Assessing Cytotoxicity of Topical Antimicrobial Agents Used In Burn Care to Human Keratinocytes With a Different Bioassay

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The cytotoxicity of topical antimicrobial agents to cells in the epidermis is important when treating skin grafts, especially cultured grafts. This study evaluates the cytotoxicity of various topical agents to cultured human keratinocytes (HK) by employing a direct, easily reproducible bioassay in a serum-free system.

The Neutral Red Bioassay™ (Clonetics) was used to test all agents. Neutral red (3-amino-7-dimethylamino-2-methylphenazine hydrochloride) is a water-soluble dye which passes through intact membranes of viable cells and is concentrated in the lysosomes. Neutral red uptake is proportional to number of viable cells.

Twenty-five hundred HK, growing in serum-free KGM™ (MCDB 153), were placed into 96-well tissue culture plates and incubated for 3 days. Agents diluted in KGM were then placed into the wells and incubated for 2 additional days. Medium was removed and neutral red was added for 3 hours. The cells are then washed, fixed, and dye was extracted. The intensity of red was measured at 540 mm. Osmolarity and pH were recorded for each agent. Serial dilutions (2 log conc. at half log intervals) of each agent are expressed relative to highest dose tested, X. Results are presented as: 1) percentage uptake vs control (KGM - antibiotics) and 2) NR-50 the concentration of test agent which reduces proliferation by 50%. Students' t test was used and statistical significance assumed if $p<.05$(*).

% NEUTRAL RED VS CONTROL

Agent (Conc. X)	**Dilution:**	**X**	**$X^{-0.5}$**	**X^{-1}**	**$X^{-1.5}$**	**X^{-2}**	**NR-50**
Gentamicin (1mg/ml)		19*	32*	90	85	117	.25 mg/ml
GU Irrigant		30*	76*	93	75*	80	0.7%
Neomycin (0.4 mg/ml)		77*	87	90	83	88	>.4 mg/ml
Polymyxin B ($2x10^3$ U/ml)		″	″	″	″	″	″
Polysporin		7*	4*	30*	91	72*	7%
Bacitracin (500 U/ml)		50*	108	100	96	92	5000/ml
Polymyxin B (10^4 U/ml)		3*	9*	62*	112	77	12000/ml
Sulfamylon (0.85%)		0*	3*	69*	125	133	1.6 mg/ml
Betadine (5%)		3*	5*	0*	90	117	0.22%
Acetic Acid (0.25%		3*	9*	141	137	84	0.5%
Mod. Dakins (2.5%)		7*	62*	91	90	74*	1%

The data shows that each agent tested has toxicity at clinically relevant doses. These results parallel our previous findings employing a more difficult direct cell proliferation study and therefore should be considered when assessing agents for toxicity to human keratinocytes.

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