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Correlation between gastric irritancy and anti-inflammatory activity of non-steroidal anti-inflammatory drugs

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Gastric irritancies and anti-inflammatory potencies of 25 commercially available non-steroidal anti-inflammatory drugs (NSAIDs) have been measured in the rat. When irritancy is measured as the dose required to produce a specified level of gastric mucosal damage, it is found that irritancy increases with anti-inflammatory potency. However, when irritancy is measured as the level of gastric mucosal damage at the anti-inflammatory ED50 (which is a clinically realistic measure) then irritancy *decreases* as anti-inflammatory potency increases. Hence it should be possible to design high-potency, low-irritancy NSAIDs.

It has been postulated (Rainsford 1975) that the gastric irritation produced by non-steroidal anti-inflammatory drugs (NSAIDs) increases as their anti-inflammatory activity increases, and this may be due (Vane 1971) to both types of effect resulting from inhibition of prostaglandin biosynthesis. The existence of such a relationship may well account for the view that there is little point in searching for more potent NSAIDs as they are likely to be highly irritant to the gastric mucosa.

We would dispute this viewpoint, on the basis of the evidence presented in this paper. As part of a study of quantitative relationships between structure and activity of NSAIDs we have measured the gastric irritancy and anti-inflammatory activity of 25 such drugs.

Materials and methods

Materials. Drug samples were kindly donated by respective pharmaceutical companies, and were used as received.

Methods. Anti-inflammatory potency and gastric irritancy were determined in male Wistar rats, 200–250 g. The animals were fasted for 24 h before use, but had free access to water. They were dosed orally with an homogenized suspension of drug in water containing 1 drop of Tween 80 per 5 ml. Thirty minutes after dosing, 0·1 ml of carrageenan (1% in aqueous NaCl isotonic with body fluid) was injected into the right hind paw. Paw volume was measured (by mercury displacement) initially between 30 and 60 s after injection and finally at 4 h after injection.

Four dosage levels were used, with four animals at each level, and eight as controls. Each test was then duplicated.

After paw volume had been measured, each animal was killed by cervical dislocation, and the stomach lining examined for damage. A lesion index was obtained by visual assessment, according to the following scales: Sloughing of epithelium, slight reddening 0–10, localised areas of reddening 11–20, pin-point areas of bleeding 21–30, haemorrhagic lesions, larger areas of bleeding 31–50, profuse bleeding, general breakdown of epithelium 51–100+.

Since gastric irritancy is usually determined separately from anti-inflammatory activity, we also measured the gastric irritancy of six of the drugs in the absence of carrageenan challenge, so that the effect (if any) of the challenge on gastric irritancy could be assessed.

All correlations were calculated on meaned results.

Results and discussion

The anti-inflammatory potencies and gastric irritancies of the drugs are given in Table 1. The anti-inflammatory ED50 is the dose required to produce a 50% reduction in paw volume increase, relative to the control. We have used two indicators of gastric irritancy. The first (dose required to produce a lesion index of 10, or ID10) is similar to that used by Rainsford (1981), which was the dose required to produce 10 gastric mucosal lesions in stressed rats. We believe our index to be more sensitive and accurate since it takes into account the size and nature, as well as the number, of lesions. However, our test measures short-term irritancy, whereas that of Rainsford was designed to measure longer-term irritancy.

ID10 was used rather than, say, ID50 because many of the less irritant drugs did not produce high irritancy scores even at high dosages; in addition, it was felt desirable to use a score level that did not require the use of dosages greatly in excess of the effective anti-inflammatory dose.

In agreement with the findings of Rainsford (1981), we have observed that gastric irritancy increases with anti-inflammatory potency. For the 25 drugs examined, the relationship is:

$$\log(1/ID10) = 0{\cdot}739 \log(1/ED50) + 0{\cdot}185 \quad (1)$$

$$n = 25 \quad r = 0{\cdot}604 \quad s = 0{\cdot}638 \quad F_{1,23} = 13{\cdot}2$$

(probability = 0·0015)

where r is the correlation coefficient, s is the standard error and F is the variance ratio.

Rainsford's (1981) results give a similar correlation:

$$\log(1/ID10) = 0{\cdot}647 \log(1/ED35) - 0{\cdot}527 \quad (2)$$

$$n = 25 \quad r = 0{\cdot}602 \quad s = 0{\cdot}728 \quad F_{1,23} = 13{\cdot}2$$

(probability = 0·0015)

* Correspondence.

Table 1. Anti-inflammatory potencies and gastric irritancies of NSAIDs.

Drug	Anti-inflammatory ED50 (mmol kg^{-1})	Dose (mmol kg^{-1}) to produce lesion index of 10 (ID10)	Lesion index at anti-inflammatory ED50 (LI50)
	(numbers in brackets are 95% confidence limits*)		
Alclofenac	0·28 (0·21 –0·37)	0·29 (0·22 –0·34)	9·8 (7·2–11·2)
Aspirin	1·2 (0·94 –1·6)	0·46 (0·39 –0·61)	13·5 (11·3–20·2)
Azapropazone	0·23 (0·21 –0·39)	2·9 (1·6 –3·3)	6·3 (3·4– 7·0)
Benoxaprofen	0·0083 (0·0025–0·18)	0·11 (0·009 –0·30)	2·7 (0·2– 7·8)
Diclofenac	0·018 (0·014 –0·024)	0·0059 (0·0002–0·015)	14·5 (0·5–56·9)
Diflunisal	0·10 (0·069 –0·12)	0·17 (0·11 –0·19)	9·1 (6·0–10·2)
Fenbufen	0·052 (0·036 –0·075)	0·041 (0·023 –0·068)	11·0 (6·0–18·0)
Fenclofenac	0·19 (0·13 –0·23)	0·21 (0·19 –0·57)	9·6 (8·3–16·3)
Fenoprofen	0·33 (0·30 –0·45)	0·12 (0·079 –0·15)	15·2 (10·1–18·9)
Feprazone	0·040 (0·016 –0·050)	1·30 (0·45 –1·72)	4·3 (1·5– 5·7)
Flurbiprofen	0·013 (0·011 –0·016)	0·015 (0·0063–0·031)	16·6 (7·3–35·5)
Flufenamic acid	0·014 (0·011 –0·017)	0·071 (0·056 –0·100)	2·8 (2·2– 3·1)
Ibuprofen	0·13 (0·10 –0·16)	0·096 (0·053 –0·13)	12·6 (6·9–16·6)
Indomethacin	0·02 (0·018 –0·026)	0·039 (0·029 –0·055)	6·9 (6·6– 9·7)
Ketoprofen	0·020 (0·0047–0·025)	0·0059 (0·0027–0·0099)	18·2 (8·4–26·4)
Mefenamic acid	0·19 (0·13 –0·25)	0·19 (0·13 –0·25)	10·0 (9·7–13·1)
Monoflunisal	0·13 (0·11 –0·15)	0·65 (0·53 –0·73)	4·4 (3·5– 4·8)
Nabumetone	0·10 (0·028 –0·24)	0·55 (0·24 –0·70)	2·0 (0·7– 2·5)
Naproxen	0·012 (0·0087–0·016)	0·055 (0·044 –0·081)	2·3 (1·8– 3·4)
Oxyphenbutazone	0·15 (0·053 –0·18)	0·12 (0·11 –0·13)	10·5 (9·9–11·2)
Phenylbutazone	0·17 (0·13 –0·22)	0·32 (0·30 –0·43)	8·7 (4·7–11·5)
Piroxicam	0·0030 (0·0023–0·0042)	0·0075 (0·0069–0·0099)	4·8 (4·0– 6·4)
Sulindac	0·010 (0·0074–0·014)	0·019 (0·015 –0·020)	5·8 (3·4– 7·8)
Tolmetin	0·081 (0·058 –0·132)	0·082 (0·052 –0·14)	9·8 (6·4–16·2)
Zomepirac	0·32 (0·20 –0·51)	0·070 (0·04 –0·09)	15·9 (5·9–17·1)

* Calculated by the method of Snedecor & Cochran (1938).

Since Rainsford's parameters are different from ours, the constant terms in equations 1 and 2 are different; two compounds (meclofenamic acid and piroxicam) were omitted from the correlation analysis on Rainsford's results, since they were obvious outliers. Nineteen compounds were common to the two studies.

The correlation is significant at the 99·8% level, although the correlation coefficient is rather low. Considering the different chemical classes of compounds involved, this is perhaps not surprising for such a simple relationship.

Such a positive correlation between irritancy and anti-inflammatory potency tends to discourage the search for more potent NSAIDs. However, what is important clinically, so far as gastric irritancy is concerned, is not the dosage required to produce a certain level of irritancy, but rather the level of irritancy produced at the clinically effective dose. In terms of the present work, this translates to the lesion index found at the anti-inflammatory ED50, and this (termed LI50) is the second indicator of gastric irritancy shown in Table 1. In making this statement, we do not necessarily imply a strong correlation between carrageenan-induced rat paw oedema tests and clinical potency.

The correlation between LI50 and ED50 for all compounds is:

$$\log \mathrm{LI50} = -0{\cdot}194 \log (1/\mathrm{ED50}) + 1{\cdot}121 \quad (3)$$

$$n = 25 \quad r = 0{\cdot}429 \quad s = 0{\cdot}267 \quad F_{1,23} = 5{\cdot}2$$

(probability = 0·0324)

Four compounds in particular (diclofenac, flurbiprofen, ketoprofen and nabumetone) are outliers in the relationship, the first three being much more irritant, and the last much less irritant, than predicted by equation 3. We have sought, but failed to find, a common reason for these outliers. Their structures are quite dissimilar; only one of them (nabumetone) is a prodrug; only one (ketoprofen) has a steeper anti-inflammatory dose-response curve; and they show no unusual atomic charge distributions.

If these four outliers are omitted from the correlation, we then obtain, as depicted graphically in Fig. 1:

$$\log \mathrm{LI50} = -0{\cdot}303 \log (1/\mathrm{ED50}) + 1{\cdot}211 \quad (4)$$

$$n = 21 \quad r = 0{\cdot}771 \quad s = 0{\cdot}169 \quad F_{1,19} = 27{\cdot}9$$

(probability < 0·0001)

That is, in rats, as anti-inflammatory potency increases, gastric irritancy (as measured by LI50) decreases. Thus the viewpoint that increasing potency will increase irritancy is not necessarily correct, and it could be inferred that the search for more potent NSAIDs can continue with the possibility of unacceptably high gastric irritancy being less of an inhibiting factor.

The negative correlation shown by equation 4 also suggests that the mechanism by which gastric irritancy is caused may be different from the mechanism by which inflammation is inhibited by these drugs.

Since LI50 is calculated using both irritancy and

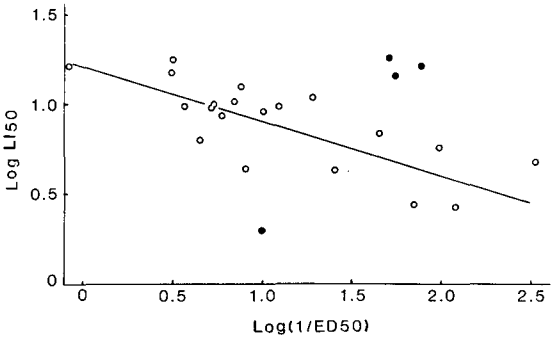

FIG. 1. Correlation of lesion index with anti-inflammatory potency. Compounds shown thus (●) were excluded from the correlation expressed by equation 4.

potency, it can be regarded as a type of therapeutic index. In fact, it correlates well with ID10/ED50, which is similar to the index devised by Boyle et al (1982) for NSAIDs. The relationship, excluding three compounds (azapropazone, feprazone and mefenamic acid) which are obvious outliers, is:

$$\log ID10/ED50 = -1{\cdot}636 \log LI50 + 1{\cdot}534 \quad (5)$$

$$n = 22 \quad r = 0{\cdot}924 \quad s = 0{\cdot}205 \quad F_{1,20} = 116{\cdot}5$$

(probability $< 0{\cdot}0001$)

Effect of carrageenan on gastric irritancy

Our reasons for assessing both anti-inflammatory activity and gastric irritancy in the same animals were two-fold: firstly, anti-inflammatory drugs are used clinically when inflammation is present, so that it is more realistic to assess irritancy following inflammatory challenge; secondly, it is more economical in the use of animals.

It is interesting to note, from Table 2, that the carrageenan challenge *reduces* gastric irritancy; the effect is quantified in equation 6:

$$\log LI50 \text{ (with carrageenan)} = 0{\cdot}867 \log LI50 \text{ (without carrageenan)} - 0{\cdot}103 \quad (6)$$

$$n = 6 \quad r = 0{\cdot}959 \quad s = 0{\cdot}104 \quad F_{1,4} = 46{\cdot}3$$

(probability = 0·0024)

Table 2. Effect of paw carrageenan challenge on gastric irritancy.

Drug	LI50 with carrageenan	LI50 without carrageenan
Aspirin	13·5	25·0
Fenclofenac	9·6	19·9
Feprazone	4·3	5·9
Flurbiprofen	16·6	25·6
Naproxen	2·3	3·3
Piroxicam	4·8	12·3

Thus the obtaining of two different biological activities from the same treatment of the same animals, thereby saving greatly on animal usage, is justified. A combined assay of ulcerogenic and anti-oedemic activity has previously been reported (Rainsford & Whitehouse 1977).

The reduction of gastric irritancy by carrageenan challenge in the paw is interesting. The effect may be an example of counter-irritation (Parente et al 1979) whereby the inflammatory reaction to the carrageenan generates endogenous anti-inflammatory materials that could reduce irritancy. Another possibility is that there is a lack of resources in the animal to mount limitless inflammatory response to challenge, so that the production of inflammation in the paw reduces the response to irritation elsewhere. Thirdly, increased prostaglandin production due to the carrageenan challenge could inhibit gastric acid secretion. These possibilities require further elucidation.

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