observed. At 24 months of age, the albumin synthesis increased, but the protein synthesis was decreased by the treatment so that their ratio increased. At 31 and 36 months of age, both albumin and protein synthesis were decreased. The decrease in albumin synthesis at 36 months between the control and treated rats was greater than the decrease in protein synthesis. Consequently, the ratio albumin vs protein synthesis by the hepatocytes of the 36-month-old treated rats is smaller than that of the control rats.

Since the BSP storage capacity of old hepatocytes was unchanged and the albumin synthesis was even decreased by meclofenoxate treatment, the conclusion can be drawn that meclofenoxate at the doses and times of treatment studied does not stimulate the functional capacities of old hepatocytes.

Protein-synthesizing activity was studied in hepatocytes isolated from rats of various ages with or without treatment with 80 mg meclofenoxate/day per kg body weight for 2 weeks. The substance did not influence protein synthesis in hepatocytes isolated from 3- and 12-month-old rats, but drastically decreased the synthesis of protein by hepatocytes isolated from 24-, 31- and 36-month-old rats. A comparable effect was observed for the liver-specific function of albumin synthesis. The absence of any stimulating effect of meclofenoxate treatment was also observed for another liver-specific function, viz. the BSP storage capacity. Therefore, the conclusion can be drawn that meclofenoxate at the doses and times of treatment studied does not stimulate the functional capacities of old hepatocytes.

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# Antioxidant properties of some chemicals vs their influence on cyclooxygenase and lipoxidase activities

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Stimulation of cyclooxygenase by a number of chemical compounds has been reported [1–6]. This stimulation is well expressed in the absence of hydroquinone as a cofactor in the enzymic preparation [3] and requires high concentration of the substrate [6]. In the presence of hydroquinone [3] and at low concentration of the substrate [6, 7] the stimulators may act as inhibitors of cyclooxygenase. A suggestion has been put forward that stimulators of cyclooxygenase are scavangers of free radicals [6]. Indeed, antioxidants are supposed to act as cofactors for cyclooxygenase [8]. Also aminopyrine—a stimulator of cyclooxygenase [3]—was reported to be an antioxidant [9].

In this study we compare antioxidant effects of several chemicals with their influence on cyclooxygenase activity.

The compounds used were paracetamol (Galena, Poland), chlorpromazine (Polfa, Poland), eicosa-tetraynoic acid (TYA) (Hoffman La Roche, Switzerland), caffeic acid (Fluka, Switzerland), phenylhydrazine (Poch, Poland), compound BW 755 (Wellcome Research Laboratories, U.K.), compound KD 785 (Chinoin Laboratories, Hungary), aspirin (Polfa, Poland) and indomethacin (Polfa, Poland).

The effect of investigated compounds on non-enzymic and enzymic lipid peroxidation was assessed according to the principles previously described [9]. Non-enzymic lipid peroxidation was assayed as the amount of malondialdehyde (MDA) that had been formed during incubation of boiled rat liver microsomes in the presence of ascorbic acid after subtraction of the amount of MDA formed during incubation of boiled microsomes without ascorbic acid. Enzymic lipid peroxidation was measured as the amount of MDA formed during incubation of lyophilized native rat liver microsomes in the presence of NADPH after subtraction of the amount of MDA that had been formed during incubation of boiled microsomes with NADPH. The incubation mixture contained 0.8 ml of microsomal suspension (equivalent to 1 mg of protein), 0.1 ml of ascorbic acid solution (non-enzymic lipid peroxidation) or NADPH (enzymic lipid peroxidation), and 0.1 ml of aqueous solution of a tested compound or 0.1 ml of water. The final concentration of both ascorbic acid and NADPH was 200 $\mu$M. Samples were incubated for 90 min (non-enzymic lipid peroxidation) or 60 min (enzymic lipid peroxidation) at 37°. The oxidation of lipids was stopped by the addition

of 0.5 ml of 20% trichloracetic acid. Samples were centrifuged at 10,000 *g* and the supernatant was boiled with 0.5 ml of 0.67% thiobarbituric acid solution for 20 min (pH of this mixture was 1). After cooling, samples were diluted with 1 ml of water and extinction was read at 535 nm against blank samples. Per cent of inhibition of lipid peroxidation by the investigated compounds was calculated. In the case of non-enzymic lipid peroxidation, several concentrations of investigated compounds were tested and the $IC_{50}$ from regression lines was calculated. In the case of enzymic lipid peroxidation, the investigated compounds were used at an approximate $IC_{100}$ calculated from the regression lines for inhibition of non-enzymic lipid peroxidation. Cyclooxygenase activity in ram seminal vesicle microsomes was assayed by a radiochemical method as described previously [6].

Table 1 presents a summary of data from the literature and from this paper on the influence of nine chosen compounds on cyclooxygenase activity at high and low concentrations of arachidonic acid, as well as their effect on lipoxidase activity. The results obtained in this paper for the effect of chlorpromazine, caffeic acid and paracetamol on cyclooxygenase activity at low concentration of the substrate are shown in Table 2.

To examine the influence of these compounds on non-enzymic and enzymic lipid peroxidation we had to investigate the concentration-dependent effect of the peroxidation inducers, namely ascorbic acid and NADPH. Ascorbic acid was an inducer of non-enzymic lipid peroxidation with maximum activity at a concentration of 400 $\mu$M. The amount of MDA (in nmole/mg protein) produced on the influence of 50, 100, 200, 400, 800 and 1600 $\mu$M of ascorbic acid was: $6.9 \pm 0.4$; $10.4 \pm 0.4$; $14.2 \pm 1.1$; $16.0 \pm 1.3$; $12.9 \pm 1.3$; $0.9 \pm 0.1$, respectively. Therefore, in further experiments ascorbic acid was used at a submaximal concentration of 200 $\mu$M in order to study the effect of investigated compounds on non-enzymic lipid peroxidation. This peroxidation was not influenced either by aspirin or by indomethacin at concentrations ten times higher than those inhibitory for cyclooxygenase (Table 1). On the other hand, BW 755C, KD 785, phenylhydrazine, caffeic acid, chlorpromazine, eicosa-tetraynoic acid and paracetamol inhibited non-enzymic lipid peroxidation and the slopes of regression lines were extremely steep (Fig. 1).

The enzymic lipid peroxidation was stimulated by NADPH at a concentration range 100–800 $\mu$M. Apart from phenylhydrazine and eicosa-tetraynoic acid, the other five investigated compounds nearly completely inhibited enzymic lipid peroxidation, when used in $IC_{100}$ concentration for non-enzymic lipid peroxidation. The detailed data for this inhibition were as follows: BW 755C 100% inhibition, KD 785—$81.2 \pm 8.5\%$, chlorpromazine—$93.0 \pm 4.0\%$, paracetamol—$99.7 \pm 2.3\%$, phenylhydrazine—$10.0 \pm 0.6\%$, eicosa-tetraynoic acid—$5.5 \pm 4.0\%$, cafeic acid—$97.0 \pm 3.0\%$.

Two classical inhibitors of cyclooxygenase (aspirin and indomethacin) had no antioxidant properties either against enzymic or non-enzymic lipid peroxidation. At moderate concentrations these compounds were found to be ineffective as inhibitors of lipoxidases (Table 1). The effect of aspirin on cyclooxygenase depends on acetylation of the active site of the enzyme [10]. Probably, also in the case of the indomethacin this effect is specific for cyclooxygenase and excludes inhibition of lipoxidases.

Two other tested compounds, namely phenylhydrazine and eicosa-tetraynoic acid, strongly inhibited cyclooxygenase as well as lipoxidase activities [11, 12]. The effect on both enzymes has been explained in the case of eicosa-tetraynoic acid by inhibition of the first step of arachidonic acid oxidation (removal of the $\omega$ 8-hydrogen), which is common for these two enzymes [12]. The antioxidant properties of phenylhydrazine and TYA were observed only in the case of non-enzymic lipid peroxidation. It seems that this kind of peroxidation is essential for inhibiting the first step of arachidonic acid oxidation.

Five of the tested compounds (BW 755C, KD 785, chlorpromazine, caffeic acid and paracetamol) stimulate cyclooxygenase at high and inhibit at low substrate concentrations. All these compounds, with the exception of paracetamol, are inhibitors of lipoxidases. Inhibition of both kinds of lipid peroxidation is the common feature of these compounds. Deby *et al.* [13] postulated that all antioxidants are free radical scavengers. On the basis of our results it seems that the compounds in question act as free radical scavengers and this is the reason for the stimulation of cyclooxygenase at high substrate concentration. We postulate that free radical scavengers facilitate initiation of cyclooxygenation at high substrate concentration and, then, are unable to prevent inactivation of the enzyme. This conclusion is drawn on the basis of our recent results. Stimulation of cyclooxygenase is strongest in the first seconds following addition of the substrate, and then a rapid decrease in reaction velocity occurs.

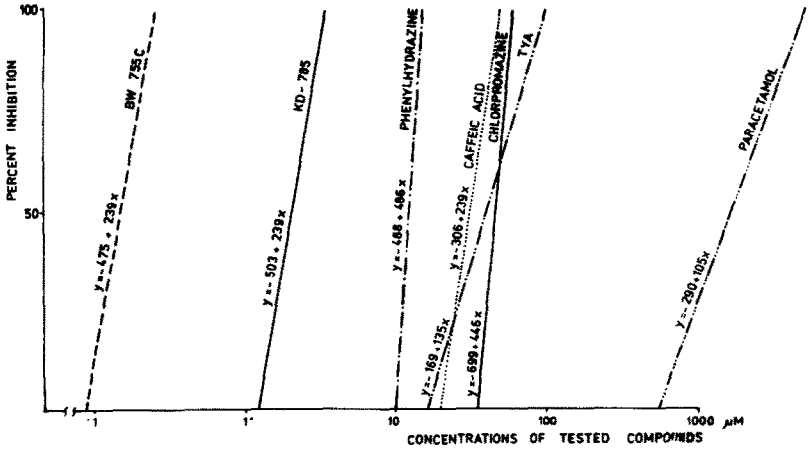


Fig. 1. Regression lines for inhibition of non-enzymic lipid peroxidation in rat liver microsomes. Boiled rat liver microsomes (equivalent to 1 mg protein) were incubated for 90 min at 37° with 200 $\mu$M ascorbic acid. The amount of malondialdehyde was then estimated using the thiobarbituric acid method. The number of experiments for each compound was 6–21.

Table 1. The influence of tested compounds on cyclooxygenase and lipoxidase activities

| Compound | Influence on enzymic activity: Cyclooxygenase, 100 μM AA: Conc. (μM) | Effect (%) | Ref. | Cyclooxygenase, 1.6 μM AA: Conc. (μM) | Effect (%) | Ref. | Lipoxidases, Soybean: Conc. (μM) | Effect (%) | Ref. | Platelets: Conc. (μM) | Effect (%) | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BW 755C | 30 | +150 | 6 | 30 | −35 | 6 | 11 | −50 | 6 | 1.7 | −50 | 6 |
| KD 785 | 300 | +200 | 6 | NT | — | — | 555 | −50 | 6 | 1510 | −50 | 6 |
| Caffeic acid | 1000 | +400 | 5 | 1000 | −5 | * | 1000 | −34 | 5 | 1000 | −57 | 5 |
| Chlorpromazine | 1000 | +100 | 4 | 1000 | −87 | * | 1000 | −90 | 13 | 1000 | −53 | 13 |
| Paracetamol | 1000 | +50 | 3 | 1000 | −41 | * | 1000 | 0 | 14 | 1000 | 0 | 14 |
| Phenylhydrazine | 1.3 | −50 | 10 | NT | — | — | 0.3 | −50 | 10 | 0.04 | −50 | 10 |
| Eicosa-tetraynoic acid | 1.0 | −50 | 11 | NT | — | — | 1 | −28 | † | 1 | −94 | † |
| Aspirin | 1000 | −50 | † | NT | — | — | 1000 | 0 | † | 1000 | −15 | † |
| Indomethacin | 10 | −90 | † | NT | — | — | 10 | 0 | † | 10 | 0 | † |

* See text.
† Unpublished data.
AA = arachidonic acid; NT = not tested.

Table 2. Radiochemical studies on the influence of 1000 μM of chlorpromazine, caffeic acid and paracetamol on arachidonic acid metabolism in the presence of 1.6 μM of the substrate

| Compound | *n* | Per cent radioactivity determined as: 6-oxo $PGF_{1\alpha}$ | PGF 2α | $PGE_2$ | $PGD_2$ | AA |
|---|---|---|---|---|---|---|
| None | 6 | 6.5 ± 1.6 | 18.5 ± 3.6 | 18.8 ± 2.9 | 9.6 ± 1.0 | 12.8 ± 0.9 |
| Chlorpromazine | 3 | 0.88 ± 0.16* | 0.99 ± 0.16* | 2.36 ± 0.31† | 1.32 ± 0.17* | 88.7 ± 1.65† |
| Caffeic acid | 3 | 11.8 ± 0.9‡ | 29.0 ± 2.6 | 14.6 ± 5.2 | 4.1 ± 0.2* | 17.5 ± 6.7 |
| Paracetamol | 3 | 5.7 ± 2.2 | 18.0 ± 4.6 | 8.8 ± 1.0‡ | 4.1 ± 1.0* | 48.3 ± 7.9† |

* $0.01 > P > 0.001$.
† $P < 0.001$.
‡ $0.05 > P > 0.01$.

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