Biochemical Pharmacology, Vol. 34, No. 1, pp. 148–149, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

Salazosulfapyridine and non-steroidal antiinflammatory drugs do not inhibit soybean lipoxygenase

(*Received* 7 *March* 1984; *accepted* 30 *July* 1984)

Indomethacin [1] was found to inhibit selectively prostaglandin but not lipoxygenase pathway of arachidonic acid metabolism. Recently Sircar *et al.* [2] claimed that indomethacin and other anti-inflammatory drugs had inhibited soybean lipoxygenase. The same authors suggest [3] that inhibition of lipoxygenase pathway by salazosulfapyridine may explain the usefulness of this drug in ulcerative colitis. In these experiments linoleic acid was used as a substrate for soybean lipoxygenase and the increase of absorbtion at 234 nm was measured during oxidation.

We wanted to find out whether the difference in the influence of anti-inflammatory drugs on soybean lipoxygenase might be the result of various methods of estimation of the enzyme activity.

Soybean lipoxygenase (EC 1.13.1.13) activity was measured polarographically using arachidonic acid (100 μM) as a substrate [4]. In another set of experiments linoleic acid (100 μM) was used as a substrate and the composition of the reaction mixture was exactly the same as in Sircar's experiments [2]. It contains 2% of aethanol and 4.3% of propylene glycol. Soybean lipoxygenase concentration was 2.5 μg/ml. In both kinds of experiments samples were incubated for 3 min in an oxymeter chamber, then acidified to pH 3 and extracted twice with aethyl acetate. Organic layer was evaporated to dryness and analysed by HPLC method as described previously [5]. Retention time of 15-HETE obtained by incubation of arachidonic acid with soybean lipoxygenase was 19 min 30 sec as verified by comparison with standard. In the case when linoleic acid was used as a substrate the peak of retention time of 17 min 30 sec was taken as that corresponding to 13-hydroxyoctadecadienoic acid. We have no standard of this last compound. The following drugs were used: naproxen (Syntex U.S.A.), salazosulfapyridine (Salazopyrine–Pharmacia, France), mefenamic acid (Parke Davis, U.S.A.) BW 755C (Wellcome Research Laboratories, Beckenham, U.K.), paracetamol (Galena, Poland), indomethacin (metindol), ibuprofen, aspirin (polopirin), phenazon (antipirin), phenylbutazone (butapyrazol) (all from Polfa, Poland), soybean lipoxygenase 80,000 units/mg (Sigma, St. Louis, MO. U.S.A.), arachidonic acid (Sigma), linoleic acid (BDH, Poole, U.K.).

The comparison of IC_{50} values for inhibition of soybean lipoxygenase by anti-inflammatory drugs [2] with the influence of the same drugs on the soybean lipoxygenase activity in our experiments is shown in Table 1. From ten drugs tested only BW 755C inhibited the enzyme activity. Other drugs did not significantly change enzyme activity or even stimulate it.

Salazosulfapyridine and naproxen at a concentration of

Table 1. The comparison of the influence of some anti-inflammatory drugs on soybean lipoxygenase

Compound	IC_{50} from [2] (μM)	Concentration used in our experiments (μM)	Oxygen consumption by the sample (μmoles of oxygen/min/mg of enzyme)
None			3.4 ± 0.12
Indomethacin	87.5	100	3.6 ± 0.4
Ibuprofen	575	50	3.4 ± 0.3
		1000	4.5 ± 0.8
Aspirin	430	10	3.3 ± 0.3
		100	3.3 ± 0.4
		1000	3.3 ± 0.2
		10,000	3.6 ± 0.5
Phenazon	146	175	3.4 ± 0.4
Paracetamol	201	200	3.3 ± 0.2
		1000	2.9 ± 0.2
Naproxen	16	20	3.4 ± 0.05
		100	4.2 ± 0.5
Phenylbutazone	123	200	5.4 ± 0.3
Mefenamic acid	133	200	4.3 ± 0.3
Salazosulfapyridine	66.2	1	3.4 ± 0.3
		10	3.8 ± 0.3
		100	4.5 ± 0.6
		1000	4.6 ± 0.3
BW 755C	65	10	*2.4 ± 0.3
		30	*0.81 ± 0.2
		60	*0.75 ± 0.3

In our experiments soybean lipoxygenase was used at the concentration of 5 μg/ml, arachidonic acid at the final concentration of 100 μM in 0.1 M, phosphate buffer pH 7. Enzymic activity was measured polarographically. Results were analysed using Student's *t*-test. Control is the mean of 24 experiments, each result at least three experiments.

S.E. is shown in the table.

* Statistically significant ($P < 0.001$) decrease of enzymic activity as compared with control.

100 μM did not inhibit oxygen consumption at pH 9 when linoleic acid was used as a substrate (100 μM). Oxygen consumption by the control sample in the presence of linoleic acid was 4.8 ± 0.75 μmoles/min/mg of enzyme (N = 5). In the presence of 100 μM of naproxen it was 3.8 ± 0.57 (N = 3) and in the presence of 100 μM salazosulfapyridine 5.4 ± 1.6 μmoles/min/mg of enzyme (N = 4).

Salazosulfapyridine and naproxen did not inhibit 15-HETE formation from arachidonic acid as examined by the HPLC method. The high of the peak corresponding to 15-HETE in samples incubated with 100 μM of salazosulfapyridine was 192 ± 31% (N = 3) of control, and in samples containing 100 μM of naproxen, 120 ± 17% (N = 3) of control. Neither of these two compounds inhibited formation of the peak taken as 13-hydroxyoctadecadienoic acid when linoleic acid was the substrate for soybean lipoxygenase. In the presence of 100 μM of salazosulfapyridine the high of this peak was 125 ± 46% (N = 3) of control and in the presence of 100 μM of naproxen 117 ± 33% (N = 3) of control.

Our results are in agreement with those of Downing [1] who found out that anti-inflammatory drugs were relatively selective inhibitors of cyclooxygenase but not of lipoxygenase pathway of arachidonic acid metabolism. The K_m value calculated from our experiments for soybean lipoxygenase and arachidonic acid [4] was 60 μM. It is in the same range (25 μM) as previously described [6]. Also our IC_{50} value for BW 755C [7] was in the same range as in Sircar's experiments [2]. We cannot explain the reason for the discrepancy between the results of Sircar *et al.* [2, 3] and ours. Anti-inflammatory drugs used at higher concentrations than those used by Sircar [2, 3] did not influence soybean lipoxygenase activity at all. It is likely that the only difference between Sircar's and our experiments is the ratio of enzyme to substrate. Sircar [2] did not specify the amount of enzyme he had used. The amount which "gives measurable readings" in u.v. experiments may be different from the amount we used.

Department of Pharmacology
N. Copernicus Academy of Medicine
Grzegórzecka 16
Cracow
Poland

EWA MARCINKIEWICZ
ZOFIA DUNIEC
JADWIGA ROBAK

REFERENCES

1. D. T. Downing, *Prostaglandins* **1**, 437 (1972).
2. J. C. Sircar, C. F. Schwender and E. A. Johnson, *Prostaglandins* **25**, 393 (1983).
3. J. C. Sircar, C. F. Schwender and M. E. Carethers, *Biochem. Pharmac.* **32**, 170 (1983).
4. J. Robak and Z. Duniec, *Biochim. biophys. Acta* **620**, 59 (1980).
5. J. Swies, J. Robak, L. Dabrowski, Z. Duniec, Z. Michalska and R. J. Gryglewski, *Pol. J. Pharmac. Pharm.* **36**, 455 (1984).
6. M. J. Gibian and R. A. Galaway, *Biochemistry* **15**, 4209 (1976).
7. J. Robak and Z. Duniec, *Biochem. Pharmac.* **31**, 1955 (1982).