In vitro Evaluation of Phototoxic Properties of Proton Pump Inhibitors, H₂-receptor Antagonists and Statins

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Proton pump inhibitors and H₂-receptor antagonists are widely used in the treatment of peptic ulcers and gastric acid-related diseases, because of their acid production inhibitory properties. Another group of frequently prescribed drugs, statins are used to significantly decrease the serum levels of cholesterol, thus stabilizing atherosclerotic plaques. Statins, as well as proton pump inhibitors and H₂-receptor antagonists, are generally well tolerated. However, there are some case reports of photosensitive side-effects (1–12): ranitidine has been shown to induce increased photosensitivity, whereas pantoprazole, lansoprazole and omeprazole have been associated with the development of cutaneous lupus erythematosus, a known photosensitive skin disease. Furthermore, statins mainly induced chronic actinic dermatitis. We therefore assessed the *in vitro* phototoxic potential of several proton pump inhibitors, H₂ antagonists, and statins, using a photohaemolysis test. This assay is an established *in vitro* method used in isolated erythrocytes for measurement of possible phototoxic effects. The effects of anti-oxidative substances (e.g. ascorbic acid, Trolox®, a water-soluble derivative of vitamin E) can also be assessed easily.

MATERIALS AND METHODS

Tests were performed with the following compounds: omeprazole (Sigma-Aldrich, Schnelldorf, Germany), esomeprazole (kindly donated by AstraZeneca, Mölndal, Sweden), pantoprazole (Altana Pharma, Konstanz, Germany), lansoprazole, nizatidine, ranitidine, cimetidine, famotidine, lovastatin (Sigma-Aldrich), pravastatin (Dragenopharm, Tittmoning, Germany). The spectral analysis data of these components were taken from the collection "UV and IR Spectra of Pharmaceutical Substances and IR Spectra of Pharmaceutical and Cosmetic Excipients" (ECV Editio Cantor Verlag, Aulendorf, Germany). For inhibition tests additionally ascorbic acid and Trolox® (Sigma-Aldrich) were used.

The test substances were dissolved in appropriate solvents (methanol or ethanol) if required and further diluted in TCM buffer (NaCl 35.0 g; Tris 15.0 g; KCl 1.5 g; MgCl₂ × 6H₂O, 1.0 g; CaCl₂ × 2 H₂O 0.75 g; aqua dest. ad 5000 ml; pH 7.4; 280 mOsm/kg). In order to improve dissolution some suspensions were sonicated in the bath-type ultrasonic cleaner Sonorex Super RK 510 H (Bandelin, Berlin, Germany) for 25 min.

Irradiation was performed with the following ultraviolet (UV) A-rich (*i*) or UVB-rich (*ii*) lamps:
- Sellas 4000 (Sellas Medizinische Geräte GmbH, Gevelsberg, Germany), emitting in the region of 323–399 nm (maximum between 350 and 390 nm). Irradiance was 68 mW/cm² UVA at a distance of 40 cm.
- TL 20 W/12 light bulbs (Philips, Hamburg, Germany) with a main emission range of 275–365 nm (maximum approxima-

tely 315 nm). Irradiance was 1.45 mW/cm² UVB, and 0.65 mW/cm² UVA at a distance of 40 cm.

UVA or UVB intensities or doses were measured by an integrating instrument (UV-Meter, VARIOCONTROL, Waldmann, Schwenningen, Germany).

The photohaemolysis test was performed as described previously (13). Briefly, three times washed fresh human erythrocytes from healthy donors (normal UV sensitivity, no intake of photosensitizing drugs or vitamins) were suspended at a dilution of 1:200 in TCM buffer containing 0.03% human albumin. A 0.4 ml volume of this erythrocyte suspension and a correspondingly prepared erythrocyte-free sample were incubated with 0.1 ml of the test substance preparations at three concentrations (1.0, 0.1 or 0.01 mmol/l) for one hour at 37ºC. Co-incubation of 0.1 ml omeprazole (1.0 mmol/l) with 0.1 ml ascorbic acid and/or Trolox® (1.0, 0.1 and 0.01 mmol/l) was done in additional experiments using a 0.2 ml volume of the erythrocyte suspension. Both substance-free erythrocyte samples (blanks) as well as samples containing the test substances (including erythrocyte-free controls) were exposed to 0, 10, 20, 40, 50 or 60 J/cm² UVA (Sellas 4000) or to 0 (0), 400 (0.18), 800 (0.36) or 1600 (0.72) mJ/cm² UVB (J/cm² UVA) from TL 20 W/12 light bulbs. During irradiation, samples were kept in a shaking bath at 37ºC. To obtain total haemolysis (100%) erythrocytes were exposed to distilled water. After an incubation period of 30 min in the dark, supernatants were recovered by centrifugation. The released haemoglobin in the supernatants was determined as cyan-methaemoglobin after incubating the samples for 15 min with Drabkin's solution (Sigma-Aldrich) for 15 min. Haemolysis was determined by reading the absorbance at 550 nm with a Opsys Microplate reader (DYNEX Technologies Inc., Berlin, Germany) and calculated on basis of the absorbance data according to the formula:

$$Haemolysis = 100 \times \frac{test\ sample - blank - erythrocyte\text{-}free\ sample}{Total\ haemolysis - blank}$$

In order to exclude equivocal results, only haemolysis >5% was regarded as a meaningful positive finding. Results are given as median of 3 independent experiments with erythrocytes from 3 different donors.

RESULTS

UVB-induced photohaemolysis was caused by omeprazole as well as esomeprazole and pantoprazole, each at a concentration of 1.0 mmol/l. Omeprazole caused photohaemolysis rates up to 69.1%, esomeprazole up to 29.5%, and pantoprazole up to 34.2% at 1600 mJ/cm² UVB (Fig. 1). Photohaemolysis was not found with UVA irradiation or with lower concentrations of the substances. None of the other 7 tested compounds induced significant haemolysis with UVA or UVB irradiation.

Co-incubation of ascorbic acid at a concentration of 1.0 mmol/l with omeprazole inhibited UVB-induced

photohaemolysis completely. The same effect was seen after incubation with Trolox® at a concentration of 1.0 mmol/l and after incubation with both antioxidants.

Lower concentrations of both antioxidants together exceeded the effects of the single substance at all UVB-irradiation doses, e.g. ascorbic acid alone at a concentration of 0.1 mmol/l reduced photohaemolysis from 49.3% to 36.1% at 1600 mJ/cm^2, Trolox® alone from 49.3% to 27.1%, whereas the combination of both antioxidants decreased the photohaemolysis from 49.3% to 12.0% (medians).

DISCUSSION

Omeprazole, pantoprazole and esomeprazole exerted considerable phototoxic effects in this *in vitro* assay, photohaemolysis occurring only by exposure to the UVB-rich source. This is of particular interest, as the majority of photosensitizers have their action spectrum in the UVA range (13). UVB-sensitivity of the tested substances may be due the absorbance ranges with a maximum absorbance of omeprazole at 302 nm, of pantoprazole at 291 nm and esomeprazole at 203 nm.

Omeprazole has been found to accumulate in the skin and lead to hyperpigmentation in sun-exposed areas (14). Furthermore, cutaneous discoid lupus erythematosus has occurred after taking pantoprazole, lansoprazole or omeprazole (5–7). Systematic analysis of the photosensitive potential of pharmaceutical substances supports these clinical findings. Omeprazole, having its absorption peak in the UVB wavelength region, showed phototoxic potential leading to peroxidation of lipids. Furthermore, generation of superoxide O_2^- after UVA/UVB exposure was demonstrated (15). According to our study, omeprazole, esomeprazole and pantoprazole induced photohaemolysis based on lipid peroxidation after UVB exposure. As photosensitivity is attributed to lupus erythematosus, increased UVB-photosensitivity by proton pump inhibitors might have contributed to the occurrence of this disease.
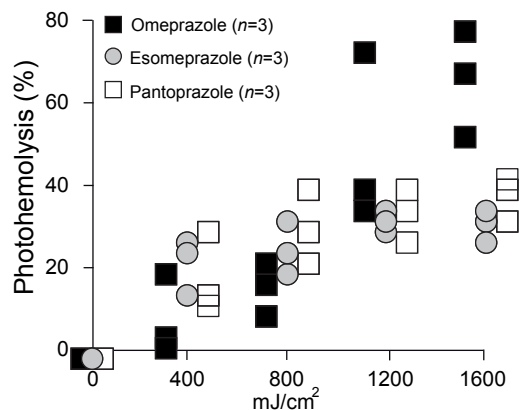

Fig. 1. Photohaemolysis caused by omeprazole, esomeprazole and pantoprazole, each at a concentration of 1.0 mmol/l, and irradiation rich in ultraviolet B (UVB). Single values of 3 independent individuals are given.

The H$_2$-receptor antagonist ranitidine has been reported to induce a UVA-triggered florid photosensitive eruption. A chronic actinic dermatitis was demonstrated by a biopsy (1). Also, an UVB-photosensitivity proven by lowered UVB-minimal erythema dose (MED) has been noticed (2). On the other hand, cimetidine showed a protective effect on the delayed phase of a hematoporphyrin-induced phototoxicity by reducing erythema and oedema (16). Our *in vitro* photohaemolysis test did not demonstrate phototoxic properties of H$_2$-receptor antagonists.

Phototoxic side-effects are described during treatment with statins. Simvastatin seemed to induce a delayed photosensitivity reaction with decreased MED after UVA exposure (8) and chronic actinic dermatitis with decreased MED in the 300–400 nm range (9) or persistent UVB sensitivity (10). Pravastatin induced a lichenoid dermatitis with hyperpigmentation on the face, and upper back, and minimal expression on buccal mucosa (10), suggesting a phototoxic effect. A recent report described systemic photosensitivity induced by simvastatin and pravastatin that presented as photo-distributed erythema multiforme. The diagnosis was confirmed by a marked reduction in the UVB-MED or both UVA and UVB-MED (12).

Neither pravastatin nor lovostatin showed any phototoxic potential in our photohaemolysis test. Yet the complex mechanism of drug-induced hyperpigmentation cannot be simulated with a photohaemolysis test.

The protective effect of ascorbic acid and tocopherol against haemolysis induced via oxidative stress has been demonstrated previously (17). Even after oral intake of ascorbic acid and d-α-tocopherol, phototoxic lysis of erythrocytes could be reduced (18) and UVB-sensitivity decreased in humans (19).

The photoprotective effect of ascorbic acid and tocopherol was also shown for omeprazole-induced UVB-phototoxicity in this study. We could demonstrate protective effects of single antioxidants and a superior effect by combining ascorbic acid and tocopherol. As proton pump inhibitors are in long-term use and may lead to an increase in UV-caused skin damage there is need for prevention. In addition to sunscreens, different sun-protecting measures should be considered, including application of antioxidants during the intake of omeprazole.

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