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Photodegradation and *in vitro* phototoxicity of aceclofenac

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Received August 9, 2005, accepted December 8, 2005

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Pharmazie 62: 337–341 (2007) *doi: 10.1691/ph.2007.5.5139*

Aceclofenac (Airtal®) (**1**) is a photoallergic and phototoxic anti-inflammatory and analgesic agent. This drug is photolabile under aerobic and anaerobic conditions. Irradiation of an ethanol-solution of aceclofenac under oxygen or argon at 290–320 nm affords via decarboxlation compound **2** as the main isolated and spectroscopically identified photoproduct, besides hydroxylamine derivates **3** and **4**. A radical intermediate was evidenced spectrophotometrically with GSH and DTNB, as well as by the dimerization of cysteine. Red blood cell lysis photosensitized by **1–4** was investigated. Furthermore, in a lipid-photoperoxidation test with linoleic acid the *in vitro* phototoxicity of aceclofenac was also verified. The photoinduced generation of peroxide by compound **1** was determined during the irradiation in presence of NADPH by chemiluminescence. In relation to the photoallergic activity of this drug, the interaction of aceclofenac with human serum albumin (HSA) has been studied through fluorescence spectroscopy. No photoinduced binding was observed after irradiation of compounds **1** in the presence of human serum albumin.

1. Introduction

Aceclofenac [[2-(2′,6′-dichlorophenyl)amino]phenylacetoxyacetic acid] (**1**), is a non-steroidal anti-inflammatory drug with analgesic properties, which is widely used in tablets and intramuscular injections (Grau 1991; Brogden 1996; Legrand 2004). This compound is capable of initiating adverse light-induced biological effects. Some cases of photodermatitis from this drug have been reported (Ludwig 2003). Recently, cases of photoallergic reactions triggered by aceclofenac have also been reported (Goday Bujan 2001). The possibility that photoproducts of the drug are involved in the photosensitivity, photoallergic or phototoxic reactions is now being studied by us. It is presumed that there must be a relationship between photochemical behavior and phototoxicity. In this context, very little is known as yet about the interrelation between photochemistry and its phototoxicity. This fact has prompted us to examine the photolysis of **1**. These studies have led to the isolation of the photoproducts of **1** and their characterization by spectroscopic means. The formation of **2** probably involves the generation of a radical intermediate. The latter could be detected when the irradiation was carried out in presence of either reduced glutathione or cysteine used as radical scavengers (Costanzo 1989). The present work also reports the phototoxic effects of **1** and its photolysis products on human erythrocytes, out of which compounds **2–4** (Scheme) turned out to be less effective than **1** in their photohemolytic properties (Costanzo 1989) and the *in vitro* study of the photooxidant properties of aceclofenac by chemiluminescence (Mouithys-Mickalad 2000).

2. Investigations and results

The photolysis of **1** (absorption maxima at 205 and 284 nm in methanol) was carried out under UV-B light (290–320 nm) and a decrease was followed by monitoring the disappearance of these bands, at 20 min intervals. The results are shown, for a methanolic solution (1×10^{-4} M) of **1**, in Fig. 1. No difference in the photoproduct yields or in the velocity of the reaction was found in the photolysis under either O_2 or argon.

The photolysis reaction of **1** is shown in the Scheme. The major photoproduct of **1** was **2** (yield = 89%). The formation of photoproduct **2** is compatible with an initial excitation of aceclofenac after light absorption, followed by a

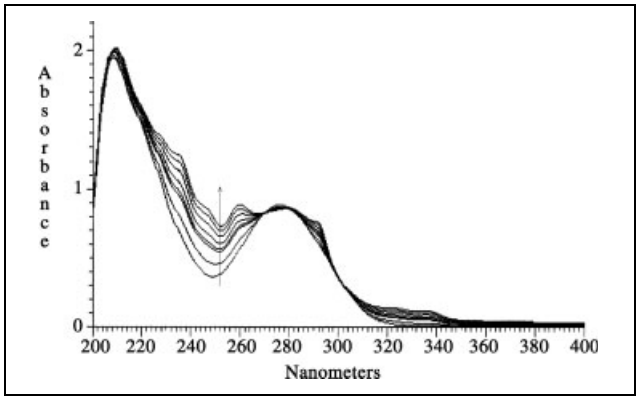
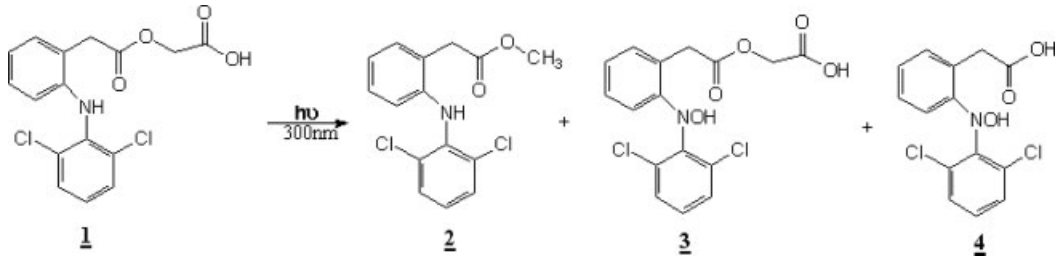


Fig. 1: UV monitoring of the photolysis of aceclofenac by irradiation with UV-B light at intervals every 2 min.

Scheme



cleavage of the CH_2-COOH bond (decarboxylation) which would give a radical intermediate that via hydrogen abstraction could give rise to this compound. The methyl ester derivate **2** formed in the photolysis of **1** was compared with the compound described in the literature, used as a compound for determination by gas liquid chromatography of this drug and their metabolites, as well as in the synthesis of new prodrugs of its degradation product, diclofenac (Sastry 1988; Poiger 2001; Sharma 2003).

The formation of compounds **3** and **4** (yield = 7 and 4% respectively) could come from the hydroxylation of the amino group and subsequent hydrolysis of the resulting molecule. Similar compounds arising from the hydroxylation of the diclofenac have been previously reported in the literature (Bort 1999).

Our investigation has indicated that **1** undergoes photohemolysis after 20 min irradiation ($\lambda = 290-320$ nm) at any of the concentrations tested under either aerobic or anaerobic conditions. Nevertheless, oxygen markedly enhances lysis. Typical experiments are illustrated in Fig. 2. No lysis was observed either when cells were irradiated for 20 min in the absence of drugs or photoproducts, or when they were incubated for 2 h in the dark at 37 °C with 100 μg/ml of the drug.

The possibility that singlet oxygen was produced during the irradiation of **1** in the red blood cell (RBC) solution was examined. In fact, this compound did not produce singlet oxygen as was shown when the photolysis of **1** was carried out in the presence of the usual singlet oxygen and superoxide anion scavengers such as 2,5-dimethylfuran (DMF), sodium azide (NaN_3) and of superoxide dismutase (SOD) respectively used during the photohemolysis test. No changes were observed during the photohemolysis test of **1** in the presence of the reactive oxygen species (ROS) scavengers as $^1O_2$ and $^{\cdot-}O_2$.

An indication of the free radical formation was given by the capacity of **1** to induce dimerization of reduced glutathione (GSH) under the same conditions of irradiation, as detected by UV-Vis spectrophotometry (Figure 3) by titration with 5,5′dithiobis(2-nitrobenzoic acid) (DTNB) at 412 nm, and the presence of cysteine dimers as detected by mass spectroscopy.

It may be inferred that the phototoxicity mechanism for aceclofenac most probably involves reactions of a free radical intermediate and stable photoproducts with cellular components. Future studies will include development of devices to discriminate between effects due either to stable photoproducts or to short-lived intermediates as well as the use of cultures and co-cultures of different human cells mimicking the human skin.

We are currently investigating the toxic effects of the isolated photoproducts using *in vitro* as well as *in vivo* models. In the near future patients photosensitive to aceclofenac will be patch-tested with these photoproducts.

The observed photohemolysis induced by aceclofenac might reflect extensive photoperoxidation of the membrane lipids (Fig. 4). When phosphate buffer saline (PBS) solutions of linoleic acid were irradiated in the presence of **1** significant amounts of dienic hydroperoxides were formed, as evidenced spectrophotometrically by the appearance of a new UV-absorption band at 233 nm (Recknagel and Glenden 1984).

The chemiluminescence (CL) observed both in the processes induced by H_2O_2 as well as by ferrous ion in luminol was used to evaluate the generation of radical hydroxyl (OH) of **1**. In the former, a quantitative increase was observed when **1** was irradiated in the presence of nicotinamide adenine dinucleotide phosphate (NADPH). The CL activity generated by luminol reflects the release of reactive oxygen species (ROS) specially $^{\cdot}OH$ (Lundqvist and

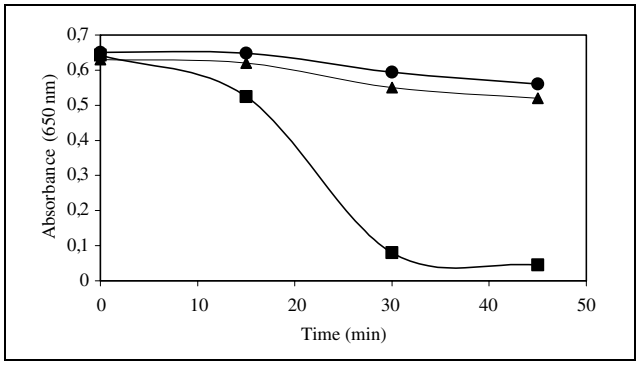


Fig. 2: Photohemolysis of RBC (3.3×10^6 cells/ml): ■ photosensitized with UV-B and aceclofenac (**1**); ▲ in the presence of radical scavengers [GSH] = 1.0×10^{-4} M and ● control in darkness. Each point represents the mean ± SEM (less than 5%) derived from four observations

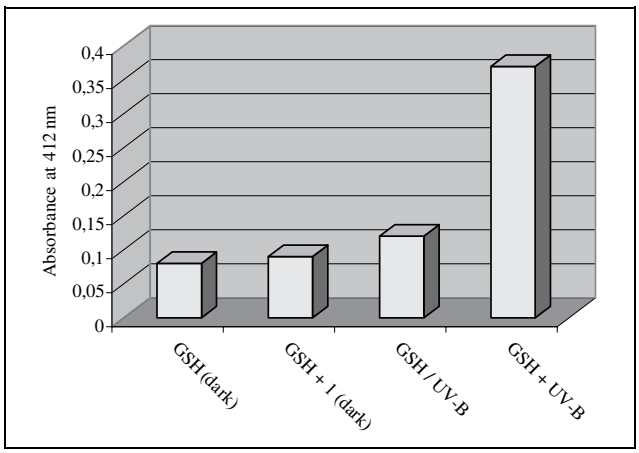


Fig. 3: Photoinduced dimerization of GSH (DTNB titration at 412 nm) in presence of aceclofenac (**1**)

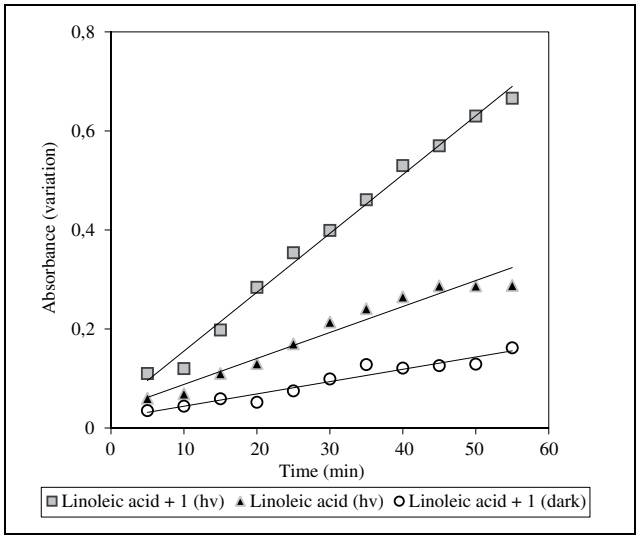


Fig. 4: Photoperoxidation of linoleic acid (10^{-3} M) sensitized by aceclofenac (**1**)

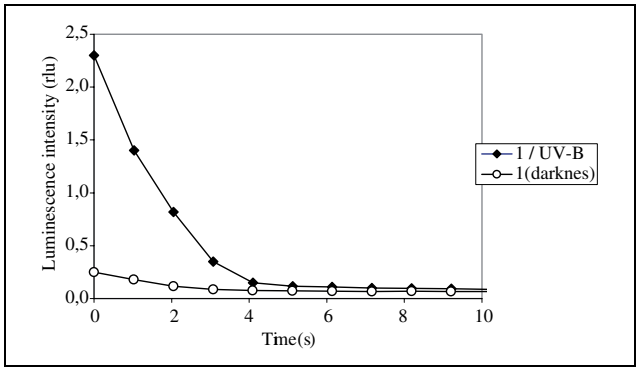


Fig. 5: Chemiluminescence generated by H_2O_2 and luminol after the irradiation of aceclofenac in the presence of NADPH. Data are the mean and SEM, ($n = 4$, $p < 0.05$ vs. control; analysis of variance)

Dahlgren 1996). Hydroxyl radical was also generated by the addition of a freshly prepared $FeSO_4$ solution to a mixture containing luminol as measured by chemiluminescence. These results are in agreement with previous observations with other drugs (Yildiz and Demiryürek 1998) where the addition of a ferrous ion salt to buffered solutions generates the hydroxyl radical-mediated oxidative reactions. The increase of the production of CL of aceclofenac is shown in Fig. 5.

No photoinduced binding was observed after irradiation of **1** in the presence of human serum albumin.

3. Discussion

Irradiation of a methanol solution of **1** produces three photoproducts, one major product, compound **2**, is generated by photodecarboxylation and the two minor products **3** and **4** by photooxidation of the amino group. **1** undergoes photodecarboxylation by homolytic rupture of the $COOH\text{-}CH_2O$ bond from its singlet state.

After establishing the photochemistry of **1** in solution, it appeared interesting to evaluate its *in vitro* phototoxicity and to extend this study to its photoproducts **2**, **3** and **4**. The photohemolysis of red blood cells was chosen as a suitable test for this purpose. Our results showed that only **1** was able to photosensitize the lysis of red bood cells. The addition of GSH as a radical scavenger resulted in an enhanced lifetime of erythrocytes, which is in favor of photohemolysis occurring via a radical-chain mechanism. The photohemolysis assay, as well as an *in vitro* phototoxicity test, has evidenced the involvement of radical-mediated cellular membrane damage in the skin photosensitization by **1**. Lipid photoperoxidation certainly correlates with the damage produced in cell membranes and thus with the observed photohemolysis. It was also possible to prove the generation of hydroxyl radicals during the irradiation of the **1** by means of chemiluminescence methods. The results reported in the present paper may be very useful from the medical standpoint. The methods used here may not only help to elucidate the photobiological action of **1** but also that of other photoreactive pharmaceutical drugs. That these compounds are not bound to HSA could be of physiological significance as this protein could be the initial target of these drugs in the organism. This result can be incongruous with the reported cases of photoallergy.

4. Experimental

4.1. Chemicals

Aceclofenac (**1**) was extracted from Airtal® (Leti laboratory) by means of a Soxhlet extractor with ethanol and recrystalized from the same solvent. The purity was 99.2% as determined by ^{1}H NMR-spectroscopy and UV-Vis spectrometry. 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB), reduced glutathione (GSH), sodium azide (NaN_3), lysis buffer (NH_4Cl, $KHCO_3$, EDTA), histidine, nicotinamide adenine dinucleotide (NADH) and cysteine were commercially obtained from Aldrich (Steinheim, Germany), while superoxide dismutase (SOD) was purchased from Sigma (St. Louis, MO, USA). Human serum albumin (HSA) was purchased from Sigma. All analytical or HPLC grade solvents were obtained from Merck (Darmstadt, Germany).

4.2. Photolysis

Photolysis of **1** was carried out in methanol solution (1.50 mmol in 50 ml) at 20 °C during 6 h in a Rayonet photochemical chamber reactor (model RPR-100, Southern New England Ultraviolet Company-USA) equipped with 16 phosphorus lamps with an emission maximum in UV-A between 320–400 nm and UV-B 290–320 nm (23 mW/cm^2 of irradiance as measured with a UVX Digital Radiometer, Melles Griot, USA). The distance between the light sources to the test aliquots was 10 cm. The temperatures detected in the cuvette during a standard 1 h irradiation were no higher than 28 °C. In the determination of quantum yields the photolysis was limited to less than 10% to minimize light absorption and reaction of photoproducts. The photon flux incident on 3 ml of solution in quartz cuvettes of 1 cm optical path was measured by means of a ferric oxalate actinometer and was of the order of 10^{15}–10^{16} quanta s^{-1} (Vargas 2002).

Either oxygen or argon was bubbled through the reaction mixture throughout the whole irradiation process. The photodegradation reaction was followed by means a Perkin Elmer 559 UV-visible spectrophotometer, a Milton-Roy Spectronic 3000 array instrument (Milton Roy Company-USA), ^{1}H NMR as well as by TLC and HPLC (Water Delta Prep 4000 equipped with a 3.9 × 300 mm, 10 µm Bondapak C18 column using a CH_2Cl_2/MeOH binary solvent system) (El Kousy 1999). After the irradiation was stopped the solvent was evaporated under reduced pressure (14 Torr) and the residue was purified by chromatography on a silica gel (230 mesh) column. The elution was carried out by means of solvent mixtures (dichloromethane/methanol) (3 : 1 vol/vol). The structures of the isolated products were elucidated by ^{1}H NMR and ^{13}C NMR (Bruker Aspect 3000, 300 and 100 MHZ respectively), IR (Nicolet DX V 5.07) and mass spectra (Varian Saturn 2000) in connection with a Varian chromatograph equipped with a 30-m capillary (CP-Sil, 8CB-MS). Elemental analyses were performed by the "Laboratorio Nacional de Análisis Químico" – IVIC – Centro de Química, Caracas, Venezuela in a Fisions Instrument EA-1108. The results were in an acceptable range.

Photoproduct **2** (yield: 88%): IR (KBr): 3050, 2960, 1770, 1735, 1580, 1490, 1454, 1383, 1230, 1205, 1183, 1090, 810, 750, 735 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 8.74$ (m, 2 H, aromatic-H), 8.03 (s, 1H, N–H), 7.49 (m, 1 H, aromatic-H), 7.19 (m, 1 H, aromatic-H), 6.93 (m, 1 H, aromatic-H), 6.85 (m, 1 H, aromatic-H), 6.83 (m, 1 H, aromatic-H), 3.94 (s, 2 H, C–CH_2–COO–), 3.67 (s, 3 H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 178.00$ (s, C=O), 142.60 (s, aromatic-C), 141.20 (s, aromatic-C), 129.70 (d, aromatics-2 CH), 128.80 (d, aromatic-CH), 127.89 (d, aromatic-CH), 127.30 (s, aromatics-2 C–Cl), 124.20 (d, aromatic-CH), 118.00 (d, aromatic-CH), 116.10 (s, aromatic-C), 108.79 (d, aromatic-CH), 51.70 (q, CH_3), 36.50 (t, CH_2). MS: m/z (%) = 313 (7), 311 (56), 309 (100), 276 (25), 274 (82), 242 (44), 214 (55), 178 (20).

$C_{15}H_{13}Cl_2NO_2$

Photoproduct **3** (yield: 7%): IR (KBr): 3450, 3055, 3000, 2700, 2500, 1745, 1617, 1530, 1495, 1454, 1090, 930, 810, 743, 732 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): $\delta = 8.72$ (m, 2 H, aromatic-H), 8.48 (br.s, 2 H, −COOH and N−OH), 7.70 (m, 1 H, aromatic-H), 7.23 (m, 1 H, aromatic-H), 6.99 (m, 1 H, aromatic-H), 6.92 (m, 1 H, aromatic-H), 6.89 (m, 1 H, aromatic-H), 4.13 (s, 2 H, $-CH_2-COOH$), 3.77 (s, 2 H, CH_2-CO). ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 170.50$ (s, C=O), 168.10 (s, C=O), 144.80 (s, aromatic-C−N), 138.90 (s, aromatic-C−N), 132.30 (s, aromatic-2 C−Cl), 128.40 (d, aromatics-2 CH), 127.50 (d, aromatic-2 CH), 127.47 (d, aromatic-CH), 122 (s, aromatic-C), 118.10 (d, aromatic-CH), 110.40 (d, aromatic-CH), 69.40 (t, CH_2), 36.00 (t, CH_2). MS: m/z (%) = 373 (13), 371 (68), 369 (100), 355 (12), 279 (22), 215 (47), 214 (90), 213 (38), 150 (12), 78 (10), 51 (10). 42 (4).
$C_{16}H_{13}Cl_2NO_5$
Photoproduct **4** (yield: 5%): IR (KBr): 3500, 3050, 3000, 2690, 2500, 1740, 1094, 1430, 930, 810, 752, 733 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): $\delta = 9.50$ (br.s, 2 H, −COOH and N−OH), 8.72 (m, 2 H, aromatic-H), 7.90 (m, 1 H, aromatic-H), 7.40 (m, 1 H, aromatic-H), 7.10 (m, 1 H, aromatic-H), 6.95 (m, 1 H, aromatic-H), 6.90 (m, 1 H, aromatic-H), 3.40 (s, 2 H, $-CH_2-COO$). ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 175.00$ (s, C=O), 145.00 (s, aromatic-C−N), 137.20 (s, aromatic-C−N), 133.00 (s, aromatic-2 C−Cl), 127.52 (d, aromatics-2 CH), 128.40 (d, aromatic-CH), 127.50 (d, aromatic-2 CH), 126.20 (d, aromatic-CH), 123.50 (s, aromatic-C), 117.00 (d, aromatic-CH), 109.00 (d, aromatic-CH), 35.00 (t, CH_2). MS: m/z (%) = 315 (8), 313 (59), 311 (100), 278 (40), 277 (35), 276 (80), 241 (5), 213 (56), 177 (24), 150 (15), 78 (4), 64 (8).
$C_{14}H_{11}Cl_2NO_3$

4.3. Photoinduced hemolysis of RBC by aceclofenac

A red blood cells (RBC) suspension from three different samples of freshly obtained human erythrocytes was prepared by washing them four times with tenfold volume of a phosphate-buffered saline solution (PBS) pH 7.4 (0.01 M phosphate buffer and 0.135 M NaCl), centrifuging the cells each time at $2500 \times g$ for 15 min and carefully removing the supernatant. For the photohemolysis experiments RBC were diluted in PBS containing the compounds **1**, **2** or **3** so that the resultant suspension had an optical density (OD) of 0.4–0.8 at 650 nm. An OD value of 0.5 corresponded to 3.3×10^6 cells ml^{-1}. The photon flux incident on the cuvettes (measured as before) was 2×10^{16} photon s^{-1}. Hence samples received, on the average 12.9 J cm^{-2} in one hour.
The hemolysis rate was determined by measuring the decreasing optical density (OD) at 650 nm, since the OD is proportional to the number of intact RBC (Valenzeno 1985). Compound **1** and the isolated photoproducts **2** and **3** were added to the RBC solutions and irradiated at concentrations of 20–80 μg ml^{-1} under aerobic (oxygen) as well as under anaerobic (argon) conditions in a Rayonet photochemical reactor equipped with 16 phosphor lamps with an emission maximum in UV-A and separately in UV-B for periods ranging between 10–100 min. The photohemolysis experiments were carried out also in the presence of 1.0×10^{-5} M of SOD and NaN_3 as superoxide and singlet oxygen quenchers, and 1.0×10^{-4} M GSH as radical scavengers. The hemolysis rate was determined by measuring the decreasing OD at 650 nm, since the optical density is proportional to the number of intact RBC. Control experiments performed in the dark did not show OD changes. All the data are the average (mean arithmetic) of the values obtained repeating the experiments three times.

4.4. Photosensitized oxidation of glutathione and cysteine by aceclofenac

To determine the photoinduced oxidation of glutathione in the presence of aceclofenac, it was irradiated in presence of compound **1**. Detection of glutathione depletion was carried out with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) and performed by means of similar assays as those described in Beutler's text book (Beutler 1984). However, the red cells used as a blank were hemolyzed with a "lysis buffer" (NH_4Cl, $KHCO_3$, EDTA, pH 7.5). The same experiment was used to determine the photoinduced oxidation of cysteine in the presence of aceclofenac. Similar control experiments were carried out without irradiation of the samples.
In a separate experiment, in order to detect the probable formation of a radical intermediate, aceclofenac (**1**) (0.5 mmol in 50 ml H_2O) was irradiated under the conditions described above, in the presence of equimolar quantities of reduced glutathione (GSH) or cysteine as radical scavengers.

4.5. Photosensitized peroxidation of linoleic acid

Linoleic acid 10^{-3} M in PBS phosphate buffered saline solution PBS (10 mM KH_2PO_4 and 150 mM NaCl) in water, pH 7.2, with Tween 20 (0.1%), was irradiated in the presence of compound **1** and in a pre-irradiated solution of **1** (10^{-5} M). The formation of dienic hydroperoxides was monitored by UV-spectrophotometry, through the appearance and progressive increase of a new band at $\lambda = 233$ nm (Recknagel and Glenden 1984).

4.6. Chemiluminescence experiments

Chemiluminescence (CL) was generated in cell-free systems; H_2O_2-induced CL (as a blank): H_2O_2 (3.5 mM) was added to phosphate buffered saline solution (PBS, 10 mM KH_2PO_4 and 150 mM NaCl, pH 7.2) and luminol (250 μM, prepared daily in 2 M NaOH and diluted with PBS). The aceclofenac-induced CL at different concentrations was dispense after irradiation with 2 phosphorus lamps with a emission maximum in UV-B 290–320 nm in presence of NADH. The generated CL at 37 °C was measured continuously for 10 min in a Luminoskan Ascent luminometer (ThermoLabsystems, Finland) in a 96-well Thermo Labsystems Microtiter plate. (Lundqvist 1996; Vargas 2003; Yildiz 1998).

4.7. Titration of the aceclofenac solutions with HSA

Titration of the aceclofenac solutions (1.0×10^{-4} M) with HSA was performed by addition of appropriate aliquots of an aqueous-buffered PBS (10 mM KH_2PO_4 and 150 mM NaCl) HSA stock solution at 1.0 mM (pH 7.4) concentration directly to the absorbance or fluorescence cell so that the final protein concentration was in the range from 0 to 5.0×10^{-4} M. The solutions were allowed to incubate in the dark for 20 min. Then, samples were irradiated in 1-cm^2 Suprasil quartz cells under the above mentioned conditions for various time periods. Control included drug protein mixtures kept in the dark and HSA solutions irradiated for the same periods of time. The drug was separated from the protein using a Sephadex G-25 column equilibrated with PBS. The photobinding was monitored by fluorescence spectroscopy (Moreno 1999).

4.8. Statistical treatment of results

At least three independent experiments were performed except where indicated otherwise. The results are expressed as a mean ± S.E.M. derived from 3–4 observations. The level of significance accepted was $p \leq 0.05$.

Acknowledgements: This research was supported by a grant from "Consejo Nacional de Investigaciones Científicas y Tecnológicas" CONICIT-Venezuela (S1-2502 and S1-960011724), Laboratorio Nacional de Análisis Químico, proyecto N° Lab. 1998003690 and Fundación Polar.

References

Beutler E (1984) Red Cell Metabolism. A manual of biochemical methods, Grune & Stratton eds. New York, 3rd ed.
Bort R, Mace K, Boobis A, Gomez-Lechon MJ, Pfeifer A, Castell JV (1999) Hepatic metabolism of diclofenac: role of human CYP in the minor oxidative pathways. Biochem Pharmacol 58: 787–796.
Bort R, Ponsoda X, Jover R, Gomez-Lechon J, Caltell JV (1999) Diclofenac toxicity to hepatocytes: a role for drug metabolism in cell toxicity. J Pharmacol Exp Therap 288: 65–72.
Brogden RN, Wiseman LR (1996) Aceclofenac. A review of its pharmacodynamic properties and therapeutic potential in the treatment of rheumatic disorders and in pain management. Drugs 52: 113–124.
Costanzo LL, De Guidi G, Condorelli G, Cambria A, Fama M (1989) Molecular mechanism of drug photosensitization-II. Photohemolysis sensitized by ketoprofen. Photochem Photobiol 50: 359–365.
Costanzo LL, De Guidi G, Condorelli G, Cambria A, Fama M (1989) Molecular mechanism of naproxen photosensitization in red blood cells. J Photochem Photobiol B Biol 3: 233–235.
El Kousy NM (1999) Spectrophotometric and spectrofluorimetric determination of etodolac and aceclofenac. J Pharm Biomed Anal 20: 185–194.
Goday Bujan JJ, Garcia Alvarez-Eire GM, Martinez W, del Pozo J, Fonseca E (2001) Photoallergic contact dermatitis from aceclofenac. Contact Dermatitis 45: 170.
Gollnick K, Griesbeck A (1983) [4 + 2]-Cycloaddition of singlet oxygen to 2,5-dimethylfuran: isolation and reactions of the monomeric and dimeric endoperoxides. Angew Chem Int Ed Engl 22: 726–728.
Grau M, Guasch J, Montero JL, Felipe A, Carrasco E, Julia S (1991) Pharmacology of the potent new non-steroidal anti-inflammatory agent aceclofenac. Arzneim-Forsch 41 : 1265–1267.
Legrand E (2004) Aceclofenac in the management of inflammatory pain. Expert Opin Pharmacother 5: 1347–1357.
Ludwig C, Brinkmeier T, Frosch PJ (2003) Exudative erythema multiforme with transition to a toxic epidermal necrolysis after taking aceclofenac (Beofenac). Dtsch Med Wochenschr 128: 487–490.
Lundqvist H, Dahlgren C (1996) Isoluminol-enhanced chemiluminescence: a sensitive method to study the release of superoxide anion from human neutrophils. Free Rad Biol Med 20: 785–792.
Moreno F, Cortijo M, González-Jiménez J (1999) Interaction of acrylodan with human serum albumin. A fluorescence spectroscopic study. Photochem Photobiol 70: 695–700.
Mouithys-Mickalad AM, Zheng SX, Deby-Dupont GP, Deby CM, Lamy MM, Reginster JY, Henrotin YE (2000) In vitro study of the antioxidant properties of non steroidal anti-inflammatory drugs by chemiluminescence and electron spin resonance (ESR). Free Radic Res 33: 607–621.

Poiger T, Buser HR, Müller M (2001) Photodegradation of the pharmaceutical drug diclofenac in a lake: pathway, field measurements, and mathematical modeling. Environ Toxicol Chem 20: 256–263.

Recknagel RO, Glenden EA (1984) In Oxygen radicals in biological systems, in: Packer L (ed) Methods in Enzymology; V. 105, Academic Press: New York, p. 331.

Sastry CH, Rao, AR, Gopala Krishna CV, Murthy AG (1988) Gas liquid chromatographic determination of diclofenac sodium in tablets. Ind J Pharmac Sci 50: 175–178.

Sharma V, Khan MS (2003) Prodrugs and mutual prodrugs: synthesis of some new pyrazolone and oxadiazole analogues of a few non-steroidal anti-inflammatory drugs. Pharmazie 58: 99–103.

Valenzeno DP, Trank JW (1985) Measurement of cell lysis by light scattering. Photochem Photobiol 42: 335–339.

Vargas F, Fraile G, Velásquez M, Correia H, Fonseca G, Marín M, Marcano E, Sánchez Y (2002) Studies on the photostability and phototoxicity of Aloe-emodin, emodin and rhein. Pharmazie 57: 399–404.

Vargas F, Rivas C, Díaz Y, Contreras N, Silva A, Ojeda L, Velásquez M, Fraile G (2003) Antioxidant properties of dipyridamole as assessed by chemiluminescence. Pharmazie 58: 817–823.

Yildiz G, Demiyürek AT, Sahin-Erdemli I, Kanzik I (1998) Comparison of antioxidant activities of aminoguanidine, methylguanidine and guanidine by luminol-enhanced chemiluminescence. Br J Pharmacol 124: 905–910.