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Factors influencing the inhibitory action of anti-inflammatory drugs on carrageenin induced oedema

ANN Y. GREEN, D. GREEN, PATRICIA A. MURRAY AND A. B. WILSON

Wyeth Institute of Medical Research, Huntercombe Lane South, Taplow, Maidenhead, Berkshire

Summary

1. In a series of experiments carried out over $2\frac{1}{2}$ years, the inflammatory response of the rat hind paw to injected carrageenin was relatively constant, but the anti-inflammatory activity of phenylbutazone was subject to wide variation.

2. Phenylbutazone, aspirin and indomethacin were all well absorbed after oral administration, and the resultant plasma drug concentrations were closely similar to those produced when the drugs were administered intraperitoneally.

3. The anti-inflammatory effect of the drugs on carrageenin oedema was variable and poorly correlated with log dose and plasma concentration.

4. There was a pronounced and linearly correlated increase in the anti-inflammatory activity of phenylbutazone with increasing ambient temperature in the range 5–30° C.

5. Variations in relative humidity at constant temperature (20° C) did not influence the anti-oedema potency of phenylbutazone.

6. For the precise evaluation of anti-inflammatory activity in carrageenin oedema tests, it is concluded that accurate control of temperature is essential.

Introduction

Since the first description of the method by Winter, Risley & Nuss (1962), production of an inflammatory oedema in the rat paw by injected carageenin has been widely adopted as an experimental model for the evaluation of potential anti-inflammatory drugs. Carrageenin is preferred to several other inflammatory agents because the oedema produced is less influenced by non-specific factors such as vasodilatation, ganglion blockade or diuresis (Garattini, Jori, Bernardi, Carrara, Paglialunga & Segre, 1965) and because the activity of anti-inflammatory drugs in this test correlates well with their anti-inflammatory activity in man (Kampmann & Frey, 1966). Despite these advantages of carrageenin, a wide variability in the anti-oedema activity of standard anti-inflammatory drugs occurs both between and within laboratories. This paper describes experiments made to identify the major sources of variation.

Methods

Routine single dose experiments with phenylbutazone

Phenylbutazone was used as a standard for comparison during $2\frac{1}{2}$ years of routine experiments to evaluate the potential anti-inflammatory activity of new chemicals.

Female rats of a Sprague Dawley derived strain, weighing 120–160 g, were used for all experiments. They were randomly distributed into control and test groups, each group containing six animals. One control group was treated orally with 100 mg/kg of phenylbutazone (Butazolidin, Geigy) as a standard anti-inflammatory agent and a second control group was given the suspending medium alone (10 ml/kg of a solution of 0·5% carboxymethylcellulose, 0·1% Tween 80, 0·9% sodium chloride in distilled water). The remaining groups of animals were used to assess the activity of the new compounds.

One hour after the oral treatments had been administered, an inflammatory oedema was induced in both hind paws by injection of 0·05 ml of 1% carrageenin gel (Viscarin, Marine Colloids Inc.) into the plantar aponeurosis, the volume of the paws being measured plethysmometrically (Green & Haines, 1966) within 30 s of this injection. Three hours later, the hind paw volumes were redetermined and the mean changes in paw volume for the control ($\bar{V}_c$) and test ($\bar{V}_t$) groups were calculated. Percentage inhibition of the carrageenin oedema in the treated groups was then calculated from:

$$100\ (1-\bar{V}_t/\bar{V}_c)$$

Experiments to investigate the relationships between dose, plasma concentration and anti-inflammatory response

Groups of six rats were used as before but were kept overnight without food but with free access to drinking water. Next morning, each group was dosed either orally or intraperitoneally with one of four concentrations of phenylbutazone (4·5, 13·5, 40 and 120 mg/kg); indomethacin (0·5, 1·5, 4·5 and 13·5 mg/kg); or aspirin (9, 27, 80 and 240 mg/kg). The control groups were given the suspending medium by the same routes of administration. Subsequently, the rats were injected with carrageenin and anti-inflammatory activity was evaluated as described above.

After the final paw volume determination, the animals were deeply anaesthetized with ether and exsanguinated via the inferior vena cava. The plasma concentration of salicylate was determined as described by Chirigos & Udenfrend (1959), indomethacin was determined using the method described by Hucker, Zacchei & Cox (1963), and phenylbutazone by the method of Burns, Rose, Chenkin, Goldman, Schulert & Brodie (1953). The complete experiment was repeated on three separate occasions.

Effect of variations in laboratory temperature and humidity

Groups of rats were fasted overnight in the normal accommodation at 20–22° C and 50–60% relative humidity. Next morning the animals were moved to a controlled environment laboratory, which had been preset and allowed to stabilize within a range of temperature ±1° C and relative humidity ±5%. In one series of experiments, the influence of environmental temperature between 5° C and 30° C was assessed, the relative humidity being maintained at 50%. In a second series,

the relative humidity was varied between 40 and 90% at a constant room temperature of 20° C.

All test animals received 100 mg/kg of phenylbutazone orally and a concurrent vehicle treated control group was run for each set of environmental conditions. At the end of the experiments, samples of soft tissues from the plantar surfaces of the skinned hind paws were homogenized in 0·1 N sodium hydroxide (9 ml/g of tissue) and phenylbutazone was extracted and estimated by the method of Burns *et al.* (1953). In all other respects the experimental procedure of the paw oedema test was identical to that already described.

Results

Routine single dose experiments with phenylbutazone

Figure 1 summarizes the results of 2½ years of experiments with phenylbutazone and shows the wide variation in the response to a standard dose of 100 mg/kg, the effect ranging on different occasions from 0% to 71% inhibition of the carrageenin oedema.

Figure 2 shows the mean percentage increases in paw volumes in control and phenylbutazone treated animals during the 2½ years. The results indicate that the observed variation in the percentage increase in paw volume in phenylbutazone treated animals was greater in extent and varied independently of the inflammatory response to carrageenin.

Experiments to investigate the relationships between dose, plasma concentration and anti-inflammatory response

The most striking feature of these experiments was the variability of the anti-inflammatory activity of phenylbutazone, indomethacin and aspirin. Comparison of the results obtained on the three separate occasions shows large differences in potency and, in many cases, no clear relationship between dose and anti-oedema effect (Fig. 3).

In contrast to the variability of the anti-oedema response, the concentrations of phenylbutazone, indomethacin and aspirin in plasma 4 h after dosing were similar

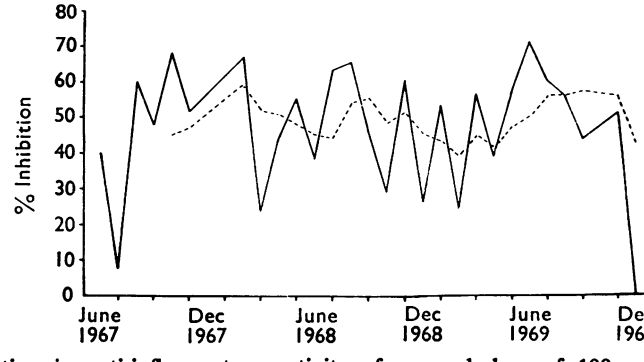

FIG. 1. Variation in anti-inflammatory activity of an oral dose of 100 mg/kg of phenylbutazone in experiments made at one-monthly intervals during a 2½ year period. Each point on the continuous line is the result of one experiment; each point on the broken line is the mean of experiments made during the 5 previous months.

whether the drugs were administered orally or intraperitoneally (Fig. 3) and there was good correlation between dose and plasma concentration ($r>0{\cdot}83$; $P<0{\cdot}01$ in each case). Table 1 lists group mean values selected from consecutive experiments which illustrate the wide variability occurring in the anti-oedema response to a fixed dose of the standard drugs despite the uniformity of the circulating plasma drug concentration.

In view of the similarity in plasma concentrations produced by oral or intraperitoneal administration, all the results obtained with each dose of drug by both routes of administration were pooled to give groups of thirty-six animals. Statistical evaluation of this pooled data showed a significant linear correlation of anti-oedema effect with log dose for each of the three drugs used.

Effect of variation in environmental temperature and humidity

There was a small but statistically significant increase in the inflammatory response to injected carrageenin of 0·27% of the initial paw volume per °C rise in temperature over the range 5–30° C. Over the same range, the inhibitory activity of a fixed dose of phenylbutazone increased by 2·5% for each °C rise (Fig. 4) and

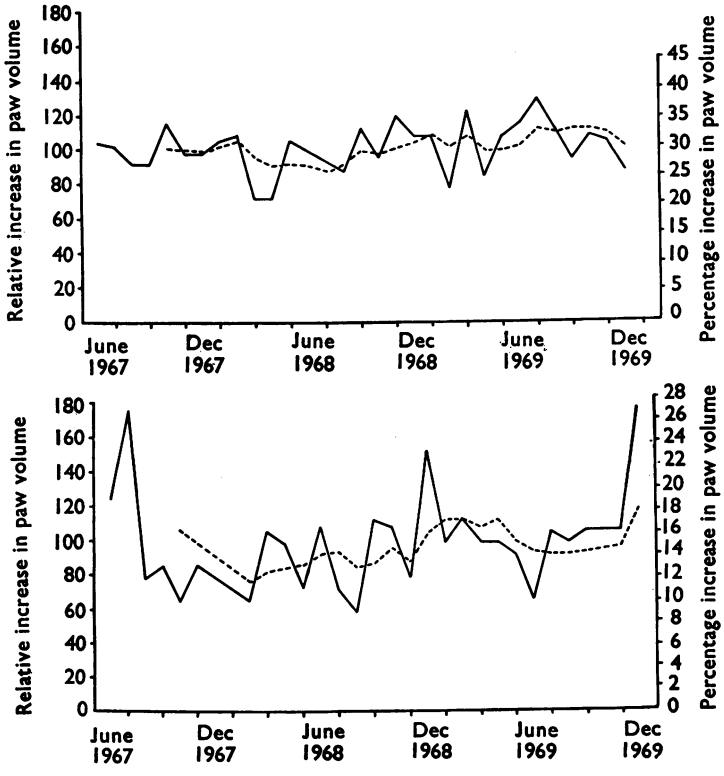

FIG. 2. Variation in paw volume increases in response to injected carrageenin in control (upper figure) and phenylbutazone treated (lower figure) groups during a 2½ year period. Each point on the continuous line is the result of one experiment; each point on the broken line is the mean of experiments made during the 5 previous months. The grand mean of the paw volume increases of all experiments was assigned an arbitrary value of 100. The left vertical scale shows percentage deviations of results about this mean. The right vertical scale shows the percentage increase in initial paw volume.

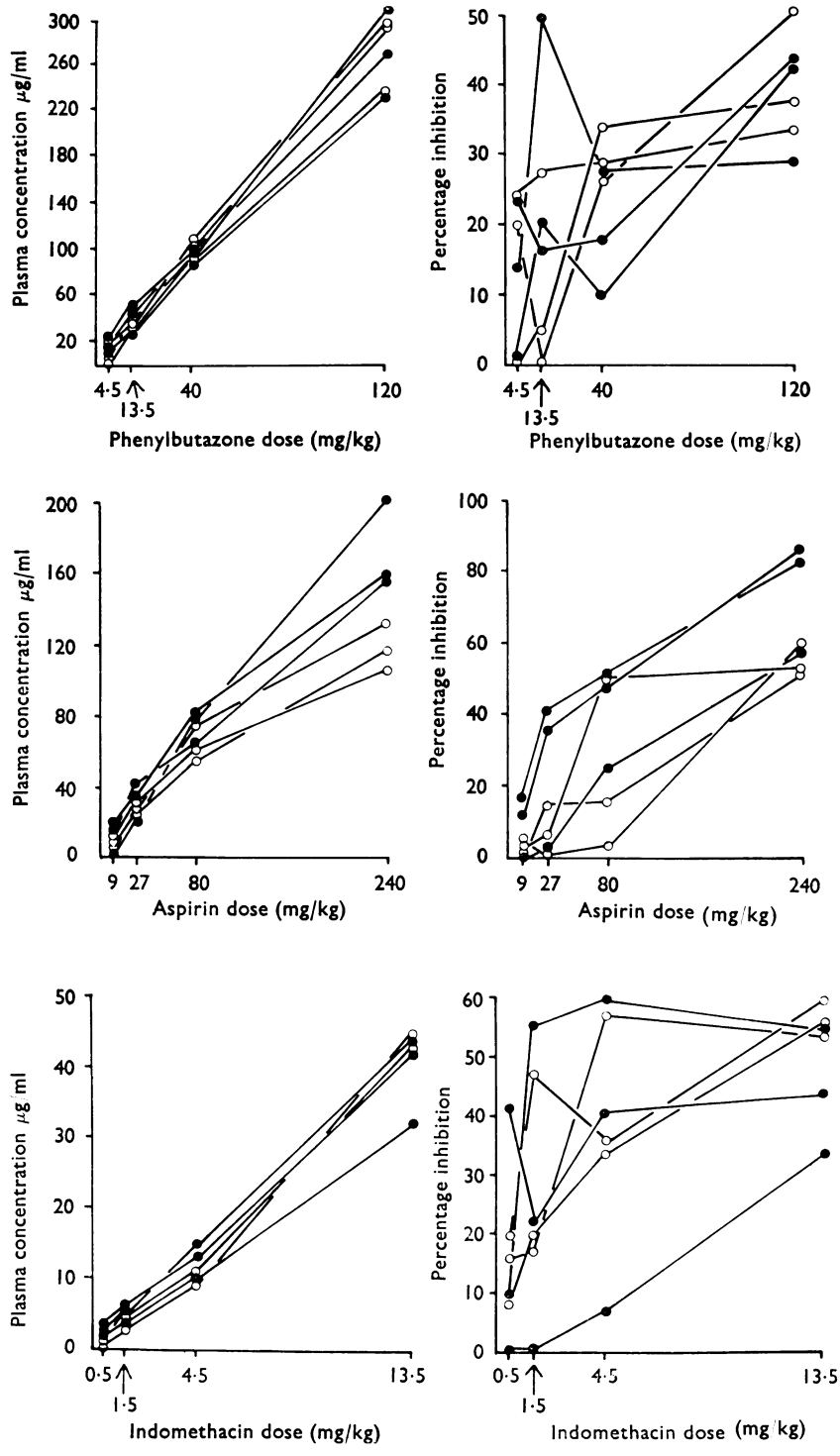

FIG. 3. Scatter diagrams showing the relationship between dose and anti-inflammatory effect, and between dose and plasma concentration for phenylbutazone, aspirin and indomethacin, after oral (○——○) and intraperitoneal (●——●) administration. Individual points represent the means of observations on six animals in three separate but identical experiments.

this increase showed a significant linear correlation with temperature ($r=0{\cdot}87$; $P<0{\cdot}01$). Analysis of the concentration of phenylbutazone in the inflamed tissue of the hind paws failed to show significant correlation between tissue concentration and antioedema effect ($r=0{\cdot}56$; $P>0{\cdot}05$) or between tissue concentration and temperature ($r=0{\cdot}50$; $P>0{\cdot}3$).

At a fixed temperature of 20° C no significant correlation was found between anti-oedema response and relative humidity over a range 40–90%.

Discussion

The response to injected carrageenin provides a convenient experimental model of inflammation which has been widely used for evaluating anti-inflammatory drugs. However, it is a common experience that inhibition of the carrageenin response by a fixed dose of anti-inflammatory drug and estimates of the relative potencies of different anti-inflammatory drugs are rather variable.

In an attempt to identify the source of the variability, data on the response to carrageenin in control and phenylbutazone treated animals were collected over 2½ years. Although there was some variability in the volume of oedema elicited by carrageenin in control animals, this was not related to the much greater variability in the anti-oedema response to a single dose of phenylbutazone.

TABLE 1. *Mean values selected from consecutive experiments illustrating the wide variability in the anti-oedema response to a fixed dose of the standard drugs despite relatively reproducible plasma drug concentrations*

Drug	Dose (mg/kg)	Plasma drug concentration (μg/ml)	% Inhibition
Phenylbutazone	120	279	51
	120	236	12
Indomethacin	4·5	14·8	60
	4·5	13·5	7
Aspirin	80	62·7	51
	80	63·6	25

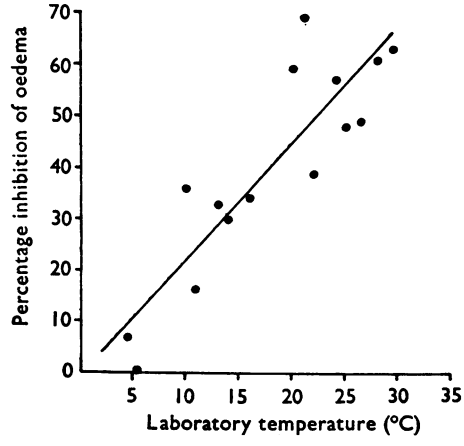

FIG. 4. Relationship between ambient temperature and anti-inflammatory activity of an oral dose of 100 mg/kg of phenylbutazone. The best-fit straight line shows a 2·5% increase in anti-inflammatory activity per °C rise in laboratory temperature.

Additional evidence on the variability of the anti-oedema response was obtained from a comparison of the dose-response relations of phenylbutazone, indomethacin and aspirin repeated on three separate occasions. With each drug, a statistical evaluation of the total pooled data from thirty-six animals at each dose showed a significant correlation of anti-oedema effect with log dose. However, the data obtained from the separate experiments using groups of six animals were, in most cases, too variable to establish a dose related anti-oedema effect either for the oral or intraperitoneal routes of administration. This finding agrees with the work of Walz, Di Martino, Griffin & Misher (1970) who recently reported that after oral administration of phenylbutazone, indomethacin, aspirin or hydrocortisone to groups of six rats, the dose-response curves and relative anti-oedema potencies of the drugs were not reproducible from day to day.

One possible explanation for the observed variation in the anti-oedema response after oral administration of phenylbutazone, indomethacin or aspirin is erratic or incomplete absorption of the drugs from the gastrointestinal tract. However, this explanation can be rejected in view of the finding that equivalent plasma concentrations were produced by oral or intraperitoneal administration of the three drugs.

The constancy of the plasma drug concentrations in conjunction with the reproducibility of the inflammatory response to carrageenin suggests that the observed variability in anti-oedema response is due to variability of some factor concerned with the development of the anti-inflammatory effect. One such factor could be a change in local circulation at the inflamed site consequent upon changes in the environmental conditions. Our experiments have shown that although changes in the relative humidity did not influence the response to phenylbutazone, there was a pronounced and highly significant increase in anti-inflammatory effect with increasing temperature. In an attempt to identify more precisely the mechanism of this effect, the amounts of phenylbutazone present in the inflamed tissues were measured 4 h after drug administration. Although it had seemed likely that the temperature related increase in anti-inflammatory effect might be associated with a difference in phenylbutazone levels, our findings showed that tissue concentrations were not correlated with anti-oedema effect.

For the precise evaluation of anti-inflammatory activity it is concluded that accurate control of temperature is essential and that rooms with locally variable temperature should be avoided. Provided that temperature is controlled, regulation of humidity is not important.

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