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Photochemical decomposition of chloramphenicol in a 0.25% eyedrop and in a therapeutic intraocular concentration

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Summary

It has been postulated that in the metabolism of chloramphenicol, nitroso compounds can be formed in the 'predisposed host'. This would be the cause of non-dose-related aplastic anemia. Solid proof for this metabolic formation in vivo has not been given. However, on exposure to sunlight under in vivo-related circumstances, relevant to the eye under treatment, we found beside *p*-nitrobenzaldehyde, *p*-nitrobenzoic acid and *p*-nitrosobenzoic acid. The latter even up to 45 mol% of the starting amount of chloramphenicol. This photoreaction is not restricted to the eye but may also occur in the skin.

Introduction

From the time of its isolation in 1947 by Burgholder, chloramphenicol (CAP) has been used extensively in the treatment of typhoid and meningitis and as a broad spectrum antibiotic in the topical treatment of microbial infections of the eye.

CAP is used systemically at a daily dose of 1.5–3 g, and locally as a 0.25% eyedrop. In this form it is used against superficial corneal infections. Therapy duration varies between 14 days to several months. Various side-effects from CAP treatment are known but the most serious complication is a non-dose-related aplastic anaemia which is usually irreversible and often appears only after the drug therapy has been discontinued (Bartlett 1967). Aplastic anaemia is found in 1 per 25,000 to

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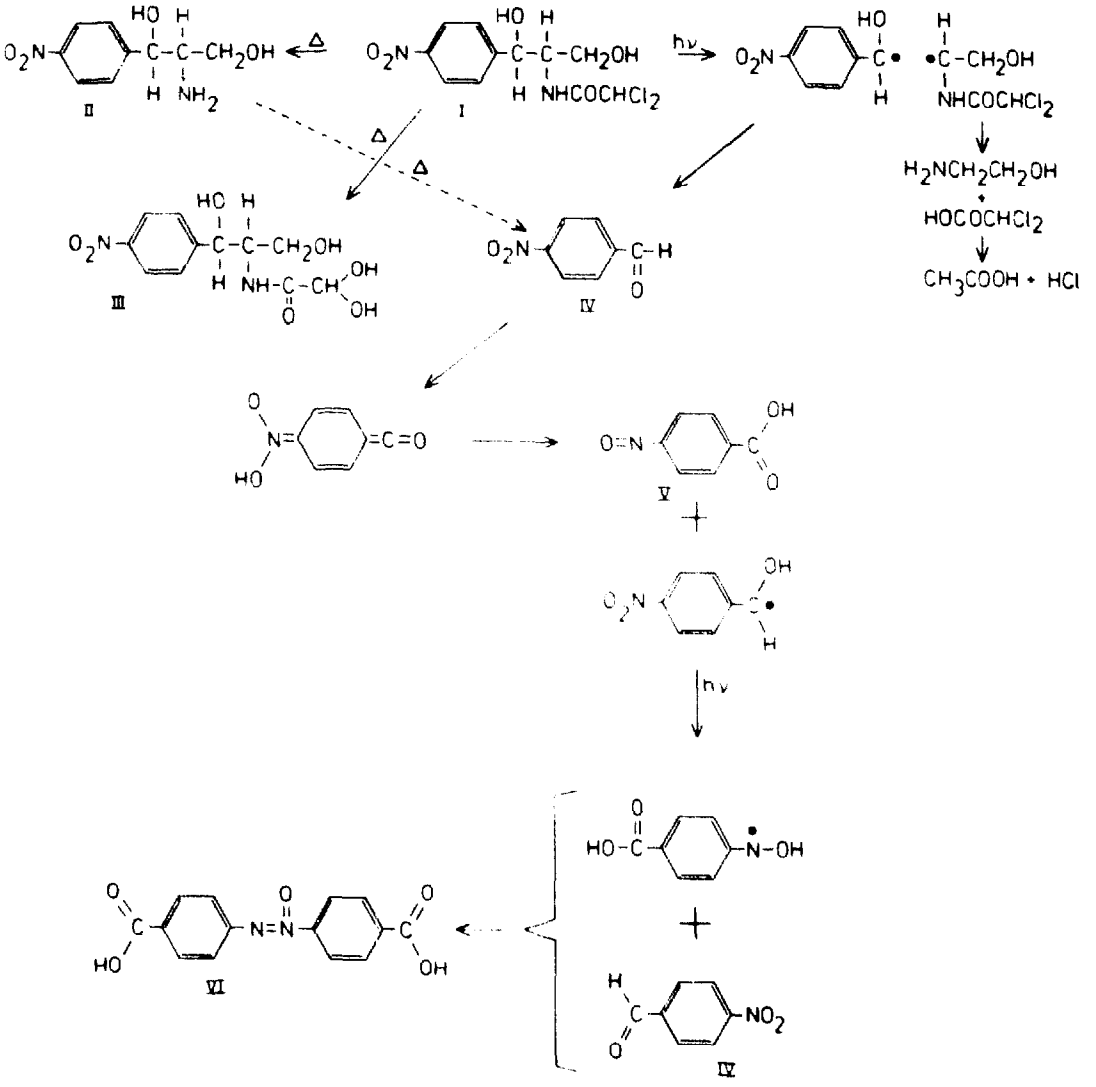
40,000 patients (Wallerstein, 1969). This side-effect is not restricted to systemic treatment but has also been observed with the use of eyedrops (Rosenthal, 1965; Carpenter, 1975; Abrams et al., 1980; Fraunfelder et al., 1982; Fraunfelder and Bagby, 1983).

Nitroso compounds possibly formed in the metabolism of CAP have been and still are suspected to be the cause. In vitro it has been shown that nitroso-chloramphenicol can induce effects on human bone marrow cells that might give rise to an aplastic anaemia syndrome in vivo (Yunis et al., 1980).

Yunis postulated that CAP-induced aplastic anaemia can occur in the predis-



Scheme 1. Photochemical and thermochemical decomposition of CAP according to Mubarak et al. (1982) and Boer et al. (1983), respectively. I = chloramphenicol; II = chloramphenicol base; III = chloramphenicol alcohol; IV = *p*-nitrobenzaldehyde; V = *p*-nitrosobenzoic acid; VI = 4,4′-azoxybenzoic acid.

posed 'host' who provides the milieu for the metabolic transformation of the *p*-NO_2 group of the drug, to toxic intermediates. Although observations may support his concept, solid proof for the metabolic formation in vivo of toxic intermediates has not been given. Concerning the photochemical and thermochemical non-enzymatic decomposition of CAP in solution, products have been identified (Mubarak, 1982). As can be seen in Scheme 1, unstable and reactive products are not found with thermochemical decomposition (*p*-nitrobenzaldehyde is not always found and may be an artefact as a consequence of incomplete exclusion of light (Kieviet et al., submitted). However, with light, reactive products such as *p*-nitrobenzaldehyde (PNB) and even a nitroso compound are formed. Such products may lead to toxic effects if they are present in the applied drug or if they are formed in the body during exposure to sunlight. In the latter case radicals (Scheme 1) may contribute to the ultimate effect. Concerning the photochemical instability it is only strongly advised to prepare the drug with exclusion of light as far as possible and to store it in the dark.

In the present study we paid attention to a CAP eyedrop. If photoproducts in the eyedrop are investigated, it may be considered of even more importance to look for photoproducts which can be formed when CAP solutions in intraocular concentrations are exposed to sunlight, as is normally the case. For that reason, besides the eyedrop, with and without preservative, we investigated the photochemical decomposition of CAP in a concentration relevant to the eye.

Materials and Methods

Water was demineralized and distilled in an all-glass apparatus before use. Organic solvents were 'chemically pure' and used after distillation. Chloramphenicol (Brocacef), *p*-nitrobenzaldehyde (*p*-NB; p.a. Merck) and *p*-nitrobenzoic acid (*p*-NBA; p.a. Flucka) were used as purchased. *p*-Nitrosobenzoic acid (*p*-NOBA) was synthesized as described by Shih (1971). The CAP eyedrop was prepared according to F.N.A. (Formularium der Nederlandse Apothekers) prescription (per 100 ml: 250 mg CAP, 1.5 g boric acid, 300 mg borax and 4 mg phenyl mercuric borate) and exposed to sunlight as such (1.0 mW/cm^2 at 360 nm). All exposures were performed in glass vials, diameter 11.5 mm). The solar intensity was determined by means of a UVX Digital Radiometer (Ultraviolet products inc.).

Simultaneously the same eyedrop without the preservative, phenylmercuric borate, was exposed. Samples were taken every minute during 5 min and were analyzed by HPLC.

Furthermore a buffered solution (0.05 M phosphate, pH 7.0) of CAP in a concentration of 10 mg/l was exposed to sunlight (1.4 mW/cm^2 at 360 nm). Samples were taken every 5 min during 45 min and analyzed by HPLC.

HPLC analyses was performed with a Spectra Physics SP 740 B solvent delivery system equipped with a Spectra Physics fixed-wavelength detector (254 nm) Injections were done by means of a Kontron MSI-660 autosampler in which the content of the vials could be processed in the dark. The column was a Chrompack 5 RP8;

15 × 0.4 cm i.d. with (1.2 ml/min) methanol/0.01 N perchloric acid (25 : 75) as eluent. But for the column length and the flow speed, this is the HPLC system used for the analysis of CAP and metabolites as described in a previous communication (Lee, 1981).

Results and Discussion

From Fig. 1 it becomes clear that under the given circumstances only sunlight of 285 nm and longer will contribute substantially to the photodecomposition of the CAP molecule. The results from exposure to sunlight with an intensity, as we measured on a normal summer day in Holland, are reproduced in Fig. 2. As can be concluded from the graph, more than 7 mol% of CAP is converted into *p*-NB within 5 min of exposure. Other products were not detectable with the HPLC system used.

Mercuric phenyl borate (or acetate) is often used as a preservative (Martindale 28th edn.; British Pharm. Codex, etc.) in the formulation of the eyedrop. As this compound may effect the photochemical reaction, its influence was investigated but proved to be small.

The warning to prepare the CAP eyedrop in diffuse light and to store it in the dark is certainly not groundless. However, the relative speed of the reaction makes one hope that this advice is followed promptly by the pharmacist and last but not least by the user. The product, *p*-NB can cause eye damage to experimental animals (Sax, 1968) which can be considered as a questionable property for an eyedrop. Although stability of the preparation is certainly better in coloured glass vials, exposure of CAP to light can hardly be avoided as soon as the eyedrop is applied to the eye. The concentration in the aqueous humor of the eye after topical application is approximately 5–15 mg/l. Systemic application can lead to an aqueous humor concentration of 5–30 mg/l (Bartlett, 1982).

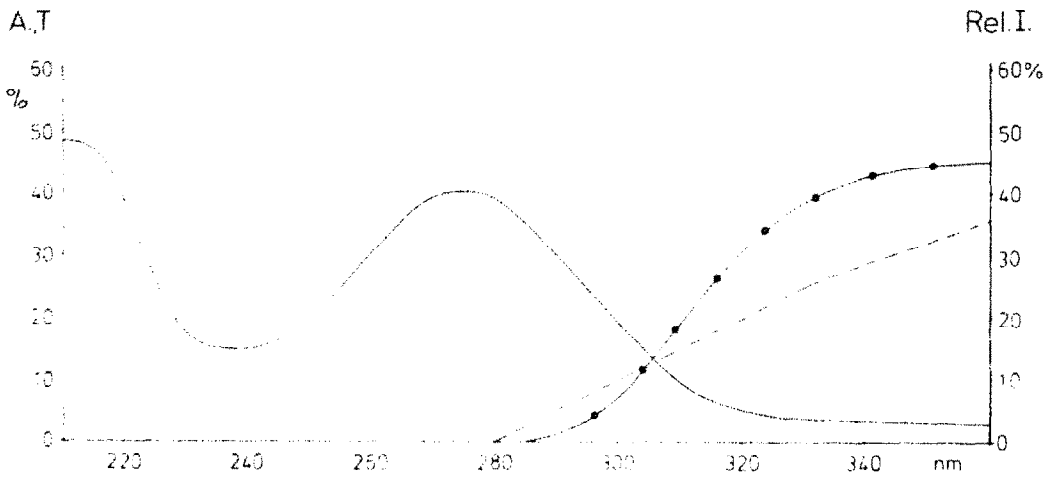


Fig. 1. ———, absorption spectrum of CAP (10^{-3} M); ●———●, transmission spectrum of vials used; - - - - - -, relative intensity of the sun (at 500 nm put at 100%).

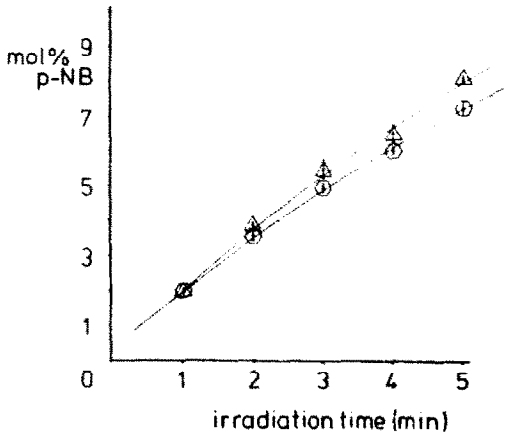


Fig. 2. Formation of *p*-nitrobenzaldehyde (*p*-NB) during exposure of the eyedrop to sunlight ($\lambda > 285$ nm). Calculation of mol% *p*-NB on starting amount of CAP (mol) which was put at 100%. ⊕ = with; and ▲ = without phenyl mercuric borate. (Each point is the mean of two observations; vertical lines represent deviation of the average.)

A single dose of chloramphenicol, once entered into the aqueous humor, is transported eventually to other parts of the body but the maximum concentration in the eye is maintained constant for about 90 min (Honegger, 1961). In the exposure of the 0.25% eyedrop to sunlight, the only product found was *p*-NB, if the irradiation took not longer than 10 min. However, when buffered solutions of CAP in a concentration occurring in the eye during therapy, are exposed to sunlight for a period of time in which CAP is assumed to be present in constant concentrations, product formation is more complex.

Fig. 3 demonstrates that, from a concentration of 10 mg/l of CAP, more than 80% is degraded by sunlight within 45 min. 30% is converted into *p*-NB. This compound has reached a maximum of 35 mol% at 25 min. Besides *p*-NB, *p*-NBA and *p*-NOBA also appear to be formed. The last compound even up to 45 mol% after 45 min. (The formation of *p*-NBA was not mentioned by Mubarak but has been noticed by Shih.) Thus the possibility that nitroso compounds in the eye can be formed under the influence of sunlight is clearly demonstrated. In both topical and systemic application of CAP, the conditions for the formation can be met. The data obtained should be considered in connection with the strong suspicion against nitroso compounds and their part in the development of aplastic anaemia.

Furthermore it should be noted that the risk of formation of nitroso compounds by light will certainly not be limited to the eye but may also take place in the skin. This is supported by the known formation of vitamin D_3 in the skin and, e.g. our recent research with chlordiazepoxide. Rats treated with chlordiazepoxide and irradiated with UV-A light in a dose equivalent to sunlight at sea level, suffered from liver damage as a result of the formation of a reactive oxaziridine in the skin (Bakri et al., 1983).

Bearing in mind the relatively high dosage and concentration in the blood and the sometimes prolonged therapy, damage by nitroso compounds from CAP, formed in

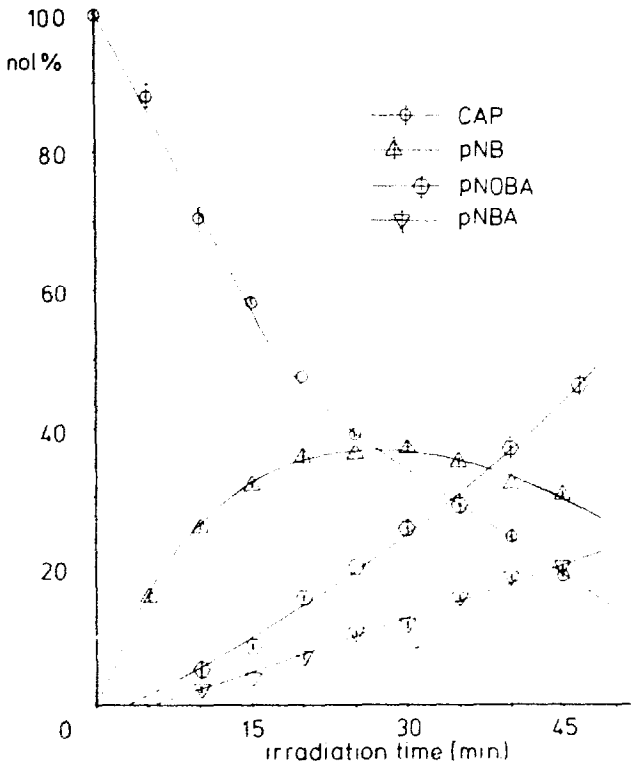


Fig. 3. Product formation and decrease of CAP during exposure of CAP (10 mg/l) to sunlight ($\lambda > 285$ nm). Starting quantity of CAP (mol) put at 100%; calculation of CAP and products at time t based on this amount. (Each point is the mean of two observations; vertical lines represent deviation of the average.) CAP = chloramphenicol; pNB = *p*-nitrobenzaldehyde; pNBA = *p*-nitrobenzoic acid; pNOBA = *p*-nitrosobenzoic acid.

the skin or eye on exposure of the body to sunlight should be considered as a possibility. Research with the rat which is in progress now, will provide us with more information about the photoreactivity of CAP in vivo.

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