Biochemical Pharmacology, Vol. 29, pp. 3169–3173.

0006-2952/80/1201-3169 $02.00/0

INHIBITION OF FATTY ACID OXYGENASES BY ONION AND GARLIC OILS

EVIDENCE FOR THE MECHANISM BY WHICH THESE OILS INHIBIT PLATELET AGGREGATION

JACK Y. VANDERHOEK,* AMAR N. MAKHEJA and J. MARTYN BAILEY

Department of Biochemistry, George Washington University School of Medicine, Washington, DC 20037, U.S.A.

(*Received* 27 *March* 1980; *accepted* 3 *June* 1980)

Abstract—Inhibition of *in vitro* platelet aggregation by onion and garlic oils was accompanied by decreased formation of thromboxane B_2 (TXB_2) and 12-hydroxyheptadecatrienoic acid (HHT) from [1-^{14}C]arachidonic acid (AA). At intermediate concentrations (10–30 μg/ml), these oils also induced a redistribution of the products of the lipoxygenase pathway; at higher concentrations (30–60 μg/ml), they completely suppressed the formation of all oxygenase products. Measurements of oxygen consumption in antimycin-treated platelets indicated a direct correlation between inhibition of platelet fatty acid oxygenases and the anti-aggregating activity of these oils. Onion and garlic oils also inhibited fatty acid oxygenases from sheep vesicular gland preparations as shown both by decreased oxygen consumption and decreased formation of prostaglandin E_2 (PGE_2) and PGD_2 from [1-^{14}C]AA. Onion oil was approximately ten times more effective in inhibiting the platelet oxygenases than the oxygenases from the vesicular gland. In addition, these oils suppressed the soybean lipoxygenase-catalyzed oxygenation of AA. In all three enzyme systems, onion oil exhibited greater inhibitory activity than garlic oil. Many nonsteroidal anti-inflammatory and anti-thrombotic drugs act by inhibiting the cyclooxygenase component of the prostaglandin and thromboxane synthetases. Our findings thus suggest that onion and garlic contain compounds that may have serendipitous pharmacological effects.

Arachidonic acid (AA) is metabolized in platelets by two fatty acid oxygenases—a cyclooxygenase that catalyzes the formation of prostaglandin endoperoxides and a lipoxygenase that converts AA to 12-hydroperoxyeicosatetraenoic acid [1]. The prostaglandin (PG) endoperoxides are the precursors of thromboxane A_2 (TXA_2), a powerful inducer of platelet aggregation [2]. Interference with the cyclooxygenase reduces the formation of the endoperoxides and their subsequent metabolites. The inhibition of platelet aggregation by aspirin and indomethacin is presumably due to inhibition of TXA_2 biosynthesis, and these anti-aggregating agents have been shown to directly inhibit the cyclooxygenase enzyme [1]. Various workers have demonstrated that onion and garlic decrease platelet aggregation [3, 4]. We recently reported that nonpolar chromatographic extracts of onion inhibited platelet aggregation and thromboxane synthesis [5]. The results reported here demonstrate that onion and garlic oils inhibit the fatty acid oxygenases from platelets as well as those from sheep seminal vesicles and soybeans.

MATERIALS AND METHODS

Garlic oil was a gift from J. Manheimer & Co., New York, NY, and onion oil was supplied by the Polarome Corp., New York, NY. Sheep vesicular gland acetone powder was a gift from Dr. Don Wallach, The Upjohn Co., Kalamazoo, MI. Soybean lipoxygenase (165,000 units/mg) and antimycin A were obtained from the Sigma Chemical Co. (St. Louis, MO). Unlabeled AA was purchased from Nuchek Preps (Elysian, MN), and [1-^{14}C]AA was obtained from the Amersham Co. (Arlington Heights, IL). PGD_2, PGE_2, $PGF_{2\alpha}$ and TXB_2 were supplied by Dr. John Pike, The Upjohn Co. Precoated silica gel G thin-layer chromatography (t.l.c.) plates (Analtech) were purchased from the Fisher Scientific Co. (Pittsburgh, PA).

Blood, from healthy volunteers who had not received any medication known to affect platelet aggregation, was prevented from coagulating by 3.8 % sodium citrate (9:1, v/v). Platelet-rich plasma (PRP) was isolated by centrifugation for 15 min at 110 *g* at room temperature. Aggregation studies were carried out in a Sienco Dual Sample aggregometer (model DP-274E) attached to a double channel recorder. After preincubating PRP (0.3 ml) with various concentrations of onion or garlic oil (in ethanol) for 1 min at 37°, ADP (8 μM final concentration) was added to induce aggregation and the aggregation response was followed for 4 min. Thromboxane synthesis studies were carried out as described previously [5].

Oxygen consumption measurements were monitored using a Yellow Springs model 53 oxygen meter (Yellow Springs, OH) coupled to a Linear Instruments Corp. model 285 recorder (Irvine, CA). Platelet oxygenase determinations were carried out in

* Author to whom all correspondence should be addressed: Dr. J. Y. Vanderhoek, Department of Biochemistry, George Washington University, School of Medicine, 2300 Eye St., N.W., Washington, DC 20037, U.S.A.

a reaction vessel containing 3–4 mg of washed platelet suspension protein and 1.8 μM antimycin A (to block basal respiration [6]) in 2 ml of medium containing 137 mM NaCl, 2.7 mM KCl, 11 mM dextrose and 25 mM Tris–HCl (pH 7.6) at 30°. Various concentrations of onion or garlic oils in ethanol were added, and after 1 min, 36–43 μM AA (final concentration) was added to initiate the reaction. Initial velocities of oxygen consumption were determined and compared with controls in which ethanol was used instead of onion (or garlic) oils to calculate the inhibitory effectiveness of these oils. The effects of garlic and onion oils on sheep vesicular gland fatty acid oxygenase (2.5–3.5 mg) and soybean lipoxygenase (5 μg) activities were determined in 0.1 M Tris–HCl (pH 8.5) (0.67 mM phenol [7], although the phenol was deleted in the soybean measurements).

Using [1-^{14}C]AA diluted with unlabeled AA as substrate, radioactive AA metabolites were isolated from sheep vesicular gland reaction mixtures as follows. After a 10-min incubation, the reaction was stopped by the addition of 0.1 ml of 10% formic acid and was extracted with 10 ml chloroform–methanol (2:1). The mixture was vortexed and centrifuged, and the chloroform layer was separated. Authentic PGD_2, PGE_2 and $PGF_{2\alpha}$ standards were added to the chloroform solution, and the solvent was evaporated under N_2. The residues were redissolved in a small amount of chloroform, applied to silica gel G t.l.c. plates, and developed in the organic phase of ethyl acetate–isooctane–acetic acid–water (11:5:2:10, by vol.) [8]. Radioactive zones were detected by autoradiography as described previously [9], identified by cochromatography with authentic standard, scraped off, and counted by a liquid scintillation counter.

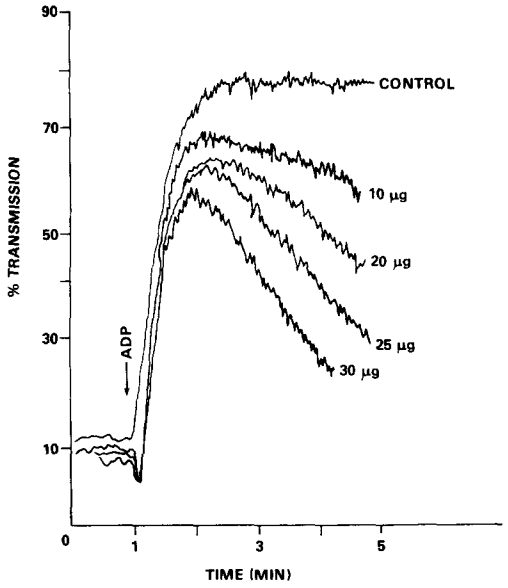

Fig. 1. Inhibition of human platelet aggregation by garlic oil. Various concentrations of garlic oil were incubated with 0.3 ml of PRP for 1 min at 37°. ADP was then added and the subsequent aggregation was followed for 4 min. The control experiment contained ethanol (1 μl) only.

RESULTS AND DISCUSSION

Garlic and onion are both members of the plant genus *Allium*. Previous studies have shown that onion chromatographic extracts exhibit anti-aggregatory activity [5]. Garlic oil, obtained by steam distillation, similarly inhibited platelet aggregation in a dose-dependent manner (Fig. 1). In addition,

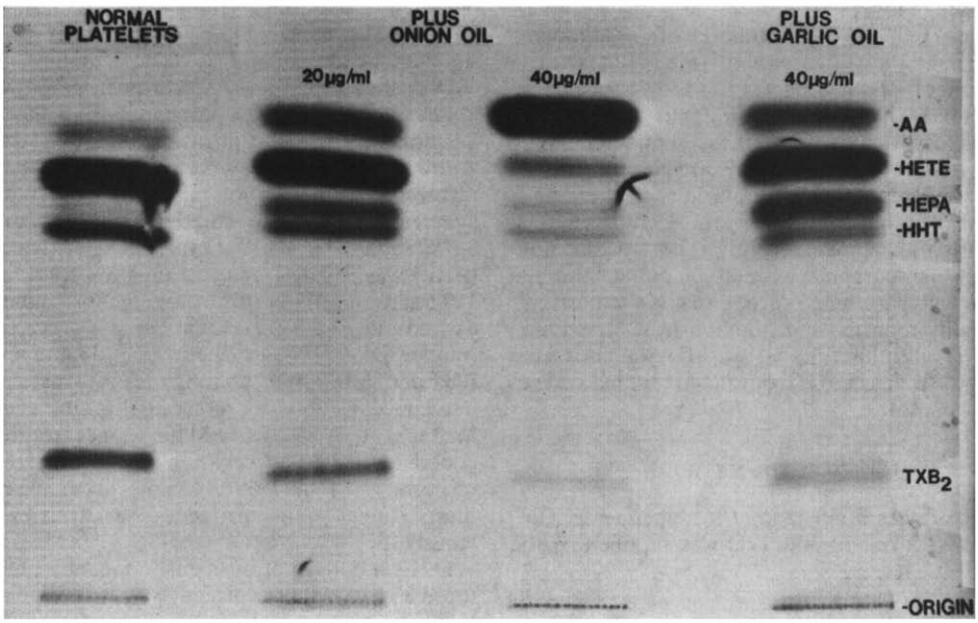

Fig. 2. Altered [1-^{14}C]arachidonic acid metabolism in human platelets treated with onion and garlic oils. Autoradiographs were obtained from the experiment described in Table 1.

Table 1. Altered [1-^{14}C]arachidonic acid metabolism in human platelets treated with onion and garlic oils*

Radioactive products	Radioactivity distribution (% of total dpm)			
	Control	Onion oil (20 μg/ml)	Onion oil (40 μg/ml)	Garlic oil (40 μg/ml)
TXB_2	12.9	6.9	2.2	5.7
HHT	12.9	8.9	2.3	7.1
HETE	56.0	55.0	4.7	45.0
HEPA	6.4†	10.6	1.9	21.4
AA (unreacted)	10.6	17.3	88.5	19.3

* Washed and resuspended human platelets (1 ml) were preincubated with ethanol or ethanolic solutions of onion or garlic oils for 2 min at 37° before the addition of [1-^{14}C]AA. The formation of radioactive metabolites was measured after 5 min as described previously [5]. The values are the percentage of total radioactivity recovered from a typical experiment.

† This value may be unusually high due to contamination with HETE, since the autoradiographs (Fig. 2) show no significant amounts of HEPA in control platelets.

onion oil, also obtained by steam distillation, inhibited platelet aggregation (I_{50} per ml PRP = 64 ± 5 μg), and the inhibitory potencies of garlic oil and onion oil were found to be the same when tested on the same platelet preparation. At levels of ADP (8 μM) that induce irreversible second phase aggregation, 55 μg of onion oil resulted in an aggregation profile similar to the monophasic reversible aggregation observed with 2.8 μM ADP. Increasing concentrations of onion oil progressively decreased the monophasic reversible response, indicating that this material can block first phase aggregation, as do certain other cyclooxygenase inhibitors. One unit of anti-aggregatory activity is defined as the amount of onion extract required to obtain 50 per cent inhibition of ADP-induced platelet aggregation in 1 ml of PRP. A typical sample of onions was found to contain 36,000 units of activity per kilogram. Of this activity, 14,000 units was attributable to material present in the oily fraction and extractable by non-polar organic solvents. (The remaining activity in the aqueous phase was due largely to adenosine and certain other water-soluble constituents.) Assuming complete absorption by blood (5000 ml), this would suggest that ingestion of about 360 g of onion could result in 50 per cent inhibition of platelet aggregation in plasma due to this oily fraction.

Because onion chromatographic extracts inhibited the conversion of [1-^{14}C]AA to TXB_2, the stable metabolite of TXA_2, in platelets [5], the effect of onion and garlic oils on platelet AA metabolism was investigated. Figure 2 and Table 1 show that the course of platelet AA metabolism depends on the concentration of the onion (or garlic) oil used. At intermediate concentrations (10–30 μg), onion oil inhibited the formation of the cyclooxygenase pathway products TXB_2 and 12-hydroxyheptadecatrienoic acid (HHT) by about 40–50 per cent and induced a redistribution of the lipoxygenase pathway products, including the accumulation of HEPA, an epoxy–hydroxy derivative of AA [10]. At higher concentrations (30–60 μg), onion oil further inhibited the production of TXB_2 and HHT and strongly

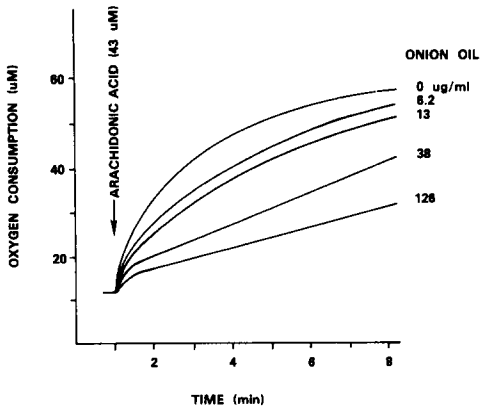

Fig. 3. Inhibition of arachidonic acid oxygenation in human platelets by onion oil. A washed platelet suspension (4 mg) in 2 ml of Tris–physiological saline buffer (pH 7.6) containing antimycin A (1.8 μM) and dextrose (11 mM) was preincubated for 1 min with different concentrations of ethanolic solutions of onion oil. Arachidonic acid (43 μM final concentration) was added and oxygen consumption was measured as described under Materials and Methods.

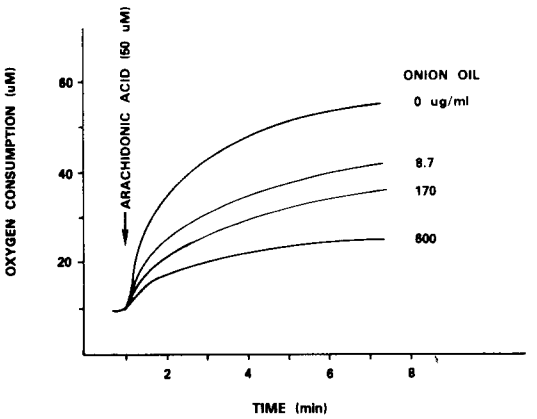

Fig. 4. Inhibition of arachidonic acid oxygenation in a sheep vesicular gland preparation by onion oil. The procedure followed was similar to that described in the legend of Table 2.

Table 2. Inhibition of prostaglandin synthetase from sheep vesicular gland by onion and garlic oils*

Radioactive products	Radioactivity distribution (% of total dpm)			
	Control	Onion oil (170 μg/ml)	Onion oil (510 μg/ml)	Garlic oil (510 μg/ml)
PGE_2	28.2	13.3	4.3	20.3
PGD_2	8.1	3.8	2.5	5.5
AA (unreacted)	40.4	69.7	83.0	59.1

* Phenol activated sheep vesicular gland acetone powder (3 mg) in 3 ml of 0.1 M Tris (pH 8.5) buffer containing 0.67 mM phenol was preincubated with ethanol (10 μl, control) or ethanolic solutions of onion or garlic oils for 1 min at 30° before the addition of [1-^{14}C]AA diluted with unlabeled AA (44 μM, final concentration). After 10 min, the reactions were stopped, worked up, and the formation of radioactive metabolites was measured as described under Materials and Methods. The values are the percentage of total radioactivity recovered from a typical experiment.

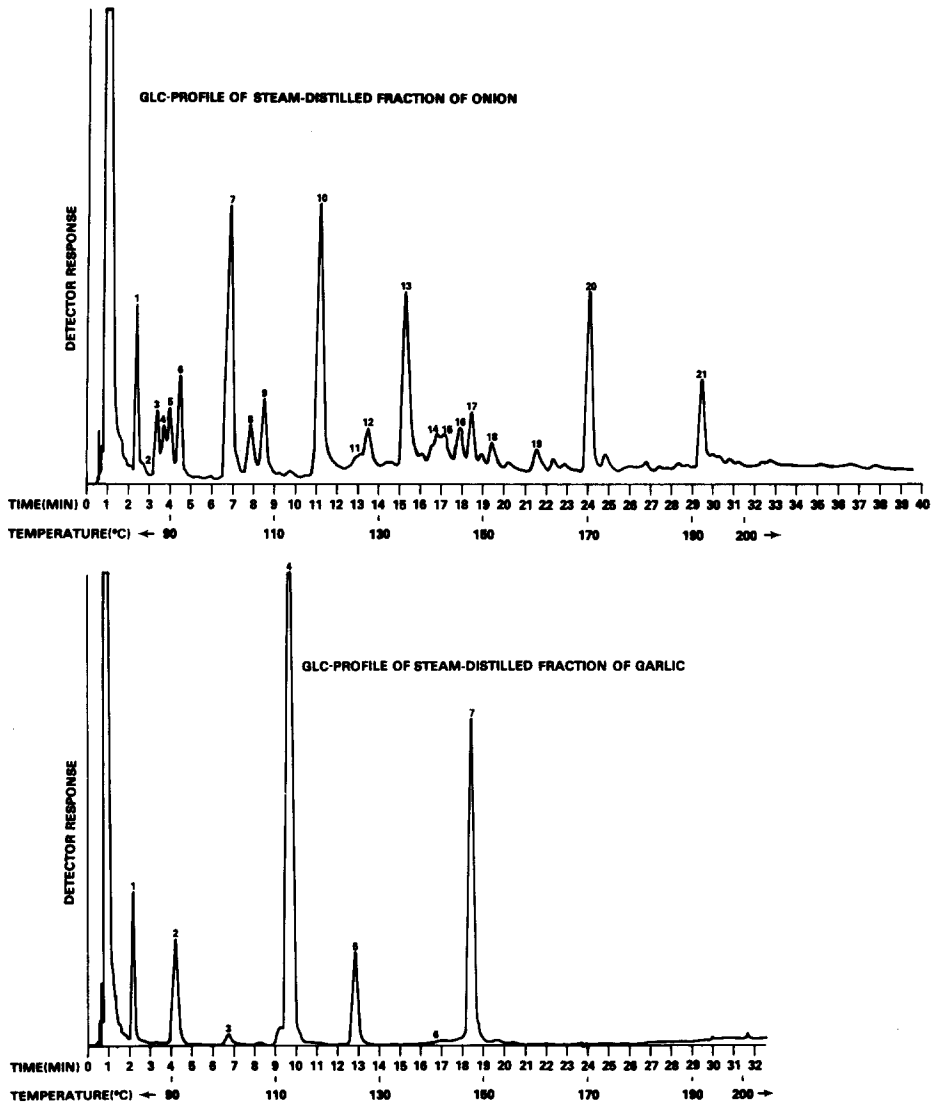

Fig. 5. Gas–liquid chromatographic profiles of onion oil and garlic oil. The glass column was packed with 10 % SP2340 on 100/120 mesh Supelcoport (Supelco, Inc., Bellefonte, PA). The helium flow rate was 75 ml/min. The structural identification of the numbered, individual components is under investigation.

Table 3. Inhibition of fatty acid oxygenases from various sources by onion and garlic oils*

Source	I_{50} (μg/ml) Onion oil	Garlic oil
Human platelets	22 ± 8	57 ± 2
Sheep seminal vesicles	200 ± 28	†
Soybean lipoxygenase	105 ± 4	156 ± 22

* Oxygenase activity was determined using oxygen consumption measurements as described under Materials and Methods. The inhibitory activity (I_{50}) is defined as the weight of oil required to inhibit the rate of oxygen consumption by 50 per cent per ml of assay medium. Each value is the mean ± S.E.M. of at least two determinations.

† The maximum attainable inhibition by garlic oil was only 40–50 per cent.

inhibited the formation of 12-hydroxyeicosatetraenoic acid (HETE) and HEPA. (We have observed that individual platelet samples differ considerably in their sensitivities to onion and garlic oils.) Table 1 also indicates that, on a weight basis, onion oil is a more effective inhibitor than garlic oil. Both oils, however, are less inhibitory than indomethacin (results not shown). It appears that onion (or garlic) oil and indomethacin influence AA metabolism quite differently because indomethacin only inhibits the cyclooxygenase enzyme [1], whereas the onion oil also affects the lipoxygenase pathway, causing either a product redistribution or product inhibition depending on the concentration of the onion oil used.

To determine which specific enzyme(s) of the AA cascade is inhibited by onion and garlic oils, we focused on the platelet fatty acid oxygenases since the cyclooxygenase and the lipoxygenase are the first enzymes involved in platelet AA metabolism [1]. Onion oil decreased both the rate and amount of oxygen consumed upon the addition of AA to a platelet suspension, as shown in Fig. 3. Similar results were obtained with garlic oil. These observations indicate that both onion and garlic oils directly inhibited the platelet fatty acid oxygenases and, thus, can account for the observed decrease in thromboxane synthesis that correlates with the anti-aggregatory activities of these oils.

To assess whether these *Allium* oils could also inhibit fatty acid oxygenases from other sources, we tested their effects on sheep seminal vesicles, a rich enzymatic source of prostaglandins [11]. Increasing amounts of onion oil progressively inhibited oxygen consumption (both rate and extent) of a sheep seminal vesicle preparation upon the addition of AA (Fig. 4), and this inhibition of the fatty acid oxygenase was further confirmed by decreased formation of PGE_2 and PGD_2 from [1-^{14}C]AA in the presence of onion oil (Table 2). We also observed that the sheep seminal vesicle preparation was much less sensitive to garlic oil; the maximum attainable inhibition of the oxygenase was only 40–50 per cent.

Because the above results were obtained with fatty acid oxygenases from animal sources, experiments were also carried out using a plant lipoxygenase. Both onion and garlic oils inhibited soybean lipoxygenase catalyzed oxygenation of AA although garlic oil was less potent. The inhibitory activities of onion and garlic oils on the fatty acid oxygenases from human platelets, sheep seminal vesicles, and soybean are summarized in Table 3. It is noteworthy that onion oil was five to ten times more effective in inhibiting the platelet oxygenases than either the sheep vesicular gland oxygenases or the soybean lipoxygenase. The different activities of onion and garlic oils probably relate to their different compositions [12] as determined by gas–liquid chromatography (Fig. 5).

A currently accepted mechanism of action of many nonsteroidal anti-inflammatory drugs is their interference with prostaglandin biosynthesis via their inhibition of the initial cyclooxygenase enzyme [13]. This mechanism can also explain the anti-aggregatory effects of certain antithrombotic drugs (e.g. aspirin, indomethacin) that decrease thromboxane formation [1]. The effectiveness of onion and garlic oils in blocking thromboxane and prostaglandin biosynthesis through their inhibition of the fatty acid oxygenases strongly suggests that their observed anti-aggregatory activities *in vitro* and possibly also *in vivo* [14] are related to this property.

Acknowledgement—The authors wish to thank Dr. C. E. Low for carrying out the gas chromatographic analyses.

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