Biochemical Pharmacology, Vol. 39, No. 3, pp. 407–413, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

DNA STRAND BREAKS PHOTOSENSITIZED BY BENOXAPROFEN AND OTHER NON STEROIDAL ANTIINFLAMMATORY AGENTS

THIERRY ARTUSO,* JEAN BERNADOU,† BERNARD MEUNIER† and NICOLE PAILLOUS*‡

† Laboratoire de Chimie de Coordination, CNRS, 205 route de Narbonne, 31077 Toulouse Cedex, France, and *Laboratoire des IMRCP, UA 470 du CNRS, Université Paul Sabatier, 118 route de Narbonne, 31062 Toulouse Cedex, France

(*Received* 6 *February* 1989; *accepted* 28 *June* 1989)

Abstract—Benoxaprofen, a non steroidal antiinflammatory drug is known to be highly phototoxic. Upon irradiation at 300 nm, benoxaprofen is shown to enhance the cleavage of ΦX 174 DNA in buffered aqueous solution (pH 7.4). A linear relationship between the number of single strand breaks and the irradiation time is found. In deaerated solutions, these breaks are three times greater in the presence than in the absence of benoxaprofen. In both cases the rate of cleavage decreases in the presence of air. The rate of DNA damage increases with the drug per base pair ratio up to approximatively 0.2 and then decreases at higher ratios. Other NSAIDs, naproxen, ketoprofen, diflunisal, sulindac and indomethacin have been tested as photocleavers of DNA by using the same experimental conditions. A comparison of the efficiency of cleavage of all these drugs (including BNP) was obtained at drug concentrations such that the light absorbance was the same. Benoxaprofen, naproxen, ketoprofen and diflunisal induce single strand breaks. Sulindac and indomethacin do not cause breaks, and they can in some conditions even act as screening agents. The most efficient of the series are naproxen and ketoprofen. In the presence of oxygen, at the same concentrations as above, the efficiency of benoxaprofen, ketoprofen and diflunisal is decreased while that of naproxen is increased. This suggests that all these compounds do not interact with DNA by the same mechanism. In the case of BNP, the mechanism of photoinduced DNA cleavage is discussed in detail. It is shown that the photoactive agent is the decarboxylated derivative of benoxaprofen, as the photodecarboxylation of benoxaprofen is much faster than the photocleavage of DNA.

The use of non steroidal antiinflammatory drugs (NSAID)§ in the treatment of rheumatism and osteoarthritis has been largely developed in the last decade. Several of these agents exhibit undesirable side-effects by increasing sensitivity of the skin to sunlight [1–3]. Among these compounds, benoxaprofen, 2-(4-chlorophenyl)-α-methyl-5-benzoxazole acetic acid, has been one of the most studied. After exposure of patients to sunlight, this drug was found to induce erythemea, weal and flare reactions, and itching, stinging and burning sensations in the skin; some patients even developed onycholysis [4–15]. These adverse effects have been attributed to phototoxicity rather than photoallergy [16, 17].

Simultaneously with erythemeas apparition, a mast cell degranulation [18, 19] and an increase of the histamine level were observed. Since mast cell degranulation is known to be modulated by phospholipids alteration, many investigations on BNP photosensitized cell-membrane damage *in vitro* have been made [12, 19–21]. *In vitro* photohemolysis experiments using various methods have shown that the phototoxic activity of BNP may be directed mainly against cellular membranes *via* radical and singlet oxygen formation. However, whether singlet oxygen is formed has been a point of controversy [20, 21]. The very reactive species so generated may also damage other biological targets, such as nucleic acids. As recently reviewed, DNA alterations mediated by phototoxic drugs have been investigated for several drugs belonging mainly to phenothiazines and tetracyclines series [22]. Here, we report on the photosensitized cleavage of bacteriophage ΦX 174 DNA by BNP. The influence of various parameters (irradiation time, presence of oxygen, substrate concentration) on the cleavage efficiency has been determined.

This study was extended to the other non-steroidal antiinflammatory drugs, such as naproxen (NP), ketoprofen (KP), diflunisal (DF), sulindac (SL), and indomethacin (IND), whose *in vivo* and *in vitro* phototoxic or non-phototoxic properties were already known [3, 23–25] (Table 1).

MATERIALS AND METHODS

Benoxaprofen was a gift of Eli Lilly (France) and

‡ Author to whom correspondence should be addressed.

§ Abbreviations used: NSAID, non steroidal antiinflammatory drugs; BNP, benoxaprofen, 2-(4-chlorophenyl)-α-methyl-5-benzoxazole acetic acid; NP, naproxen, *d*-2-(6-methoxy-2-naphtyl) propionic acid; KP, ketoprofen, 2-(3-benzoylphenyl) propionic acid; DF, diflunisal, 2-hydroxy-5-(2,4 difluorophenyl) benzoic acid; SL, sulindac,5-fluoro-2-methyl-1-[[4-(methyl sulfinyl)phenyl]methylene]-1-*H*-indene-3-acetic acid; IND, indomethacin, 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indole-3-acetic acid; EtBNP, 2-(4-chlorophenyl)-5-ethyl benzoxazole; SSB, single strand breaks; *S*, mean number of SSB per DNA molecule; bp, base pair; UV, ultraviolet; HPLC, high performance liquid chromatography; O.D., optical density.

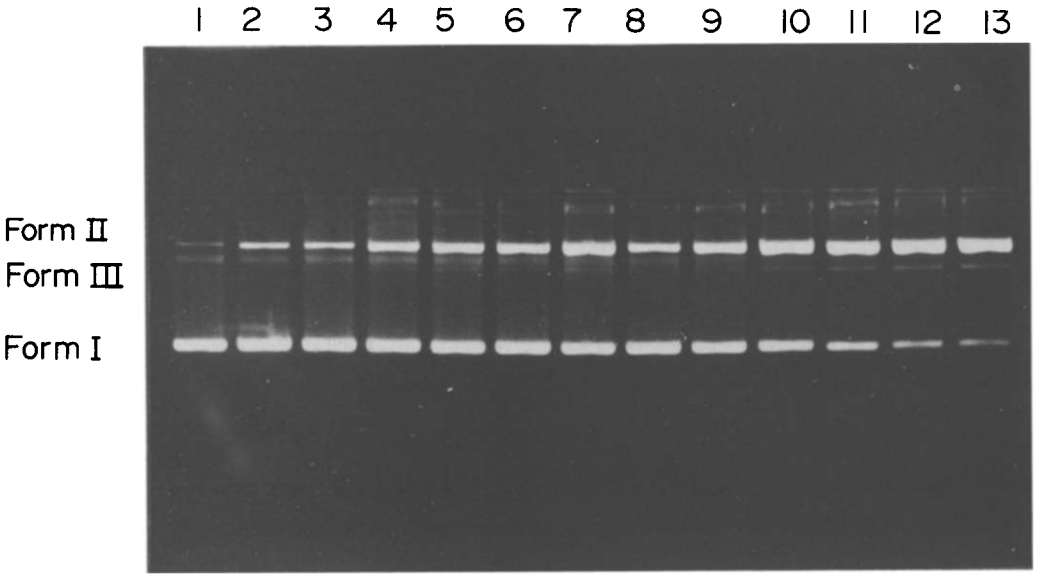


Fig. 1. Electrophoresis gel pattern showing the influence of irradiation time on the photocleavage of ϕX 174 DNA mediated by BNP in deaerated phosphate buffered solutions at 300 nm. BNP (45 μM final concentration, O.D. = 0.6) to DNA base pair ratio was 2.4. Lanes 1 to 7, control samples, DNA irradiated alone for 0 min (1), 5 min (2), 10 min (3), 15 min (4), 20 min (5), 25 min (6), 30 min (7). Lanes 8 to 13, treated samples, DNA irradiated in the presence of BNP for 5 min (8), 10 min (9), 15 min (10), 20 min (11), 25 min (12), 30 min (13). DNA migration is from top to bottom.

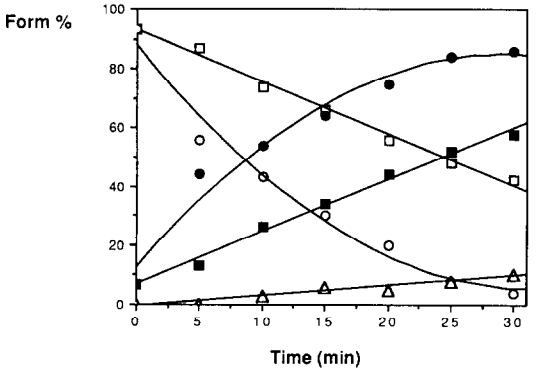


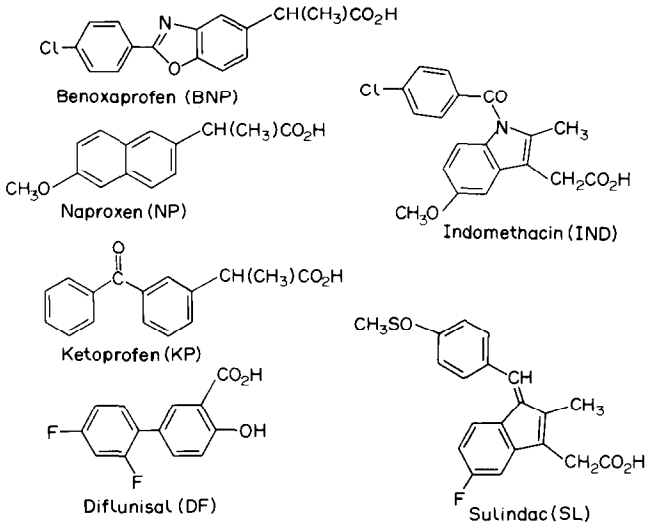
Fig. 2. Changes in ϕX 174 DNA irradiated in the presence of BNP (45 μM) in deaerated solution in function of the irradiation time. Disappearance of form I (○), appearance of form II (●) and III (△). Controls: DNA irradiated alone, form I (□) and form II (■).

was received as pills. The pills were extracted with chloroform and the product was recrystallized twice from methanol and then sublimated. Its purity was checked by chromatographic and spectrometric methods. For ketoprofen (Specia, Paris, France), naproxen (Syntex, Puteaux, France), diflunisal, sulindac and indomethacin (Merck, Clermont, Ferrand, France), pure samples were kindly provided by the manufacturers and were used without further purification.

Supercoiled ϕX 174 DNA (MW = 3.6×10^6 D, 5386 base pairs), form I, purchased from Pharmacia (Guyancourt, France), was used after dilution in 5 mM phosphate buffer (pH 7.4) containing 10 mM NaCl (final concentration: 14 nM in DNA molecule or 75.4 μM in bp). The amount of contaminating form II DNA was less than 6%, and no linear form III DNA was detected in our preparations, as checked by agarose gel electrophoresis and microdensitometry. All preparations were made using bidistilled water.

Irradiation conditions. Samples were prepared from a mixture of 10 μl of buffered aqueous solutions of DNA, 10 μl of BNP at a fixed concentration in phosphate buffered solutions, and 20 μl of phosphate buffer (0.2 M, pH 7.4). The mixture was placed in 5 mm i.d. glass tubes. For experiments in deaerated conditions, the buffered solutions with or without BNP were deaerated by bubbling with argon and then the tubes containing the mixtures were flushed with argon and capped. All the concentrations indicated in the text are final concentrations unless otherwise stated, and reported optical densities (O.D.) were obtained in a cuvette with the same pathlength as the irradiation tubes. Irradiations were carried out in a Rayonet RPR 100 reactor equipped with 16 UV mercury lamps with an output at 300 ± 15 nm. The lower wavelengths are cut out by a pyrex filter which absorbs 95% of the light intensity at 280 nm. Samples were placed in a merry-go-round apparatus and illuminated at an average temperature of 35–40°. Light energy (2.4×10^{19} quanta/l/sec) was monitored by actinometry. Irradiation times were 30 min unless otherwise specified. Following irradiation,

Table 1. Structure of NSAIDs compounds tested as DNA breakers under irradiation



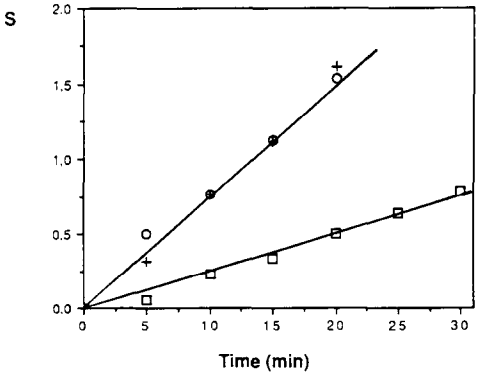


Fig. 3. Influence of the irradiation time on the mean number of SSBs per DNA molecule (S), photosensitized by BNP (○) or EtBNP (+) or without photosensitizer (□) in deaerated solutions.

10 μl of a mixture composed of 250 mM Tris buffer (pH 7.2), 75% glycerol, and 0.05% bromphenol blue was added to each sample.

Electrophoresis. After irradiation samples (20 μl) were analysed by electrophoresis on a 0.8% agarose gel in Tris-borate buffer (89 mM Tris, 89 mM boric acid, 25 mM EDTA, pH 7.4) incubated in a solution of ethidium bromide (2 mg/ml, 100 μl for 200 ml of the previous solution). Electrophoresis was performed with a Pharmacia apparatus (GNA-200) with a power supply at 25 mA for 15 hr (gel size = 20 × 20 cm).

After migration, DNA forms were detected by fluorescence using a 254 nm UV lamp. Photographies were taken with an apparatus equipped with a red filter. The negative films were scanned with a Hoefer Scientific densitometer GS300. The coefficient 1.47 was used for decreased stainability of form I DNA vs form II and III [26].

RESULTS

Strand breaks photoinduced by benoxaprofen

Strand scissions on double stranded DNA are readily quantified by following the conversion of supercoiled ΦX 174 DNA (form I) to its circular relaxed (form II) and linear (form III) forms by neutral agarose gel electrophoresis. Data shown on Figs 1 and 2 indicate that the DNA cleavage observed after irradiation at 300 nm in the absence of oxygen at pH 7.4 is greater in the presence of BNP (45 μM, 2.4 BNP molecules/base pair) than in the absence of BNP. Upon increasing the irradiation time, the percentage of form I decreases while the percentage of form II increases. In experiments performed with BNP, a small amount of the linear DNA form, form III, can be detected after 5 min of irradiation. In some experiments, small amounts of slowly migrating material is detected and we attribute this to a dimer form of DNA similar to that proposed by Decuyper *et al.* [27], in the case of DNA breakage induced by photoexcited promazine derivatives. Since the major product of photodegradation of the supercoiled form of ΦX 174 DNA is the relaxed circular form II, the photoactivation process goes mainly through single strand breaks. In these conditions and when the percentage of the detected form III is below 10%, it is possible to quantify S, the mean number of breaks per DNA molecule, by using the following equation [28]:

$$S = \ln I_0/I$$

where I_0 is the initial concentration of DNA form I and I the form I concentration after irradiation. As shown in Fig. 3, it is clear that the number of single strand breaks increases linearly as a function of time between 0 and 20 min. S is three times greater in the presence than in the absence of BNP in anaerobic conditions.

In contrast, aerobic conditions decrease the DNA cleavage for irradiations performed with or without

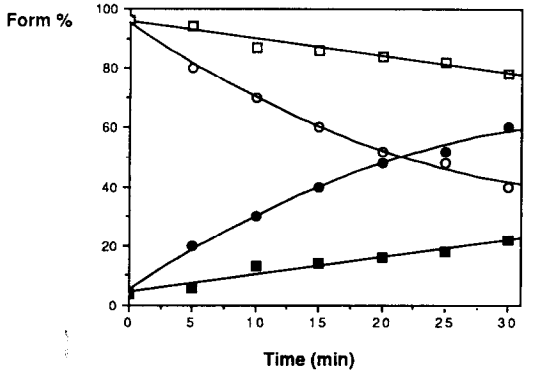


Fig. 4. Changes in ϕX 174 DNA irradiated in the presence of BNP (45 μM) in the presence of oxygen in function of the irradiation time. Disappearance of form I (○), appearance of form II (●). Controls: DNA irradiated alone, form I (□) and form II (■).

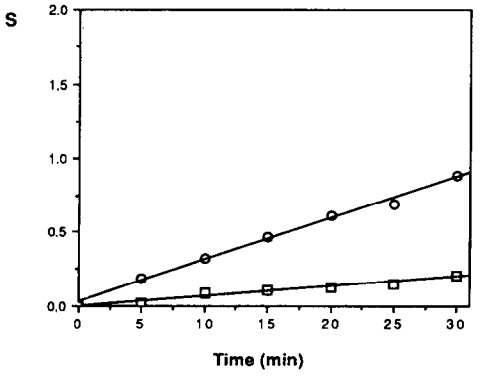


Fig. 5. Influence of the irradiation time on the mean number of SSBs per DNA molecule (*S*) photosensitized by BNP (○) or without photosensitizer (□) in aerated solutions.

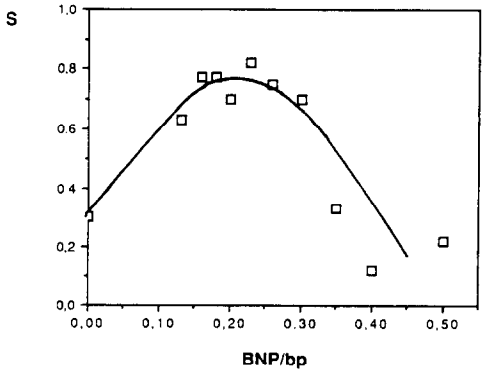


Fig. 6. Influence of the drug by base pair ratio on the mean number of SSBs per DNA molecule (*S*), photosensitized by BNP in deaerated solutions.

BNP (Fig. 4). The number of SSBs is decreased by a factor of 3 or 4 respectively (Fig. 5). It thus appears that BNP is a more efficient DNA photosensitizer in the absence than in the presence of oxygen.

It should be noted that while DNA has a very weak absorption at 300 nm, some SSBs are observed in the absence of drug, both in aerobic and anaerobic conditions. These cleavages may be attributed both at the high sensitivity of the technique used to study the DNA cleavage and to the high dose of irradiating light delivered in our sample (~36 kJ/m^2 in 30 min). Recently, Boullard *et al.* have shown by the same electrophoretic technique that similar high doses of UV light at 290 nm and 313 nm may promote SSBs in DNA [29].

The influence of drug per base pair ratio on the number of SSBs was investigated in deaerated solutions by varying the DNA concentrations (from 3.5 to 0.74 nM). The BNP concentration was unchanged in order to assure the same absorbance in all samples. As shown in Fig. 6 the DNA damages increase by changing the BNP/bp ratio up to approximatively 0.2 and decrease beyond that range. A similar effect has also been described for photochemical DNA damages induced by other compounds such as daunomycin [30] and flavines [31].

Comparative studies on DNA cleavage efficiency of various non-steroidal antiinflammatory drugs NSAIDs

By using the same experimental approach as that described above for benoxaprofen, other NSAIDs known to be phototoxic *in vitro* like naproxen (NP), ketoprofen (KP) or NSAIDs known to have little or no phototoxic effects like diflunisal (DF), sulindac (SL) and indomethacin (IND) [23], have been tested for their potential ability as photoactive DNA cleavers.

We found that in the absence of irradiation none of these antiinflammatory drugs induce DNA damage. These various drugs have very different UV spectra (Table 2). So to compare the photoactivity of these NSAIDs, DNA cleavage experiments were performed by irradiating DNA drug solutions of the same optical density at 300 nm (O.D. = 2, pathlength 5 mm). As shown in Table 3, propionic acid derivatives, namely BNP (0.148 mM), NP (5.4 mM), and KP (1.6 mM) are photoactive as seen by breaks on supercoiled ϕX 174 DNA. The most efficient are NP and KP. DF (1.5 mM) is also photoactive while SL (3.3 mM) and IND (0.77 mM) do not induce photocleavage and can even (in some conditions) promote photoprotection. In the presence of oxygen, the efficiency of BNP, KP and DF decreases while that of NP increases significantly (Fig. 7). Taken together, these results suggest that these compounds do not interact with DNA *via* the same mechanism.

DISCUSSION

BNP, a phototoxic antiinflammatory drug, can promote DNA strand breaks in conjunction with light. Several mechanisms might be involved in the cleavage processes. Oxidation reactions via a radical or a singlet oxygen pathway are often proposed.

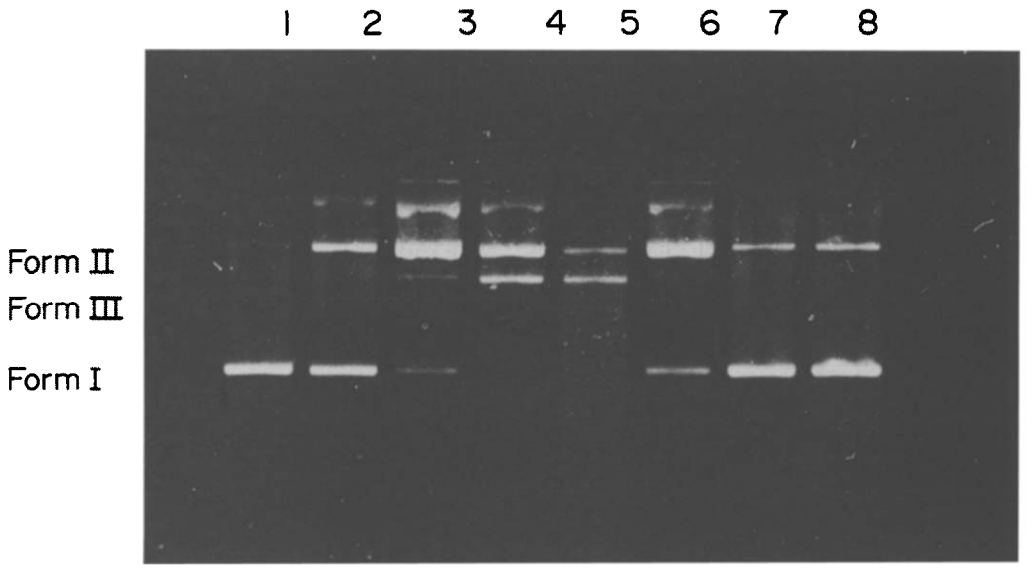


Fig. 7. Electrophoresis gel pattern showing the influence of six antiinflammatory drugs (O.D. = 2) on the photocleavage of ΦX 174 DNA in aerated solutions. Lanes 1 and 2 control samples: DNA alone (1) and DNA irradiated alone (2). Lanes 3 to 8, DNA irradiated in the presence of drug: BNP, 0.148 mM (3); NP, 5.4 mM (4); KP, 1.6 mM (5); DF, 1.5 mM (6); SL, 3.3 mM (7); IND, 0.77 mM (8). All solutions are irradiated for 30 min.

Table 2. Absorption characteristics of NSAIDs compounds in phosphate buffer (pH 7.4)

Drug	BNP	NP	KP	DF	SL	IND
λ_{max} (nm)	305	317, 330	260	306	286, 328	320
$\varepsilon_{max} \times 10^{-3}$ (/cm/mol dm^3)	29	1.52, 1.76	16	2.9	16.2, 13.5	7.1

Table 3. Relative percentage of forms I, II and III after irradiation of ΦX 174 DNA in the presence of six antiinflammatory drugs in deareated or non aerated solutions for 30 min

Lane		1	2	3	4	5	6	7	8
Drug				BNP	NP	KP	DF	SL	IND
Form I	Aerated	98	78	35	0	0	46	90	77
	Deareated	98	64	7	0	0	35	90	79
Form II	Aerated	2	22	60	44	40	54	10	23
	Deareated	2	36	82	80	20	65	10	21
Form III	Aerated	0	0	5	22	27	0	0	0
	Deareated	0	0	11	20	26	0	0	0

The concentrations of the drugs have been chosen in such a manner that all drugs have the same absorbance. Lanes 1 and 2, control samples: DNA alone before and after irradiation. Lanes 3 to 8, DNA irradiated in the presence of drug: BNP (3), NP (4), KP (5), DF (6), SL (7), IND (8). Values given in this table are calculated from three independent experiments. Data in aerated conditions correspond to densitometric analysis of electrophoresis shown in Fig. 7.

It is already known that in aqueous solutions, in the absence of DNA, BNP undergoes a photochemical decarboxylation via a radical mechanism [32]:

$$R{-}\underset{\displaystyle CH_3}{\overset{\displaystyle |}{C}}HCOOH \xrightarrow{h\nu} RCH_2CH_3 + CO_2 \cdot$$

(EtBNP)

In the presence of oxygen and light, singlet oxygen and superoxide anion have been detected [33]. The HPLC monitoring of BNP decarboxylation performed in the same conditions as those used for the anaerobic DNA cleavage studies, but in the absence of DNA indicates that all BNP was photodecarboxylated within one minute. The rate of this reaction was seen to be enhanced by the addition of DNA. It is thus clear that the photoactive species in the currently studied BNP-mediated photocleavage

of ΦX 174 DNA is the decarboxylated derivative of BNP (EtBNP). We found that EtBNP, prepared *in situ* by irradiation of BNP in anaerobic conditions before DNA addition, was able to generate SSBs, under the same experimental conditions as used for BNP, to give the same amount of DNA cleavage. Further, as can be seen in Fig. 3 the DNA break efficiency observed for BNP was reproduced with pure EtBNP (isolated after BNP irradiation and purified by recrystallization from methanol). In the absence of oxygen. EtBNP is photochemically stable. Such stability can be compared to that of 2-(4-chlorophenyl)benzoxazole which undergoes dehalogenation with very low quantum yield ($\emptyset \sim 4.10^{-5}$) upon anaerobic irradiation at 300 nm in ethanol [34]. Thus, BNP mediated DNA cleavages do not seem to be related to the formation of radical species derived from BNP or EtBNP.

Since molecular oxygen decreases the efficiency of the photocleavages, both in BNP experiments and in controls, it appears that an oxidation reaction based on generation of activated oxygen species (singlet oxygen or superoxide anion) is not the main process involved in the BNP/DNA photoreaction. Such a situation is interesting since most photosensitized reactions of DNA have been found to be oxygen dependent [35]. Nor should such a process be compared anymore to the behavior of BNP in DNA breaks generated by BNP activated leucocytes under UV radiation [36]. It has been proposed in this case that the oxidative DNA breaks observed do not result from a photo-activation of BNP but rather from the sensitizing effect of UVA on polymorphonuclearleucocytes [37].

Our experimental data are in favor of an oxygen independent mechanism, like energy transfer or electron transfer to or from nucleobases. Drug photosensitization reactions by energy transfer are known to occur *in vitro*, for example between DNA and aflotoxin B-12 [38] or griseofulvine [39]. Electron transfers have been reported between DNA and photoexcited states of antitumor drugs of the Adriamycin® class [40] or phenothiazine derivatives [41]. With out current results on DNA cleavage by NSAIDs, we are unable to distinguish between the two possible mechanisms of DNA degradation.

The comparison of the efficiency of the six different antiinflammatory drugs tested in the present work indicates that the propionic acid derivatives are the most active with respect to photocleavage of DNA. These data are in agreement with the previously described photohemolysis of human red blood cells as well as growth inhibition of *Candida albicans* showing that these propionic acid derivatives are the most phototoxic *in vitro* [23].

DF, slightly or not phototoxic according to *in vitro* conditions, can also promote DNA breaks, but with a lower efficiency. SL or IND, which are not phototoxic, are also not photoactive in the DNA cleavage. In fact, these two compounds can play the role of photoprotecting agents since DNA cleavage may be less efficient in the presence than in the absence of these drugs. Ljunggreen has indicated a similar effect in the inhibition of photohemolysis by IND [23]. IND has been shown to decrease the erythemal response in UV irradiated guinea-pigs and so it has been proposed as a sunscreening agent [42, 43], that is in agreement with the absence of photochemical reactivity [44].

As a final point, it should be noted that the order of DNA cleavage efficiency of the three derivatives of propionic acids (KP > NP > BNP) is different to that obtained in photohemolysis experiments (BNP > KP > NP). This order does not correlate with the order of the photochemical reactivity of these compounds (KP > BNP > NP) but might be related to the variable affinity of the substrate (the drug itself or its photoproducts) for the targets (membrane or nucleic acids). So, no extrapolation of the current results *in vitro* to *in vivo* can be made. For example, it is known that KP is not phototoxic *in vivo* [24], while we have shown it to have significant *in vitro* photoactivity toward DNA cleavage.

CONCLUSION

Some non steroidal antiinflammatory drugs containing a propionic acid subunit photosensitize the cleavage of double stranded DNA. These data, obtained *in vitro*, cannot be extrapolated to *in vivo* drug behavior, since many other factors (metabolism, cell penetration, membrane uptake, . . .) also mediate the metabolic properties of these NSAIDs. However the experiments performed here with purified macromolecules might be considered a useful screening method to initiate detection of potentially photoactive molecules able to promote damage to nucleic acids.

REFERENCES

1. Diffey BL, Daymond TJ and Fairgreaves H, Photosensitivity studies of non-steroidal anti-inflammatory agents. *Br J Rheumatol* **16**: 633–638, 1983.
2. Harber LC, Kockevar IE and Shalita AR, Mechanisms of photosensitization to drugs in human. In: *The Science of photomedicine* (Eds. Regan JD and Parrish JA). Plenum Press, New York, 1982.
3. Przybilla B, Ring J, Schwab U and Dorn M, Nichtsteroidale Antirheumatika mit photosensibiliserender Wirkung. *Allergol* **9**: 8–15, 1986.
4. Huskisson EC, Treatment of rhumatoid arthritis with a single daily dose of benoxaprofen. *Rheumatol and Rehabil* **18**: 110–113, 1979.
5. Mikulaschek W, Long-term safety of benoxaprofen, *J Rheumatol and Rehabil* **7** (suppl.6): 100–108, 1980.
6. Wiskemann A, Photosensibilisierung durch benoxaprofen. *Arzneimittelforsch/Drug Res* **31**: 730–732, 1981.
7. Fenton DA, Wilkinson JD and English JS, Photosensitisation to benoxaprofen not due to ultraviolet alone. *Lancet* **II**: 1230–1231, 1981.
8. Ferguson J, Addo HA, McGill PE, Woodcock KR, Johnson BE and Frain-Bell W, A study of benoxaprofen induced photosensitivity. *Br J Dermatol* **107**: 429–442, 1982.
9. Halsey JP and Cardoe N, Benoxaprofen: side-effect profile in 300 patients. *Br Med J* **284**: 1365–1368, 1982.
10. McCormack LS, Elgart ML and Turner ML, Benoxaprofen induced photo-onycholysis. *J Am Acad Dermatol* **7**: 678–680, 1982.
11. Greist MC, Ozols II, Ridolfo AS and Muus JC, The phototoxic effects of benoxaprofen and their man-

agement and prevention. *Eur J Rheum Inflam* **5**: 138–147, 1982.
12. Webster GF, Kaidbey WH and Kligman AM, Phototoxicity from benoxaprofen: *in vivo* and *in vitro* studies. *Photochem Photobiol* **36**: 59–64, 1982.
13. Diffey BL and Daymond TJ, Photosensitivity studies and benoxaprofen. *Photochem Photobiophys* **5**: 169–179, 1983.
14. Allen BR, Benoxaprofen and the skin. *Br J Dermatol* **109**: 361–364, 1983.
15. Imann WH and Rawson NSB, Side effect of benoxaprofen. *Lancet* **II**: 1336, 1984.
16. Kligman AM and Kaidbey KH, Phototoxicity to benoxaprofen. *Eur J Rheumatol Inflam* **5**: 124–137, 1982.
17. Addo HA, Ferguson J, Johnson BE and Frain-bell W, A study of benoxaprofen induced photosensitivity. *Br J Dermatol* **107** (suppl. 22): 17–18, 1982.
18. Keahey TM, Webster GF, Kaidbey KH and Lavrer RM, Photourticaria from benoxaprofen. *J Invest Dermatol* **78**: 355, 1982.
19. Deleo VA, Hanson D and Scheide S, Benoxaprofen photosensitization of phospholipase activation in mammalian cells in culture. *Toxicol Lett* **32**: 215–220, 1986.
20. Sik RH, Paschall CS and Chignell CF, The phototoxic effect of benoxaprofen and its analogs on human erythrocytes and rat peritoneal mast cells. *Photochem Photobiol* **38**: 411–415, 1983.
21. Kochevar IE, Hoover KW and Gawienowski M, Benoxaprofen photosensitization of cell membrane disruption. *J Invest Dermatol* **82**: 214–218, 1984.
22. Piette J and Van de Vorst A, DNA Strand breakage induced by photosensitization. *Radiat Phys Chem* **30**: 401–408, 1987.
23. Ljunggreen B, Propionic acid-derived non steroidal antiinflammatory drugs are phototoxic *in vitro*. *Photodermatol* **2**: 3–9, 1985.
24. Ljunggreen B and Lundberg K, *In vivo* phototoxicity of non-steroidal antiinflammatory drugs evaluated by the mouse tail technique. *Photodermatol* **2**: 377–382, 1985.
25. Przybilla B, Schwab-Przybilla U, Ruzicka T and Ring J, Phototoxicity of non-steroidal anti-inflammatory drugs demonstrated *in vitro* by a photo-basophil-histamine-release test. *Photodermatol* **4**: 73–78, 1987.
26. Bernadou J, Pratviel G, Bennis F, Girardet M and Meunier B, Potassium monopersulfate and a water soluble manganese porphyrin complex $Mn[TMPyP](OAc)_5$ as an efficient reagent for the oxidative cleavage of DNA. *Biochemistry* **28**: 7268–7275, 1989
27. Decuyper J, Piette J, Lopez M, Merville MP and Van de Vorst A. Induction of breaks in deoxyribonucleic acid by photoexcited promazine derivatives. *Biochem Pharmacol* **33**: 4025–4031, 1984.
28. Hertzberg RP and Dervan PB, Cleavage of DNA with methidiumpropyl-EDTA-Iron (II): reaction conditions and product analyses. *Biochemistry* **23**: 3934–3945, 1984.
29. Boullard A and Giacomoni PU, Effect of UV irradiation at defined wavelengths of the tertiary structure of double stranded covalently closed circular DNA. *J Photochem Photobiol B* **2**: 491–501, 1988.
30. Gray PJ, Phillips DR and Wedd AG, Photosensitized degradation of DNA by daunomycin. *Photochem Photobiol* **36**: 49–57, 1982.
31. Calberg-Bacq CM and Van de Vorst A, Induction of free radicals in DNA by proflavine and visible light: influence of oxygen and ionic strength. *Photochem Photobiol* **20**: 433–439, 1974.
32. Reszka K and Chignell CF, Spectroscopic studies of cutaneous photosensitizing agents. IV The photolysis of benoxaprofen, and anti-inflammatory drug with phototoxic properties. *Photochem Photobiol* **38**: 281–291, 1983.
33. Navaratnam S, Hughes JL, Parsons BJ and Phillips GO, Laser flash and steady state photolysis of benoxaprofen in aqueous solution. *Photochem Photobiol* **41**: 375–380, 1985.
34. Fery-Forgues S and Paillous N, Photodehalogenation and photodimerization of 2-(4-halophenyl)benzoxazoles. Dependence of the mechanism on the nature of the halogen atom. *J Org Chem* **51**: 672–677, 1986.
35. Cadet J, Berger M, Decarroz C, Wagner JR, Van Lier JE, Ginot YM and Vigny P, Photosensitized reactions of nucleic acids. *Biochimie* **68**: 813–834, 1986.
36. Schwalb G, Beyers AD, Anderson AE and Nel AE, Promotion of DNA strand breaks in cocultered mononuclear leukocytes by protein kinase C-dependent prooxidative interaction of benoxaprofen, human polymorphonuclear leukocytes and ultraviolet radiation. *Cancer Res* **48**: 3094–3099, 1988.
37. Anderson R and Eftychis HA, Potentiation of the generation of reactive oxidants by human phagocytes during exposure to benoxaprofen and ultraviolet radiation *in vitro*. *Brit J Dermatol* **115**: 285–295, 1986.
38. Neely WC, Lansdsen FT and McDuffic JR, Spectral studies on the deoxyribonucleic acid-aflatoxin B1 system. Binding interactions. *Biochemistry* **9**: 1862–1866, 1970.
39. Neely WC and Siraj MY, Binding, excitation energy transfer and photosensitization in griseofulvin-DNA mixtures. *Photochem Photobiol* **48**: 181–185, 1988.
40. Carmichael AJ and Riesz D, Photoinduced reactions of anthraquinone antitumor agents with peptides and nucleic acids bases. An electron spin resonance and spin trapping study. *Arch Biochem Biophys* **237**: 433–444, 1985.
41. Decuyper J, Piette J, Lopez M, Merville JP and Van de Vorst A, Induction of deoxyribonucleic acid breakage by photoexcited promazine derivatives. *Biochem Pharmacol* **33**: 4025–4031, 1985.
42. Lion HW, Thorbecke GJ, Baer RL and Gigli I, Effect of indomethacin on alteration of ATPase-positive Langerhans cell density and cutaneous sunburn reaction induced by ultraviolet-B radiation. *J Invest Dermatol* **81**: 455–458, 1983.
43. Lion HW, Baer RL and Gigli I, Topical indomethacin—an effective sunscreen agent. *J Invest Dermatol* **80**: 378, 1983.
44. Moore DE and Chappuis PP, A comparative study of photochemistry of the non-steroidal anti-inflammatory drugs, naproxen, benoxaprofen and indomethacin. *Photochem Photobiol* **47**: 173–180, 1988.