J. Pharm. Pharmacol. 1983, 35: 358–362
Received October 27, 1982

Penetration of nabumetone into inflammatory exudates in the rat

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The penetration of radioactivity into inflamed sites induced by subcutaneously implanted cotton pellets was studied in the rat after oral administration of [^{14}C]nabumetone. Equilibration between inflamed site and plasma concentrations was slow, maximum concentrations in the pellet granuloma being later than those in the plasma. Nabumetone itself was found only in very low concentrations at the inflamed site and was not found in plasma. The major component (70%) of the radioactivity at both sites was the metabolite 6-methoxy-2-naphthylacetic acid, a compound which has anti-inflammatory properties. Elimination of radioactivity from the plasma and inflamed site was virtually complete within 24 h of dosing. Daily dosing with [^{14}C]nabumetone confirmed that there was no progressive accumulation of radioactivity at the inflamed site.

The first seven days of granuloma formation around implanted cotton pellets in rats can be divided conveniently into two phases (Swingle & Shideman 1972; Freeman et al 1979). The initial 'acute' phase is marked by increased vascular permeability at the site of implantation, an accumulation of inflammatory exudate around the cotton pellet and an influx of polymorphonuclear leucocytes. This is followed after two to three days by the 'proliferative' phase during which mononuclear cells (primarily macrophages) predominate, cell proliferation commences and connective tissue is laid down around the pellet (Boyle & Mangan 1980).

Nabumetone, 4-(6-methoxy-2-naphthyl)-butan-2-one, is a novel non-steroidal anti-inflammatory drug (NSAID) which displays a wide spectrum of anti-inflammatory activity combined with freedom from gastric side effects (Boyle et al 1980, 1982). In contrast to aspirin, naproxen and indomethacin, it is active in reducing cotton pellet granuloma formation over a wide dosage range without toxic side effects, and in reducing cellular infiltration during both the acute and proliferative phases of granuloma formation (Freeman et al 1982). In view of this activity, radiotracer studies have been conducted to investigate the uptake of radioactivity by the rat cotton pellet granuloma following oral administration of [^{14}C]nabumetone. As far as possible, the pharmacological test conditions (Boyle et al 1982) were reproduced in these studies.

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MATERIALS AND METHODS

Chemicals

4-(6-Methoxy-2-naphthyl) [4-^{14}C] butan-2-one [^{14}C] nabumetone; specific activity 0·2, 0·5, 1·1 or 1·9 μCi mg^{-1}; radiochemical purity >99%), and 6-methoxy-2-naphthylacetic acid were synthesized in our laboratories.

Implantation of cotton pellets

Groups of female, Wistar strain rats (Charles River, U.K.), 140–180 g, were anaesthetized by injection of fentanyl and fluanisone (Hypnorm, Janssen Pharmaceutica; 0·1 ml i.m.) and diazepam (Valium, Roche; 0·1 ml of 5 mg ml^{-1} solution i.p.). Two pre-weighed sterile cotton pellets of equal weight ±1 mg (approx. 7 mm diameter and 5 mm long) cut from No. 1 dental roll (Claudius Ash, London, U.K.) were implanted subcutaneously in each rat in the mid-abdominal region, one each side of a ventral mid-line incision. In some rats used for whole-body autoradiography the pellets were implanted on the ventral midline, anterior and posterior to the incision. After the wound was closed, the animals were allowed to recover and given free access to food and water.

Dosing schedule

Nabumetone was suspended in 0·7% (w/v) aqueous methyl cellulose (BDH, Poole, U.K.) and given orally once daily at a nominal dose of 40 mg kg^{-1} beginning 1 h before pellet implantation. Each rat received either three or six doses. [^{14}C]Nabumetone was incorporated into the final dose to allow the determination of nabumetone-related material in the plasma and cotton pellet granuloma.

Collection of plasma samples and cotton pellets

At various times after dosing [^{14}C]nabumetone, rats were anaesthetized with halothane (Fluothane, ICI, U.K.), terminal venous blood samples were collected and the plasma separated and assayed for radioactivity. The rats were killed by cervical dislocation and the pellets excised, weighed, dried overnight at 80 °C and assayed for radioactivity. The wet weight of each granuloma was determined by subtraction of the original pellet weight.

Whole-body autoradiography

Six hours after administration of [^{14}C]nabumetone (spec. act. 1·9 μCi mg^{-1}) on the sixth day, rats were deeply anaesthetized (Fluothane), killed by immersion in dry ice/acetone and sectioned for whole-body autoradiography according to Kalberer (1966). Rats with cotton pellets either side of the midline were sectioned in coronal planes; rats with pellets along the midline were sectioned in parasagittal planes. Those frozen sections which included the pellets were placed in contact with X-ray film (Kodirex; Kodak, U.K.) and stored at −70 °C for 4 weeks after which time the films were developed.

Investigation of radiometabolites in the cotton pellet granuloma and plasma

Six hours after dosing [^{14}C]nabumetone plasma samples were collected, assayed for radioactivity, adjusted to pH 2 with 0·2 M citrate/phosphate buffer (one volume) and then extracted with ethanol (eight volumes). Pellets excised from the same rats immediately after plasma sampling were unrolled, extracted with methanol (3 × 5 ml) and the cotton residue assayed for radioactivity.

The plasma and pellet extracts were assayed for radioactivity, concentrated, mixed with the reference compounds nabumetone and 6-methoxy-2-naphthylacetic acid and applied to fluorescent silica gel t.l.c. plates (ART 5735; E. Merck, Darmstadt, W. Germany) which were each developed in one of the following t.l.c. solvent systems: chloroform–methanol–acetic acid (90:10:2, v/v), toluene–ethyl acetate (7:2, v/v), chloroform–methanol–acetic acid (97·5:2·5:1, v/v), chloroform.

Nabumetone and 6-methoxy-2-naphthylacetic acid were located on the t.l.c. plates by fluorescence quench under u.v. light (wavelength 254 nm). Radioactive zones on the t.l.c. plates were detected by autoradiography (Kodirex X-ray film) and these zones were assayed for radioactivity.

Determination of radioactivity

Radioactivity was measured on a Tri-Carb liquid scintillation spectrometer (Model B2450, Packard Instrument, Reading U.K.). Aliquots (0·5 ml) of radioactive extracts of pellets or plasma were assayed by direct liquid scintillation counting after the addition of 10 ml of 2-ethoxyethanol (scintillation grade; BDH) and 10 ml of a solution of Omnifluor (New England Nuclear Corp; 4 g dissolved in 1000 ml of toluene).

Dried pellets and aliquots of plasma were combusted using a Packard Sample Oxidiser and the radioactivity measured in a liquid scintillation medium consisting of 8 ml of Carbo-Sorb and 15 ml of Permafluor V (Packard).

Plastic-backed t.l.c. plates were sectioned into appropriate zones which were placed in polythene counting vials. Distilled water (0·6 ml) and 40% aqueous hydrofluoric acid (0·8 ml) were added to each vial to dissolve the silica gel, followed by 6 ml of Triton X-100 and 12 ml of Omnifluor in toluene (0·4%, w/v) before liquid scintillation counting.

RESULTS

Granuloma and plasma profiles of radioactivity

When [^{14}C]nabumetone was incorporated into either the third or sixth daily dose, similar plasma concentrations and similar pellet granuloma concentrations of radioactivity were found (Table 1). Plasma concentrations were highest at about 1 h after dosing (about 80 μg of nabumetone equivalent ml^{-1}) and declined to below 12 μg equiv ml^{-1} by 12 h. In contrast, pellet granuloma concentrations of radioactivity increased slowly, reaching a peak (approximately 24 μg equiv g^{-1} of wet granuloma material) at 4 to 6 h. Between 12 and 24 h after dosing, the granuloma concentrations were slightly higher than those in the plasma. Both the granuloma and plasma concentrations of radioactivity declined to low levels (less than 3·5 μg equiv g^{-1}) by 24 h (Table 1).

When rats with subcutaneously implanted pellets were dosed once daily with [^{14}C]nabumetone for either three or six days there was no evidence of

Table 1. Concentrations of radioactivity in the pellet granuloma and plasma of rats after incorporation of [^{14}C]nabumetone into the daily dose (40 mg kg^{-1} day^{-1}) on the third or sixth day. Values are the mean ± s.e.m. from two animals, each animal providing two pellets, implanted on the first day.

Time after dosing [^{14}C]nabumetone (h)	Third day			Sixth day		
	Wet weight* of granuloma material (mg)	Concn of radioactivity†		Wet weight* of granuloma material (mg)	Concn of radioactivity†	
		Pellet granuloma	Plasma		Pellet granuloma	Plasma
0 (pre-dose)	183 ± 6	<0·1	<0·1	174 ± 11	<0·1	<0·1
0·5	187 ± 15	3·6 ± 0·4	74·1	197 ± 11	3·7 ± 0·9	70·9
1	207 ± 19	9·6 ± 2·0	74·4	161 ± 14	12·9 ± 2·5	83·2
2	187 ± 12	18·4 ± 0·9	72·8	181 ± 19	14·1 ± 0·7	71·3
4	203 ± 9	24·0 ± 1·4	53·7	178 ± 10	21·0 ± 0·5	47·6
6	199 ± 5	23·7 ± 1·7‡	33·4‡	208 ± 15	23·2 ± 1·1‡	42·1‡
6	313 ± 25			269 ± 10		
9	285 ± 22	18·6 ± 3·0	20·8	312 ± 9	16·5 ± 0·8	18·7
12	310 ± 18	11·6 ± 0·2	4·8	286 ± 14	12·9 ± 1·0	7·7
18	313 ± 30	4·9 ± 0·4	0.7	270 ± 14	8·6 ± 0·7	1·0
24	296 ± 13	2·5 ± 0·3	0·4	292 ± 15	2·9 ± 0·1	0·4

† Concentrations are expressed in μg of nabumetone equivalent g^{-1} of wet granuloma material or ml^{-1} of plasma.
* 0–6 h and 6–24 h experiments were conducted on separate occasions; the weights of the clean pellets before implantation were 36 ± 1 mg and 45 ± 1 mg respectively.
‡ Four animals.

progressive accumulation of [^{14}C]nabumetone-related material at the inflamed site or in the plasma; the concentrations of radioactivity in the pellet granuloma at 24 h after the final dose were less than 3·5 μg equiv g^{-1} in all cases.

Whole-body autoradiography

Typical autoradiographs of whole rat sections, taken 6 h after dosing [^{14}C]nabumetone on the sixth day, are shown in Fig. 1. As judged by the relative intensity of the darkened zones, the highest concentrations of radioactivity at 6 h after dosing were in the gut, although moderate concentrations were also present in the liver, heart and lungs. The sectioned pellet granulomas can be identified in Fig. 1, located along the ventral midline of the rat (plate 1) or on either side of the ventral midline (plate 2). The darkness of the pellet image shows that radioactivity penetrated the granuloma, the image intensity being similar to that of the liver. A ring of low radioactivity can be seen immediately around the outside of each pellet. This may correspond to the layer of connective tissue which encases the five-days-old granuloma (Boyle & Mangan 1980). The area surrounding each granuloma, between the skin and the underlying abdominal wall, corresponds to a pool of inflammatory exudates and shows up in the autoradiographs as an area of relatively high radioactivity concentration.

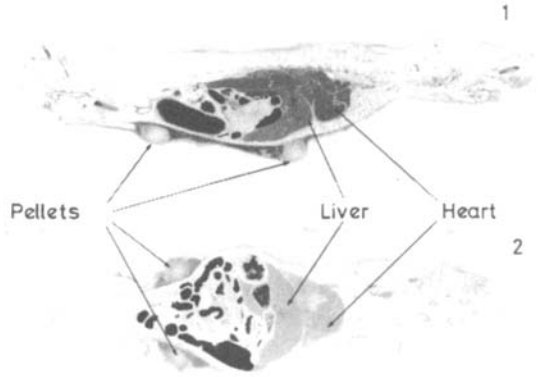

FIG 1. Whole body autoradiographs of rats sectioned 6 h after an oral dose of [^{14}C]nabumetone (40 mg kg^{-1}). Cotton pellets had been implanted five days earlier and non-radiolabelled nabumetone administered once daily at the same dose.

Nature of the radioactive material in the plasma and pellet granuloma

The radioactive materials were completely extracted from plasma and pellet granuloma obtained on the sixth day, 6 h after dosing [^{14}C]nabumetone to two rats. Thin-layer chromatography indicated that nabumetone itself was not present in the plasma (less than 0·1 μg ml^{-1}) although trace amounts were apparent in the granuloma (maximum value 0·7 μg g^{-1}). Most of the radioactivity (70%) in both plasma and granuloma co-chromatographed with 6-methoxy-2-naphthylacetic acid. Polar materials at the t.l.c. plate origin accounted for most of the remaining radioactivity.

DISCUSSION

Whole-body autoradiographs (Fig. 1) reveal that radioactivity derived from [^{14}C]nabumetone did reach the inflamed sites induced by implanted cotton pellets. However, the major component of this radioactivity was an acidic metabolite, 6-methoxy-2-naphthylacetic acid rather than nabumetone itself. Nabumetone is rapidly metabolized to 6-methoxy-2-naphthylacetic acid in animals and man and its concentration in plasma is very low after oral dosage (Haddock et al, unpublished results).

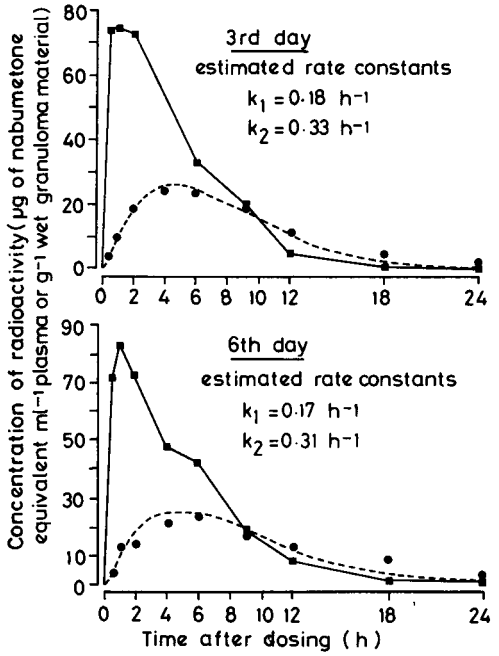

FIG. 2. Computer-predicted concentrations of radioactivity in the pellet granuloma based on a simple model. Each point represents the mean value from two rats (Table 1). ■——■ observed plasma concentrations; - - - predicted pellet granuloma concentrations; ● observed pellet granuloma concentrations.

Specific localization of acidic NSAIDS at inflamed sites in animals has been reported for naproxen and salicylate (Doherty et al 1977), phenylbutazone and indomethacin (Brune et al 1976) and for bumadizone (Benakis et al 1973). Brune et al (1976) suggest that the clinical efficacy of acidic NSAIDs, compared with their non-acidic counterparts, may be due simply to their superior ability to reach the site of inflammation in-vivo and that a pK_a value in the range 4 to 5 and an ability to bind strongly to plasma proteins are optimal requirements for a NSAID. Although nabumetone is a neutral molecule its major circulating metabolite, 6-methoxy-2-naphthylacetic acid, has a pK_a of about 5, does bind strongly (>95%) to plasma proteins and has anti-inflammatory activity in animals (Goudie et al 1978). Although the precise mode of action of nabumetone is not known, it is probable that this metabolite is responsible for the anti-inflammatory activity shown by the drug.

The concentrations of nabumetone metabolites (radioactivity) at the inflamed site were not identical to those in plasma. Composite profiles of concentration versus time (Fig. 2) suggested that entry to the inflamed site was delayed. However the data obtained during the 'acute' phase (third day) were similar to those obtained during the 'proliferative' phase of granuloma development (sixth day), indicating that cell proliferation at the inflamed site did not materially affect the uptake or release of nabumetone metabolites. The slow equilibration of radioactivity between plasma and inflamed site suggested that the inflamed site acted as a separate compartment. This situation can be represented by a simple model

$$\text{Dose in GI tract} \rightarrow \text{Plasma} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{Pellet granuloma}$$

and estimates* of the apparent first order rate constants for the transfer of materials to and from the inflamed site were used to simulate the concentration at the inflamed site from the measured plasma concentrations. The simulated profile agreed reasonably well with the observed pellet granuloma concentrations (Fig. 2) indicating that this model may be valid. The composite profile of plasma concentration was complex and could not be satisfactorily fitted to a simple pharmacokinetic model; an approximate half-life of 2–3 h was suggested by graphical methods.

* In the proposed kinetic model, assuming changes in total radioactivity concentration obey first order kinetics, the rate of change of the pellet granuloma radioactivity concentration at any time may be expressed as:

$$\frac{dCp}{dt} = k_1C_1 - k_2C_p$$

where C_1 is the plasma concentration and C_p is the pellet granuloma concentration. At the time of peak pellet granuloma concentration, dCp/dt is equal to zero. Hence, the ratio of k_1/k_2 was calculated by dividing the pellet granuloma concentration at this time by the corresponding plasma concentration. The value of k_2 was estimated as the slope of a plot of $\Delta C_p/\Delta t$ versus $(k_1C_1/k_2) - C_p$ and the approximate value of k_1 was determined from the k_1/k_2 ratio. Simulation of the pellet granuloma profile from the plasma data using these estimated rate constants was achieved using a computer.

Slow equilibration with the inflamed site has been reported for a number of acidic NSAIDs. Naproxen penetrates slowly into inflammatory exudates in the rat (Doherty et al 1977). Similarly, the penetration of other NSAIDs including aspirin (Sholkoff et al 1967), indomethacin (Emori et al 1973), ketoprofen (Mitchell et al 1975) and carprofen (Ray et al 1979) into the synovial fluid of arthritic joints in patients is delayed, peak concentrations occurring some hours after peak plasma concentrations.

In conclusion, after dosing [^{14}C]nabumetone to rats with subcutaneously implanted cotton pellets, radioactive material (predominantly the metabolite 6-methoxy-2-naphthylacetic acid) has good access to the cotton pellet granuloma during both the acute and proliferative phases of cellular infiltration in this model system. The time course of radioactivity at the inflamed site, relative to the plasma, is similar to that seen in arthritic patients after dosing with other NSAIDs. Since both the plasma and granuloma concentrations decline to low values within 24 h of dosing, the chronic anti-inflammatory action of daily administered nabumetone in the rat cotton pellet granuloma model (Freeman et al 1982) does not seem to be dependent upon the progressive accumulation of nabumetone related materials.

Acknowledgements

The authors wish to thank Mr B. Legg for his computer-based evaluation of the kinetic model. Thanks are also due to Mr D. J. D. Tidy for supplying [^{14}C]nabumetone, Dr C. J. Rose for supplying 6-methoxy-2-naphthylacetic acid, Mr J. V. Ramji for practical assistance and discussion, and to Mr K. E. Randall for the surgical implantation of cotton pellets.

REFERENCES

Benakis, A., Tsoukas, G., Glasson, B. (1973) Arzneim. Forsch. 23: 1231–1236

Boyle, E. A., Freeman, P. C., Mangan, F. R., Thomson, M. J. (1980) Int. Cong. of Inflammation, Bologna, Italy p. 103

Boyle, E. A., Freeman, P. C., Mangan, F. R., Thomson, M. J. (1982) J. Pharm. Pharmacol. 34: 562–569

Boyle, E. A., Mangan, F. R. (1980) Br. J. Exp. Pathol. 61: 351–360

Brune, K., Graf, P., Glatt, M. (1976) Agents Actions 6: 159–164

Doherty, N. S., Anttila, M., Dean, P. B. (1977) Ann. Rheum Dis. 36: 244–248

Emori, H. W., Champion, G. D., Bluestone, R., Paulus, H. E. (1973) Ibid. 32: 433–435

Freeman, P. C., Mangan, F. R., Watkins, D. K. (1979) Biochem. Pharmacol. 28: 573–578

Freeman, P. C., Mangan, F. R., Watkins, D. K. (1982) J. Pharm. Pharmacol. 34: 580–583

Goudie, A. C., Gaster, L. M., Lake, A. W., Rose, C. J., Freeman, P. C., Hughes, B. O., Miller, D. (1978) J. Med. Chem. 21: 1260–1264

Kalberer, F. (1966) Atomlight (New England Nuclear Corp.) 51: 1–7

Mitchell, W. S., Scott, P., Kennedy, A. C., Brooks, P. M., Templeton, R., Jeffries, M. G. (1975) Curr. Med. Res. Opin. 3: 423–430

Ray, J. E., Wade, D. N., Graham, G. G., Day, R. O. (1979) J. Clin. Pharmacol. 19: 635–643

Sholkoff, S. D., Eyring, E. J., Rowland, M., Riegelman, S. (1967) Arthritis Rheum. 10: 348–351

Swingle, K. F., Shideman, F. E. (1972) J. Pharmacol. Exp. Ther. 183: 226–234