

Phototoxicity of Non-steroidal Anti-inflammatory Drugs: *In vitro* Studies with Visible Light

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Photosensitivity reactions due to non-steroidal anti-inflammatory drugs (NSAID) are well-known side-effects of these agents and are usually attributed to ultraviolet (UV) radiation. Eight NSAID (carprofen, diclofenac, ibuprofen, ketoprofen, naproxen, piroxicam, phenylbutazone, tiaprofenic acid) were assessed in a photohemolysis test for phototoxic effects in the visible light range. For the studies an experimental visible light lamp was used, emitting 5% UVA at most. To control for UVA-induced effects, additional samples were exposed to appropriate UVA doses from an UVA source and as well to the visible light lamp while being covered by a GG 400 or GG 420 filter. Photo-induced hemolysis was found after exposure to the visible light lamp with carprofen, ketoprofen, naproxen, piroxicam, phenylbutazone or tiaprofenic acid; diclofenac or ibuprofen did not cause photohemolysis. Control irradiation with UVA induced minor hemolysis only with carprofen, ketoprofen and tiaprofenic acid. When the samples were exposed to the filtered visible light lamp, using the GG 400 filter, photohemolysis was found with carprofen, naproxen and phenylbutazone and, using the GG 420 filter, with carprofen and phenylbutazone. Phototoxic effects induced by visible light may be of importance with regard to clinical photosensitization. **Key words:** drug reaction; photosensitivity; photohemolysis; visible light irradiance.

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Non-steroidal anti-inflammatory drugs (NSAID) are widely used as analgesic and antirheumatic agents. Photosensitization is a well-known side effect of this group of drugs (1, 2), and it may occur due to phototoxic or photoallergic mechanisms (3). Many of the NSAID causing photosensitivity are phenylpropionic acid derivatives, e.g. benoxaprofen, carprofen, ketoprofen, tiaprofenic acid or naproxen (4–11). Other structurally unrelated NSAID, e.g. piroxicam, have repeatedly been associated with photosensitivity reactions as well (12, 13). The action spectrum of photosensitization due to NSAID was found to lie in the UVA or UVB range (14–17). However, longer wavelengths have usually not been investigated with regard to their possible relevance for the elicitation of photo-reactions. We have screened a number of NSAID (carprofen, diclofenac, ibuprofen, ketoprofen, naproxen, piroxicam, phenylbutazone, tiaprofenic acid) for phototoxic properties in the visible light (VL) range by means of a photohemolysis test using human erythrocytes.

MATERIAL AND METHODS

Lamps

Irradiation was performed with the following lamps: (1) experimental high-intensity VL metal vapour lamp 1,000 W PL (Saalmann, Herford, Germany),

emitting in the range of 360 nm–680 nm (maxima at about 400 nm–460 nm and 550 nm–620 nm); total irradiance at a distance of 50 cm was 9.5 mW/cm² with a UVA irradiance of 400 µW/cm² (4.2% of total irradiance); the spectral irradiance of the experimental lamp was measured with a double monochromator (Shimadzu, Tokyo, Japan) (Fig. 1); (2) UVASUN 5,000 (Mutzhas, Munich, Germany), emitting in the range of 320 nm–460 nm (maximum at about 375 nm); UVA irradiance at a distance of 40 cm was 51 mW/cm² measured by an integrating instrument (Centra-UV; Osram, Munich, Germany) (18).

Filters

The following filters were used: Schott glass filters (Schott, Mainz, Germany) GG 400 or GG 420 (thickness 3.0 mm ± 0.3 mm). These filters transmitted 50% of radiation at 400 nm or 420 nm, respectively, while wavelengths shorter than 390 nm (transmission 0.03%) or 410 nm (transmission 0.2%) were virtually cut off. Irradiance beneath these filters, measured with a double monochromator (Shimadzu, Tokyo, Japan), was 89% or 87% of the initial intensity.

Test substances

Tests were performed with the following NSAID: carprofen (Hoffmann La Roche, Zürich, Switzerland), ibuprofen (Serva Feinbiochemica, Heidelberg, Germany), tiaprofenic acid (Albert Roussel, Wiesbaden, Germany), ketoprofen (Bayer Leverkusen, Germany), diclofenac, naproxen, piroxicam and phenylbutazone (Sigma, St. Louis, USA). The test substances were dissolved in appropriate solvents and further diluted in a Tris-calcium-magnesium buffer (TCM buffer, pH 7.4; (19)).

Hemolysis assay (19; modified after 20)

Erythrocytes were prepared from freshly drawn human venous blood by centrifuging three times at 2,600 rpm for 10 min and washing the cells with TCM buffer after careful removal of the supernatants. Then the cells were resuspended at a dilution of 1:200 in TCM buffer containing 0.03% human albumin. Aliquots of 0.4 ml of this solution were incubated in polystyrene tissue culture dishes (plain bottom, opening area 1.75 cm²) for 30 min at 37°C in a gently shaken waterbath with 0.1 ml solution of the test substance (final concentrations 10⁻⁴ mol/l or 10⁻³ mol/l) or with buffer alone (blank). Erythrocyte-free dishes were prepared with 0.4 ml TCM buffer and 0.1 ml solution of the test substances (erythrocyte-free sample). Duplicate samples were irradiated at a distance of 50 cm beneath the VL source or at 40 cm beneath the UVA source in the waterbath. Irradiation doses were 0, 5, 10, 20 or 50 J/cm² with the 1,000 W PL lamp, 0 or 2.5 J/cm² UVA with the UVASUN 5,000 lamp; these irradiations were performed to control for UVA-induced effects from the 1,000 W PL lamp (UVA control irradiation). The dishes were covered with aluminium foil after the respective doses had been reached. The waterbath temperature during irradiation with the 1,000 W PL lamp was 37°C with 0, 5 or 10 J/cm², 38°C with 20 J/cm² and 39°C with 50 J/cm². After irradiation,

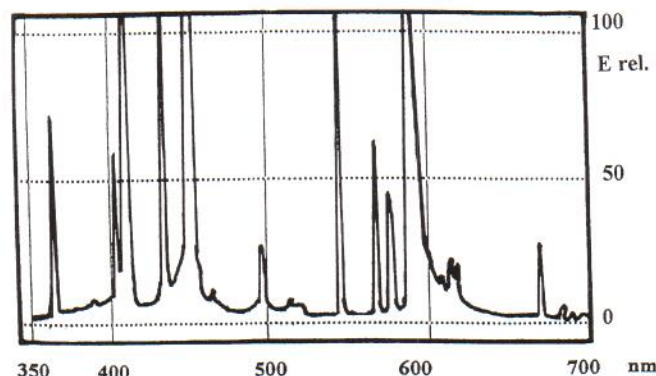


Fig. 1. Spectral distribution of the high-intensity VL lamp 1000 W PL.

incubation was continued for another 30 min. Then supernatants were recovered by centrifugation (2,600 rpm, 10 min), and aliquots of 0.15 ml were incubated with 0.05 ml of Drabkin's solution (Sigma, St. Louis, USA) in microplates for 15 min at room temperature. Absorbance was read with a microplate reader (MR 700; Dynatech, Denkendorf, Germany) against a sample containing 0.15 ml TCM buffer and 0.05 ml Drabkin's reagent (test filter: 550 nm; reference filter: 660 nm). For each irradiation dose 100% hemolysis values were obtained by adding distilled water to erythrocytes spun down from samples of the blank which had been exposed to the respective doses. Hemolysis was calculated on the basis of the absorbance data according to the formula:

$$\text{Hemolysis (\%)} = 100 \times \frac{(\text{test sample}) - (\text{blank}) - (\text{erythrocyte-free sample})}{(100\% \text{ value}) - (\text{blank})}$$

For each compound, testing was performed with erythrocytes from at least three to five donors; the results are given as medians. Throughout the testing procedure, exposure to intense ambient daylight or artificial light was avoided. In order to exclude equivocal results, only photohemolysis >5% was regarded to be a meaningful finding.

RESULTS

At 10^{-3} mol/l carprofen, ketoprofen, naproxen, phenylbutazone and tiaprofenic acid were found to induce phototoxic effects, causing photohemolysis >40% with at least one of the doses from the VL lamp. Piroxicam-dependent photohemolysis at the 10^{-3} mol/l concentration was up to 12% after irradiation with the VL lamp. Photohemolysis depended on drug concentration and radiation dose. Diclofenac or ibuprofen at the 10^{-3} mol/l concentration and all investigated agents at the 10^{-4} mol/l concentration did not cause any phototoxic effect. Details are shown in Fig. 2.

UVA control irradiation with 2.5 J/cm² UVA induced minor hemolysis only in the presence of carprofen (4.3%), ketoprofen (7.6%) or tiaprofenic acid (17.2%). When the GG 400 filter was used, photohemolysis induced by the VL lamp was found with carprofen, naproxen or phenylbutazone, when the GG 420 filter was used, with carprofen or phenylbutazone. Detailed results are given in Fig. 2.

When testing was performed as described with solvents alone, no hemolysis was found. Following irradiation with the highest doses used, pH values of the test solutions at 10^{-3} mol/l ranged between 7.2–7.5, the changes due to exposure being ≤0.3.

DISCUSSION

Six out of the eight NSAID evaluated exhibited a phototoxic action depending on the dose administered with the VL lamp. The propionic acid derivatives (carprofen, ketoprofen, naproxen, tiaprofenic acid), as well as phenylbutazone, caused a clear-cut photohemolysis, whereas with piroxicam only a minor, but still significant hemolysis was found. Irradiations with a UVA lamp to control for UVA-induced effects from exposure to the VL lamp resulted in minor hemolysis only with carprofen, ketoprofen or tiaprofenic acid. When the GG 400 filter was used, there were phototoxic effects dependent on drug concentration and radiation dose from the VL lamp with naproxen, carprofen or phenylbutazone. Using the GG 420 filter, we could demonstrate phototoxic effects with carprofen or phenylbutazone.

Phototoxic activity of the phenylpropionic acid derivatives carprofen, ketoprofen, naproxen and tiaprofenic acid has been demonstrated in vitro using cultures of *Candida albicans*, human leukocytes or erythrocytes (14, 15). Similarly, the phototoxicity of these compounds has been established in animal experiments and by phototesting in humans (16, 17,

21). In vitro results correlate well with the occurrence of clinical reactions to these compounds (6–10). However, reports about clinical photosensitivity due to the phenylpropionic acid derivative ibuprofen are rare (22).

Piroxicam is also a well-known cause of photoinduced drug reactions. In contrast to other photosensitizing NSAID, piroxicam phototoxicity could not be demonstrated in most of the in vitro assays used (14, 23). Western et al. found a piroxicam metabolite to cause phototoxic effects in vitro (24). Photoreactions due to piroxicam have been claimed to be photoallergic (14, 23, 25–27). In some clinical reports photoactive properties have been assigned to phenylbutazone, a NSAID which is better known to induce adverse skin reactions like erythema multiforme (28). With diclofenac clinical photosensitivity reactions have been rare (29).

The action spectra of photosensitizing NSAID, particularly propionic acid derivatives, were often found to be within the UVA range, rarely within the UVB range. The absorption of all NSAID tested lies mainly in the UVA/UVB region, and they have only little absorption in the VL range (14–17, 21–22). Photohemolysis results found at testing with the 1,000 W PL lamp emitting VL and infrared energy seem not to fit these absorbance characteristics. However, factors such as binding of compounds to cell membranes or specific complexing of molecules which shift the absorption spectrum may influence the phototoxicity action spectra (19, 30).

The results reported here give evidence of phototoxic effects of some of these drugs in the VL range. With carprofen and phenylbutazone photohemolysis was obtained using the GG 420 filter (transmission 0.03% at 410 nm); thus the phototoxic effects were induced by wavelengths longer than 410 nm. There were naproxen-dependent phototoxic reactions using the GG 400 filter but not using the GG 420 filter; the action spectrum seems to end in the range between 390 nm and 410 nm.

With ketoprofen or tiaprofenic acid there was no photohemolysis using the GG 400 or GG 420 filter, but a very prominent photohemolysis after exposure to the VL lamp alone emitting at most 5% UVA. UVA control irradiations induced only a minor phototoxic hemolysis due to these compounds. Evidently, the combination of VL with UVA potentiates phototoxic hemolysis. This effect might also have contributed to the phototoxic effects we could demonstrate with piroxicam. The waveband interaction between VL and UVA could be interpreted as a photoaugmentation reaction. Photoaugmentation or photoinhibition induced following UVA plus UVB or UVB plus UVA is an ambiguous phenomenon, the true mechanism of which has not yet been fully elucidated (31–37). Furthermore, it has to be considered that the rather high doses of infrared emitted by the 1,000 W PL lamp also could have contributed to the phototoxic effects observed.

When compared with the 10 J/cm² dose, there was a decrease of ketoprofen-induced phototoxic hemolysis at the irradiation dose of 20 J/cm² VL from the 1,000 W PL lamp, followed by an increase of photohemolysis at 50 J/cm² VL. This finding, which was seen with the samples from all five donors (data not shown), is difficult to explain. Perhaps it may be due to increased phototoxic degradation of hemoglobin at 20 J/cm², which could be overcome by photohemolysis at higher doses.

Further investigations are necessary to evaluate the clinical relevance of VL for drug induced photosensitivity. However, it can already be concluded from the results obtained in vitro

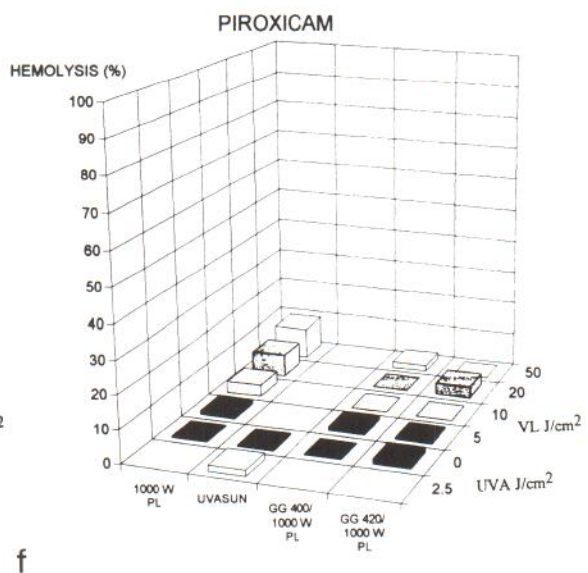
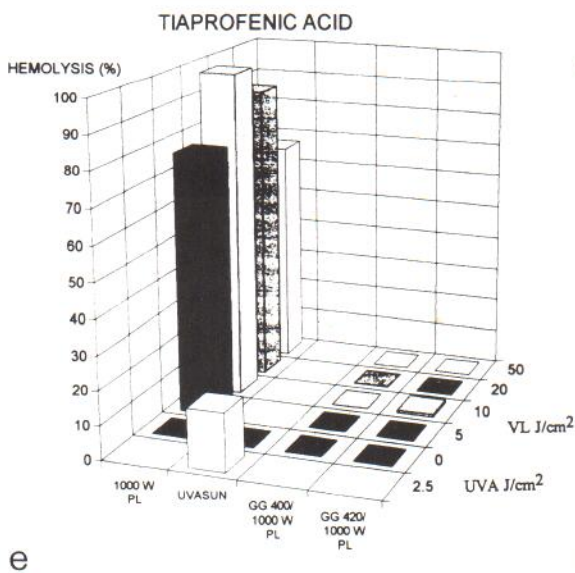
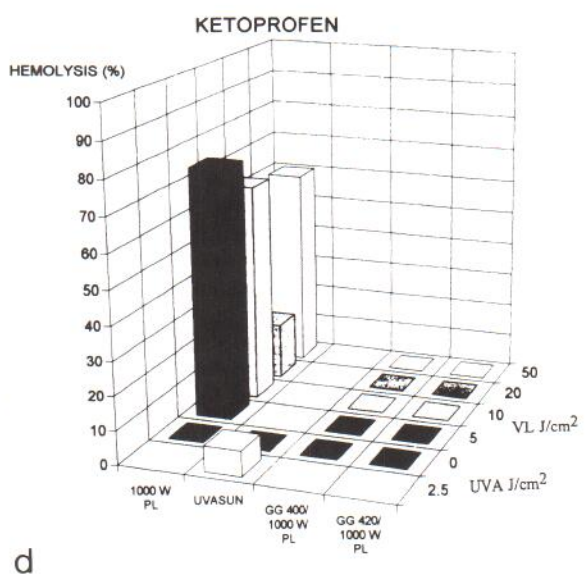
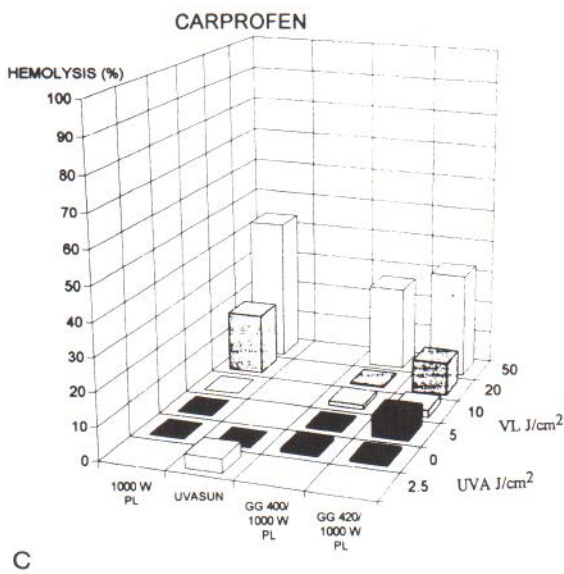
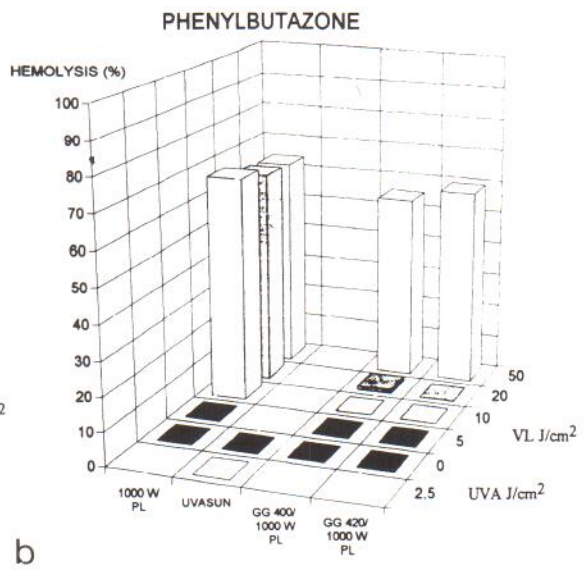
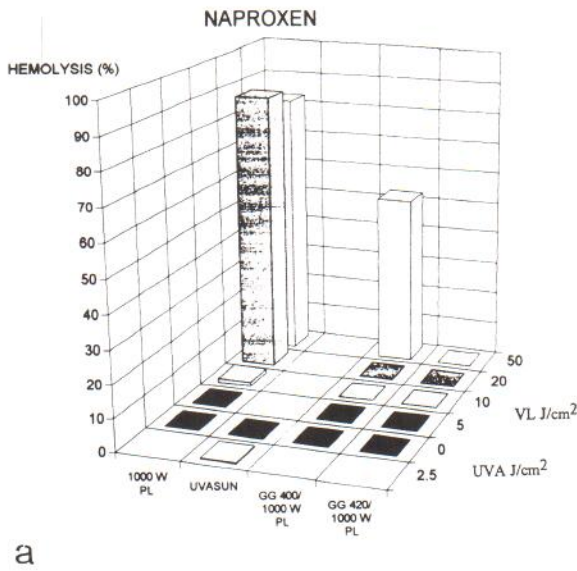


Fig. 2. (a-f) Wavelength-dependence of photohemolysis induced by naproxen, phenylbutazone, carprofen, ketoprofen, tiaprofenic acid or piroxicam (each compound 10^{-3} mol/l; results given as median), 1,000 W PL: high intensity VL lamp; UVASUN: UVASUN 5,000 lamp; GG 400 (GG 420): irradiation beneath filter GG 400 (GG 420).

that the VL range can probably be of importance with regard to certain photoreactions.

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