

INVESTIGATIVE REPORT

Evaluation of Phototoxic Properties of Antimicrobials Used in Topical Preparations by a Photohaemolysis Test

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Antimicrobials are widely used in topical formulations as preservatives or as therapeutically active agents. Photosensitization by such compounds has not yet been studied systematically. To identify possible phototoxic properties, antimicrobials (benzyl alcohol, bronopol, chloracetamide, clioquinol, diazolidinyl urea, ethylenediamine dihydrochloride, formaldehyde, glutaraldehyde, imidazolidinyl urea, sodium benzoate, propylene glycol) were evaluated *in vitro* by means of a photohaemolysis test using suspensions of human erythrocytes. Irradiations were performed with UVA- and UVB-rich light sources. In the presence of bronopol or clioquinol, there was photohaemolysis up to 78.1% or 48.5% with UVA and up to 100% or 34.3% with UVB, respectively. The phototoxic effect depended on the concentration of the compounds and the UV doses administered. None of the other substances tested caused significant photohaemolysis. It is concluded that bronopol and clioquinol exert phototoxic effects *in vitro* and thus might also cause photosensitization when used on the skin. The clinical significance of this has to be established by further work. **Key words: photosensitization; phototoxicity; preservatives; antibiotics; photohaemolysis.**

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Antimicrobials are used as preservatives in water-containing cosmetics or topically applied drugs to inhibit the growth of microorganisms, which may cause degradation of the product or endanger the health of the consumer (1). Also, such components are administered to the skin for treatment or prevention of microbial infections (2). Whereas contact allergy to preservatives and antibiotics is frequent and quite well characterized (3), only a few case reports on photosensitivity reactions due to antimicrobials have been published (4–6).

Phototoxic compounds may directly induce acute skin reactions. Even more important, long-term use of phototoxic agents has been found to be associated with an increased number of actinic keratoses, i.e. precursors

of squamous cell carcinoma (7). Photochemotherapy, using photosensitizing psoralens and UVA radiation (PUVA) to treat skin diseases, is associated with an increased number of malignant skin tumours (8). Quinolones, which have been found to be phototoxic in the photohaemolysis test (e.g. ciprofloxacin, enoxacin, ofloxacin) (9), were found to be associated with photocarcinogenesis *in vitro* (10) and *in vivo* (11, 12). Thus, although the photohaemolysis test does not show effects related to DNA damage, the phototoxicity supports photocarcinogenic risks. This is not surprising as, besides DNA damage, other mechanisms also contribute to UV-induced skin cancer (13–15). These findings prompted us to systematically assess *in vitro* the phototoxic potential of various antimicrobials used in preparations applied to the skin.

MATERIALS AND METHODS

Test substances

Tests were performed with the following compounds: benzyl alcohol, tert-butylhydroquinone, chloracetamide, chlorhexidine digluconate, diazolidinyl urea, ethylenediamine dihydrochloride, formaldehyde, glutaraldehyde, imidazolidinyl urea and propylene glycol (Sigma, St Louis, USA); bronopol, sodium benzoate, phenylmercuric acetate (Aldrich, Steinheim, Germany) and clioquinol (Merck, Darmstadt, Germany). The test substances were dissolved in appropriate solvents (methanol or ethanol) and further diluted in TCM buffer (NaCl, 7.0 g; Tris, 3.0 g; KCl, 0.3 g; MgCl₂ × 6 H₂O, 0.2 g; CaCl₂ × H₂O, 0.147 g; aqua dest. ad 1000 ml, pH 7.4; 280 mosm/kg).

UV sources

Irradiations were performed with the following UVA-rich (i) or UVB-rich (ii) lamps: (i) UVASUN 5000 (Mutzhass, Munich, Germany), emitting in the range of 320–460 nm (maximum at about 375 nm). UVA irradiance at a distance of 40 cm was 42 mW/cm², the irradiation time for the maximum dose used (100 J/cm² UVA) was 40 min; (ii) TL 20 W/12 light bulbs (Philips, Hamburg, Germany) with a main emission between 275 and 365 nm (maximum at about 315 nm). Irradiance was 1.0 mW/cm² for UVB and 0.4 mW/cm² for UVA at a distance of 40 cm; the irradiation time for the maximum dose used (3200 mJ/cm²UVB) was 53 min.

Dosimetry

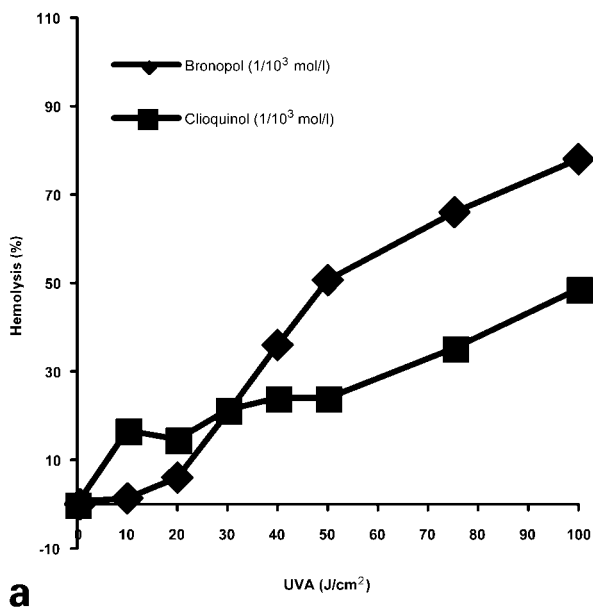
UVA or UVB intensities or doses were measured by an integrating instrument (Centra-UV, Osram, Munich, Germany).

Photohaemolysis test

The test was done as described previously (9). Briefly, suspensions of human erythrocytes or correspondingly prepared erythrocyte-free samples were incubated with the test substances at concentrations of 10^{-3} mol/l for 1 h at 37°C. Both substance-free erythrocyte samples (blanks) as well as samples containing the test substances (including erythrocyte-free controls) were exposed to 0, 10, 20, 30, 40, 50, 75 or 100 J/cm² UVA (UVASUN 5000) or to 0 (0), 400 (0.16), 800 (0.32), 1600 (0.64), 2400 (0.96) or 3200 (1.28) mJ/cm² UVB (J/cm² UVA) from the TL 20W/12 light bulbs. During irradiation, samples were kept in a shaking bath at 37°C; 100% haemolysis was obtained by exposure of the erythrocytes to distilled water. After an incubation period of 30 min in the dark supernatants were recovered by centrifugation. After a further incubation for 15 min with Drabkin's solution (Sigma), haemolysis was determined by reading of absorbance at 550 nm with an MR 700 Microplate® reader (Dynatech, Denkendorf, Germany). Haemolysis was calculated on the basis of the absorbance data according to the formula:

$$\text{Haemolysis (\%)} = 100 \times \frac{\text{test sample} - \text{blank} - \text{erythrocyte-free sample}}{\text{total haemolysis} - \text{blank}}$$

In order to exclude equivocal results, only haemolysis >5% was regarded to be a meaningful positive finding. Results are given as median of three independent experiments performed with erythrocytes from three different donors.



RESULTS

Tert-butylhydroquinone, chlorhexidine digluconate and phenylmercuric acetate induced significant haemolysis (>15%) without UV irradiation at a concentration of 10^{-3} mol/l. These substances were not further tested for phototoxic effects.

With exposure to the UVASUN 5000 lamp, bronopol as well as clioquinol, each at the 1 mM concentration, caused a UV dose-dependent haemolysis up to 78.1% and 48.5%, respectively (Fig. 1a). At 1 mM, both compounds also induced UV dose-dependent haemolysis upon irradiation with the TL 20W/12 light bulbs, reaching 100% with bronopol and 34.3% with clioquinol (Fig. 1b). All other compounds tested for phototoxicity did not yield haemolysis >5%.

It can be seen from Fig. 1 that 75 J/cm² of UVA and 2 J/cm² together with 1 mM clioquinol caused 25–30% haemolysis, whereas these UV doses together with 1 mM bronopol caused 65–90% haemolysis. This UVA and UVB exposure is equivalent, as an example, to 4 h of irradiation (2 h before and 2 h after local noon) for Central Europe (Munich) on 15 June with average cloudless conditions (16).

DISCUSSION

Clioquinol and bronopol exerted prominent phototoxic effects in this *in vitro* assay, photohaemolysis occurring

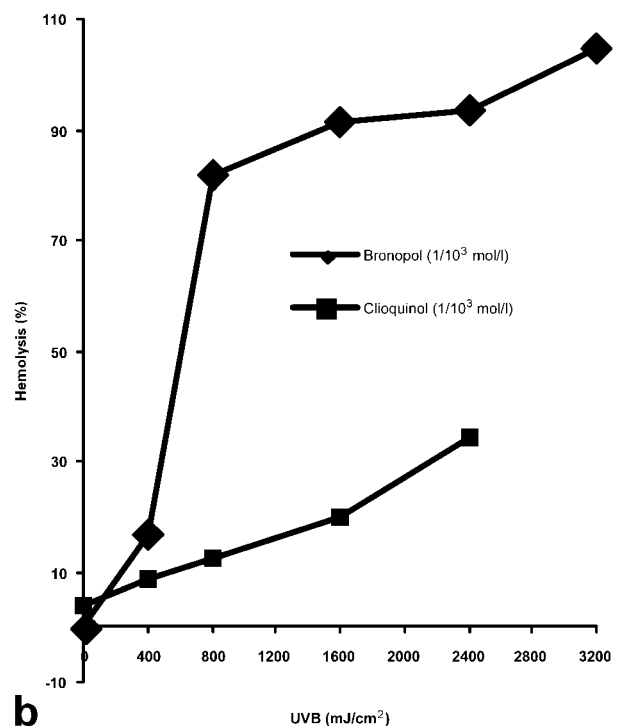


Fig. 1. Median of haemolysis induced by bronopol (10^{-3} mol/l) or clioquinol (10^{-3} mol/l) and radiation rich in UVA ($n=3$) (a) or UVB ($n=3$) (b).

with exposure to both UVB- and UVA-rich sources. The action spectrum in the UVB range is of particular interest, as the majority of photosensitizers are active in the UVA range.

Clioquinol is a quinoline compound that has antibacterial and antifungal properties. It is chemically related to the quinolones among which there are many phototoxic compounds (9). Clioquinol is used in topical preparations to treat skin infections such as infected eczema or athlete's foot and can also be present in eye or ear preparations. Positive patch test reactions to clioquinol were noted at a frequency of 0.7% in the study (17). Delayed contact allergy to a number of contact allergens, including clioquinol, was more frequent in patients with photosensitivity dermatitis/actinic reticuloid than in a comparison group (18). It may be speculated that the phototoxic action of clioquinol could be related to the development of these conditions.

Bronopol is widely used as a preservative in cosmetics and toiletries. Allergic contact dermatitis to bronopol has been reported, the sensitization rate being about 0.5% (19, 20). Previously, phototoxic or photoallergic properties of bronopol were said to have been ruled out experimentally (21). These results are in contrast to our findings. However, in the studies done by Raab (21) aqueous solutions of bronopol were exposed to a UV source and only changes of the absorption curves before and after irradiation were used as indication of photoeffects. Evidently, this procedure is insufficient to determine biological effects.

Our data suggest that possible photosensitizing effects of common contactants should be studied systemically. A further step should be to assess the clinical relevance of findings obtained by *in vitro* tests. Particularly, it would be important to study the relation of results obtained in the photohaemolysis tests to effects on DNA and on organisms *in vivo*. Nevertheless, the current knowledge that clioquinol and bronopol are phototoxic agents can increase our awareness in clinical practice, where photosensitivity reactions to topical antimicrobials may be underestimated.

As a systematic search for phototoxic compounds is quite often 'successful' (22–24), such substances seem quite abundant. However, acute phototoxic reactions are not very frequent. Nonetheless photosensitization below the threshold of overt skin disease may enhance chronic photodamage, which is primarily characterized by 'photoaging' and photocarcinogenesis (7). As the latter has become an ever-increasing health problem, the suggested association between exposure to 'common' phototoxic compounds and enhanced photocarcinogenesis demands further studies.

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REFERENCES

1. De Groot AC. Fatal attractiveness: the shady side of cosmetics. *Clin Dermatol* 1998; 16: 167–179.
2. Spann CT, Taylor SC, Weinberg JM. Topical antimicrobial agents in dermatology. *Clin Dermatol* 2003; 21: 70–77.
3. Schnuch A, Geier J, Uter W, Frosch PJ. Patch testing with preservatives, antimicrobials and industrial biocides. Results from a multicentre study. *Br J Dermatol* 1998; 138: 467–476.
4. Vilaplana J, Romaguera C. Contact dermatitis from tosylamide/formaldehyde resin with photosensitivity. *Contact Dermatitis* 2000; 42: 311–312.
5. Rivara G, Barile M, Guarrera M. Photosensitivity in a patient with contact allergic dermatitis from clioquinol. *Photodermatol Photoimmunol Photomed* 1991; 8: 225–226.
6. Shelley W. Immediate sunburn-like reactions in a patient with formaldehyde photosensitivity. *Arch Dermatol* 1982; 118: 117–118.
7. Placzek M, Eberlein-König B, Przybilla B. Association between actinic keratoses and potentially photosensitizing drugs. *N Engl J Med* 1999; 341: 1474–1475.
8. Stern RS, Bolshakov S, Nataraj AJ, Ananthaswamy HN. p53 mutation in nonmelanoma skin cancers occurring in psoralen ultraviolet a-treated patients: evidence for heterogeneity and field cancerization. *J Invest Dermatol* 2002; 119: 522–526.
9. Przybilla B, Georgii A, Bergner T, Ring J. Demonstration of quinolone phototoxicity *in vitro*. *Dermatologica* 1990; 181: 98–103.
10. Chetelat AA, Alberini S, Gocks E. The photomutagenicity of fluoroquinolones in tests for gene mutation, chromosomal aberration, gene conversion and DNA breakage (Comet assay). *Mutagenesis* 1996; 11: 497–504.
11. Klecak G, Urbach F, Urwyler H. Fluoroquinolone antibacterials enhance UVA-induced skin tumors. *J Photochem Photobiol B* 1997; 37: 174–181.
12. Mäkinen M, Forbes PD, Sternbäck F. Quinolone antibacterials: a new class of photochemical carcinogens. *J Photochem Photobiol B* 1997; 37: 182–187.
13. Schwarz T. Photoimmunosuppression. *Photodermatol Photoimmunol Photomed* 2002; 18: 141–145.
14. de Gruijl FR. Ultraviolet radiation and tumor immunity. *Methods* 2002; 28: 122–129.
15. Fisher MS, Kripke ML. Systemic alteration induced in mice by ultraviolet light irradiation and its relationship to ultraviolet carcinogenesis. 1977. *Bull World Health Organ* 2002; 80: 908–912.
16. CIE, Spectral weighting of solar ultraviolet radiation. *Comm. Internationale de l'Eclairage, Vienna, Austria. Technical Report CIE* 2003; 151. ISBN 3901906207.
17. Morris SD, Rycroft RJ, White IR, Wakelin SH, McFadden JP. Comparative frequency of patch test reactions to topical antibiotics. *Br J Dermatol* 2002; 146: 1047–1051.
18. Hannuksela M, Suhonen R, Förström L. Delayed contact allergies in patients with photosensitivity dermatitis. *Acta Derm Venereol* 1981; 61: 303–306.

19. Choudry K, Beck MH, Muston HL. Allergic contact dermatitis from 2-bromo-2-nitropropane-1,3-diol in Metrogel. *Contact Dermatitis* 2002; 46: 60–61.
20. Peters MS, Conolly SM, Schroeter AL. Bronopol allergic contact dermatitis. *Contact Dermatitis* 1983; 9: 397–401.
21. Raab W. 2-nitro-2-bromopropane-1,3-diol (bronopol) and UV-rays. *Arch Dermatol Res* 1980; 269: 211–212.
22. Eberlein-König B, Bergner T, Diemer S, Przybilla B. Evaluation of phototoxic properties of some food additives: sulfites exhibit prominent phototoxicity. *Acta Derm Venereol* 1993; 73: 362–364.
23. Diemer S, Eberlein-König B, Przybilla B. Evaluation of the phototoxic properties of some hypolipidemics in vitro: fenofibrate exhibits a prominent phototoxic potential in the UVA and UVB region. *J Dermatol Sci* 1996; 13: 172–177.
24. Eberlein-König B, Bindl A, Przybilla B. Phototoxic properties of neuroleptic drugs. *Dermatology* 1997; 194: 131–135.