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A study of the effects of nabumetone (BRL 14777), a novel anti-inflammatory drug, on cell infiltration into sterile cotton pellets implanted in rats

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Nabumetone a new non-steroidal anti-inflammatory drug is active in reducing the DNA content of implanted cotton pellets in rats on various dosing regimens. The effect of nabumetone can be seen during the early and late phases of the cellular reaction to the implant implicating effects upon polymorphonuclear leucocytes and monocytes. The levels of two lysosomal enzymes are reduced in the exudate by the drug treatment.

The attraction and accumulation of cells, particularly neutrophilic polymorphonuclear leucocytes (PMNL's), at sites of inflammation is an essential part of the host defence mechanism. However, in situations where the inflammatory stimulus is not removed, the continuing emigration and accumulation of PMNL's, lymphocytes and macrophages can lead to the release of mediators and enzymes which are responsible for the tissue damage seen in chronic inflammatory conditions (Allison et al 1978; Gordon et al 1978; Harris et al 1978; Schnyder & Baggiolini 1978).

The growth of granulation tissue around an implanted sterile cotton pellet in rats was first shown by Meier et al (1950) to be reduced by cortisone. Since then the model has been widely used to examine the effects of non-steroidal anti-inflammatory drugs with limited success and it is claimed that the test does not demonstrate satisfactorily the anti-inflammatory activity of this type of compound (Swingle 1974).

The pattern of early cellular infiltration in this model can be divided into an acute phase lasting for about 2 days, in which the PMNL's predominate, followed by a further period of several days during which the population of mononuclear cells, mainly macrophages, increases and cell proliferation commences (Freeman et al 1979). It was further shown possible to measure acid hydrolase levels in the exudate removed from the pellets by centrifugation, and that the pattern of the enzyme activity paralleled the initial PMNL peak and then was further increased during the later macrophage/monocyte infiltration. Nabumetone (4-(6′-methoxy-2′-naphthyl)-butan-2-one) has been shown to have a wide spectrum of anti-inflammatory activity combined with freedom from gastric side effects (Boyle et al 1978; Mangan et al 1981) and, in contrast to aspirin, naproxen and indomethacin, it is active in reducing granuloma formation in implanted cotton pellets over a wide dosage range without toxic side effects. We have therefore investigated the drug's effects on the early events in the developing granuloma to determine which stages are inhibited and whether levels of lysosomal enzymes are reduced in the exudate.

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MATERIALS AND METHODS

Implantation of cotton pellets

Groups of Wistar rats (OLAC, 150–170 g) were anaesthetized using fentanyl plus fluanisone (0·1 ml i.m., Janssen Pharmaceuticals) and diazepam (5 ml kg^{-1} i.p., Roche). Two pre-weighed sterile cotton pellets (weight about 30 mg) cut from No. 1 dental roll (Claudius Ash, London, U.K.) were implanted subcutaneously in each rat, one each side of a ventral mid-line incision. After closing the wound with a Michel clip, the animals were allowed to recover and given free access to food and water. For each experiment pellets were of equal weight ± 1 mg.

Nabumetone or hydrocortisone were administered orally suspended in 0·7% methyl cellulose 1 h before implantation (day 0) or at indicated times thereafter. Body weights were recorded throughout the studies and at the end of the experiment the animals were killed, either by cervical dislocation,

when the pellets were to be used for acid hydrolase measurements, or by a lethal intraperitoneal dose of pentobarbitone (Nembutal, Abbot) when the weight or DNA content of the pellets were the only parameters to be measured.

Dry weight determinations

Pellets were removed, dried overnight at 80 °C and re-weighed, granuloma formation being calculated by subtracting the original weight.

DNA determinations

Pellets were placed (2 from each animal) in 4 ml of 0·5N perchloric acid and the DNA was hydrolysed at 95 °C for 15 min, the tubes were then cooled in ice and the supernatant removed by centrifugation. DNA content was determined by the method of Burton (1956) as described by Freeman et al (1979).

Hydrolytic enzyme and protein determinations on the exudate

Pellets were removed from rats and placed in conical polyethylene supports held in polycarbonate centrifuge tubes and centrifuged at 1000 *g* for 10 min at 4 °C. The clear cell-free fluid was collected and the protein content determined by the method of Lowry et al (1951). β-Glucuronidase (β-G, EC 3, 2, 1, 31) and β-*N*-acetylglucosaminidase (NAG, EC 3, 2, 1, 30) were assayed by an auto-analyser system (Freeman et al 1979).

RESULTS

Nabumetone was given for varying periods and the DNA content of the pellets measured. Nabumetone given at −1 h and 24 h after implantation significantly reduced the DNA content of the pellets measured 2 days after implantation (Table 1). The high level of DNA on day 2 is similar to that seen previously (Freeman et al 1979) and reflects the initial large influx of PMNL's. When nabumetone was dosed 1 h before and then at 24, 48 and 72 h after implantation, and the DNA content measured at 96 h, the reduction compared with controls was not statistically significant (Table 1). Freeman et al (1979) have shown that the DNA levels and hydrolytic enzyme content fall during the 48–72 h period presumably reflecting the loss of PMNL's, and it is a period before the monocytes/macrophages start to appear in greater numbers.

In a third experiment dosing with nabumetone was delayed until 72 h post implantation and was continued daily until 144 h. Pellets were removed at 168 h. During that 72 h dosing period (days 3–6) the population of monocytes/macrophages increased, cell proliferation commences and collagen began to be deposited. Treatment with nabumetone during this phase significantly reduced DNA levels in the pellets (Table 1).

Exudates obtained by centrifugation of the cotton pellets from control and rats dosed with nabumetone (50 mg kg^{-1}) daily showed that protein content of the exudate was not reduced by treatment with nabumetone at any day after implantation (Table 2). However, the dry granuloma weight was lower than control on each day. It can also be seen that treatment with nabumetone at this dose did not affect body weight gain of the rats when they were compared with vehicle-dosed control rats (Table 2).

Table 1. The effect of nabumetone (70 mg kg^{-1} oral) dosed days 0–1, 0–3 or 3–6 on the DNA content of implanted cotton pellets.

Treatment	Duration of treatment (days)	Day of measurement	No. of rats	Mean DNA content ± s.e.m. μg in 2 pellets
Methyl cellulose	0–1	2	20	1157 ± 45
Nabumetone	0–1	2	20	***821 ± 54
Methyl cellulose	0–3	4	10	897 ± 70
Nabumetone	0–3	4	10	725 ± 104
Methyl cellulose	3–6	7	10	991 ± 54
Nabumetone	3–6	7	10	*739 ± 82

Significantly different from control groups as determined by Student's *t*-test: * $P < 0.02$, *** $P < 0.001$.

Table 2. The effect of dosing nabumetone (50 mg kg^{-1} day^{-1} oral) on protein content of exudate and dry weight of the granuloma after exudate removal.

Day after implantation	Treatment	Protein content mg/0·1 ml of exudate ± s.e.m.	Granuloma dry weight mg ± s.e.m.
1	Methyl cellulose	6·11 ± 0·12	42·33 ± 1·05
	Nabumetone	6·03 ± 0·15	*37·20 ± 1·72
2	Methyl cellulose	5·79 ± 0·17	45·70 ± 2·10
	Nabumetone	5·64 ± 0·17	*38·90 ± 2·07
3	Methyl cellulose	5·46 ± 0·23	51·89 ± 1·99
	Nabumetone	5·16 ± 0·12	***43·70 ± 1·69
4	Methyl cellulose	6·21 ± 0·26	48·00 ± 3·84
	Nabumetone	5·84 ± 0·15	*37·11 ± 2·38
7	Methyl cellulose	5·44 ± 0·76	68·60 ± 6·95
	Nabumetone	**5·74 ± 0·35	56·44 ± 7·24

Body weight (g) of the rats at the end of 7 days dosing with either vehicle or Nabumetone. Control 186·0 ± 5·04, Nabumetone 180·0 ± 4·43.

Significance of difference from control determined by Student's *t*-test: * $P < 0.05$, ** $P < 0.02$, *** $P < 0.01$.

The levels of NAG in the exudate obtained from pellets removed from rats dosed with nabumetone were lower than those from control rats on every day measured (Fig. 1a). The pattern was the same for control and drug dosed groups, a high level on day 1 was followed by a fall on days 2 and 3 and then a steep rise in activity was seen up to day 7. A second acid hydrolase β-G exhibited a similar biphasic

activity, the enzyme levels were lower than those of NAG and the effect of nabumetone was apparent only after 4 days dosing (Fig. 1b). Unfortunately, the variation in the activity of this enzyme was such that none of the decreases brought about by nabumetone were statistically significant.

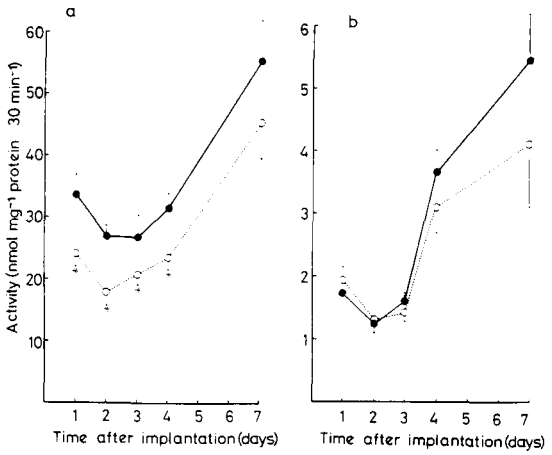

FIG. 1. The effect of nabumetone treatment on acid hydrolase levels in exudate collected by centrifugation of cotton pellets (a) N-acetyl-B-D-glucosaminidase leves (b) β-glucuronidase levels ●—● Methylcellulose ○---○ Nabumetone.

DISCUSSION

Chronic inflammatory reactions are complex and involve the participation of several cell types, enzymes and mediators. All inflammatory reactions are characterized by an early influx of PMNL's which have been shown to release acid hydrolases that can act on many tissue components (Oronsky & Perper 1975). However, in chronic inflammatory conditions, the most important cell type is thought to be the monocyte/macrophage, although the initiating role of the PMNL should not be overlooked. Of the studies of the effects of steroidal and non-steroidal anti-inflammatory drugs (NSAID's) on the release of lysosomal enzymes (see Ringrose et al 1975) and neutral proteinases (see Vassalli et al 1976) from macrophages, most have used in vitro incubations of macrophages in the presence of the drugs. Others have used the inflamed paw of the rat (Arrigoni-Martelli & Restelli 1972), and although the lysosomal enzyme content of macrophages obtained from peritoneal fluid of mice has been examined (Schnyder & Baggiolini 1978) the effects of drugs in vivo have not been studied.

The implantation into rats of sterile cotton pellets induces a pattern of cellular events typical of a developing chronic inflammatory lesion. Removal of the pellets at various stages, allows the cell content to be examined, and the hydrolytic enzyme content of the exudate measured (Freeman et al 1979).

Ford-Hutchinson et al (1978) showed that several anti-inflammatory drugs, including indomethacin, aspirin, sodium salicylate, phenylbutazone and hydrocortisone, were capable of reducing the numbers of leucocytes present in inert sponges 9 h after implantation. With aspirin and indomethacin the reduction in cell numbers was about 50%, whereas the same doses of these drugs decreased the prostaglandin content of the sponges by about 90%. Similar results were found using indomethacin and flurbiprofen, whereas benoxaprofen only showed inhibition of prostaglandin production in vivo at doses equivalent to those required to inhibit white cell migration in the model (Ford-Hutchinson et al 1977). Those observations led the authors to suggest two independent mechanisms of anti-inflammatory activity of NSAID's, one related to prostaglandin synthesis, the other to inhibition of leucocyte migration. We did not measure the prostaglandin content of the pellets but concentrated on the effects of nabumetone on the accumulation of leucocytes and monocytes/macrophages. In being able to reduce the cell content of the pellets at an early stage after implantation, nabumetone clearly shares with indomethacin, flurbiprofen and benoxaprofen the ability to reduce the influx of PMNL's into implanted material. Benoxaprofen, however, has been reported unable to modify the influx of polymorphonuclear leucocytes into the pleural cavity of the rat after carrageenan injection, although a small reduction was found after dextran injection which produces a milder inflammatory reaction than carrageenan (Meacock & Kitchen 1979).

It is possible that the injection of fluid into the pleural cavity and the implantation of inert materials subcutaneously bring about reactions of different intensities. Our finding that the DNA content of cotten pellets after nabumetone treatment is reduced by about 30%, may be compared with 50% reported by Ford-Hutchinson et al (1977) using sponges and anti-inflammatory drugs. However, a direct comparison of implanted cotton pellets and vinyl sponges showed that the DNA content of sponges was about 25% that of pellets 2 days after implantation (unpublished results). A cotton pellet appears therefore to produce a much more intense inflammatory reaction than a similar sized polyvinyl sponge. After the initial influx of leucocytes into the cotton pellets, the cell numbers decline as indicated by a decreased

DNA content. During this decline, nabumetone treatment had only a small effect on DNA content probably because it was unable to influence this change further in the same direction. This period of decline is, however, immediately followed by an influx of monocytes/macrophages on days 3–6, during which nabumetone is able to reduce the DNA content and therefore presumably the influx of these phagocytic cells.

Nabumetone thus appears capable, under the conditions of the experiments reported here, of reducing leucocyte infiltration, a property shared with indomethacin and other non-steroidal anti-inflammatory drugs. It also reduced monocyte/macrophage infiltration, a property shared with benoxaprofen, ketoprofen, phenylbutazone and indomethacin (Meacock & Kitchen 1976, 1979).

The role of acid hydrolases released from PMNL's (Oronsky & Perper 1975) and monocytes (Schorlemmer et al 1977) in tissue destruction is well established. Interference with either the synthesis or release of these enzymes would therefore help to reduce their effects on tissues. The studies of Freeman et al (1979) and the present work have shown an initial high level of lysosomal enzyme activity to be present in exudate obtained from implanted cotton pellets. The high level appears to correlate well with the initial influx of PMNL's. Nabumetone treatment significantly reduced the level of NAG during days 0–3. However the level of a second enzyme, β-G, was not reduced. During days 3–6, the period in which the monocyte/macrophage population is increasing, nabumetone reduced the level of NAG in the exudate. These were much higher than that of β-G. This different response of the two enzymes, particularly in the early stages of the reaction, days 0–3 is puzzling. Mørland & Mørland (1978) found marked differences in the pattern of enzymes released from mouse macrophages incubated with various stimulants in vitro measuring acid phosphatase, β-G and cathepsin D and suggested that this indicated separate regulation of lysosomal enzyme synthesis depending upon the type of stimulation. The cotton pellet in these experiments may afford a stimulus more suitable for the release of NAG than of β-G.

In conclusion, nabumetone administration to rats under the conditions of these experiments is capable of reducing the early PMNL infiltration of implanted sterile cotton pellets, and the later infiltration of cells many of which are monocytes/macrophages. Furthermore the level of acid hydrolases present in the exudate obtained by centrifugation of these pellets is reduced, particularly at the later stages of the reaction. However, of the two enzymes measured (NAG and β-G), only the reductions in the former are statistically significant.

REFERENCES

Allison, A. C., Ferluga, J., Prydz, H., Schlorlemmer, H. U. (1978) Agents Actions 8: 27–35

Arrigoni-Martelli, E., Restelli, A. (1972) Eur. J. of Pharmacol. 19: 191–198

Boyle, E. A., Freeman, P. C., Mangan, F. R., Thomson, M. J. (1978) Int. Cong. Inflam. Bologna, Italy, p. 103

Burton, K. (1956) Biochem. J. 62: 315–323

Ford-Hutchinson, A. W., Walker, J. R., Connor, N. S., Oliver, A. M., Smith, M. J. H. (1977) J. Pharm. Pharmacol. 29: 372–373

Ford-Hutchinson, A. W., Walker, J. R., Smith, M. J. H. (1978) J. Pharmacol. Methods 1: 3–7

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

Gordon, S., Newman, W., Bloom, B. (1978) Agents Actions 8: 19–26

Harris, E. D., Jr., Vater, C. A., Mainardi, C. L., Werb, Z. (1978) Agents Actions 8: 36–42

Lowry, O. H., Rosebrough, N. H., Farr, A. L., Randall, R. J. (1951) J. Biol. Chem. 193: 265–275

Mangan, F. R., Boyle, E. A., Thomson, M. J. (1981) XVth Int. Cong. of Rheumatology, Abst. 1451

Meacock, S. C. R., Kitchen, E. A. (1976) Agents Actions 6: 321–325

Meacock, S. C. R., Kitchen, E. A. (1979) J. Pharm. Pharmacol. 31: 366–370

Meier, R., Schuler, W., Desaulles, P. (1950) Experientia 6: 469–471

Mørland, B., Mørland, J. (1978) J. Retic. Soc. 23: 469–477

Oronsky, A. L., Perper, R. J. (1975) Ann. N.Y. Acad. Sci. 256: 233–253

Ringrose, P. S., Meriel, A. P., McLaren, M. (1975) Biochem. Pharmacol. 24: 607–614

Schnyder, J., Baggiolini, M. (1978) J. Exp. Med. 148: 435–450

Schorlemmer, H. U., Davies, P., Hylton, W., Gugic, M. Allison, A. C. (1977) Br. J. Exp. Path. 58: 315–326

Swingle, K. F. (1974) in: Med. Chem., 13, II, Scherrer, R. A., Whitehouse, M. W. Academic Press, New York, p. 33

Vassalli, J-D., Hamilton, J., Reich, E. (1976) Cell 8: 271–281