be affected. For this reason we decided to study the compound in correlation with one of the most frequent diseases in dermatology – herpes simplex. We started with the testing of the direct urotropin effect on HSV-1 and HSV-2.

We used HSV type 1 (Victoria strain) and HSV-2 (Bja strain), obtained by prof. S. Dundarov and grown in Madin-Darby bovine kidney cells (MDBK). The cells we grown in 96-well plates Corning Inc. (Corning, NY), with growth medium DMEM (Ginco, Invitrogen, Paisley, Scotland, UK), supplemented with 10% fetal bovine serum and 10 mM HEPES buffer (AppliChem GmbH, Darmstadt) and antibiotics (penicillin 100 IU/ml, streptomycin 100 μg/ml) in CO_2 thermostat (ERA cell 150, Haereus, Germany) at 37°C/5% CO_2 HSV strains were cultivated in DMEM + 5% fetal bovine serum, 10 mM HEPES buffer and antibiotics. Infectious titres of viral stocks were $10^{6.5}$ and $10^{6.75}$ $CCID_{50}$/ml, respectively.

Virus assay was carried out by the end point virus dilution (Reed and Muench, 1938). Tested monolayers were observed by light microscopy, the living cells have been subjected to a procedure for the determination of the neutral red absorption (Borenfreund and Puerner, 1985). Optical density (OD) of each well was recorded at 540 nm in Microplate Reader (Organon Teknik Reader 530, Oss, Nederland). CPE percentage was evaluated by the following formula: $\%CPE = 100-[(OD_D-OD_{100})/OD_{cc} \times 100]$, where the mean value of well OD_s of the highest virus dilution at which 100% CPE was observed ,and OD_{cc} was the mean value of OD_s in wells in the cell culture (uninfectied). 50% CPE was determined by regressive analysis.

In the study for virucidal action of urotropin the viral suspension containing 100 ID_{50} was mixed with equal volume (v/v) of urotropin. The mixture was stored at room temperature and samples for the determination of the residual infectious virus were taken after 10 min, 30 min and 60 min. The results obtained are presented in Table 1 and Table 2.

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Table 1. Effect of urotropin toward HSV-1 (Victoria strain)

Test groups	Contact 10 min		Contact 30 min		Contact 60 min	
Urotropin, μg/ml	log10CCID50/ml	Δlog	log10CCID50/ml	Δlog	log10CCID50/ml	Δlog
0 (Control)	6.6		6.2		6.2	
100	6.6	0	6.2	0	5.3	0.9
320	5.5	1.1	5.1	1.1	5.1	1.1
1000	4.8	1.8	4.8	1.4	5.0	1.2

Table 1. Effect of urotropin toward HSV-2 (Bja strain)

Test groups	Contact 10 min		Contact 30 min		Contact 60 min	
Urotropin, μg/ml	log10CCID50/ml	Δlog	log10CCID50/ml	Δlog	log10CCID50/ml	Δlog
0 (Control)	7.0		6.5		6.8	
100	6.6	0.4	7.0	0	7.0	0
320	7.0	0	7.0	0	6.8	0
1000	6.6	0.4	6.6	0	6.8	0

In conclusion

1. Urotropin shows virucidal effect toward HSV-1. This effect is dose-dependent. It is observed markedly at concentration of 1000 μg/ml and diminished at 320 μg/ml. The compound was without effect at 100 μg/ml.

2. The effect is still seen after 10-min contact and there is an insignificant diminishment along the duration of the contact.

3. Urotropin does not manifest direct virucidal effect toward HSV-2.

References

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