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PRELIMINARY COMMUNICATION

FOOT-EDEMA INDUCED BY CARBONYL COMPOUNDS ORIGINATING FROM THE PEROXIDATION OF LIVER MICROSOMAL LIPIDS

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The possibility that peroxidized lipids, or final products of lipid peroxidation, play a role in the inflammatory process has been suggested by several authors (1-4). Lipoperoxides are formed in the pathway leading to the production of prostaglandins. Prostaglandins from arachidonic acid and peroxidized arachidonic acid were found to cause platelet aggregation (5). Evidence has been presented according to which oxygen radicals are involved in the production of foot-edema in rats (6). Also it has been demonstrated (1) that superoxide anions are involved in the production of carrageenan foot-edema. It has been suggested (1) that superoxide anions formed by macrophages may interact with membrane arachidonic acid to produce prostaglandins or lipid peroxides which may increase vascular permeability. The role of superoxide anions in the initiation of lipid peroxidation has been demonstrated (7).

Previous studies from our laboratory (8-10) showed that during the course of the peroxidation of rat liver microsomal lipids products are formed which have the capacity of inducing cytopathological effects. The cytotoxic products are dialysable and can be recovered in extracts (10) obtained from the dialysate. They have the capacity of damaging cellular membranes (hemolytic effect) and of impairing the activity of membrane bound enzymes (inhibition of microsomal enzymes). A partial separation of the products present in the dialysate extract was obtained (10) by thin layer chromatography (TLC). When the lipid materials eluted from the various chromatographic bands were tested for their toxicological activity, it was found that the highest toxicological activity occurs in a well resolved band which contains most of the carbonyl functional groups detectable in the unfractionated dialysate extract (10). Studies concerned with the molecular characterization of the products contained in this chromatographic band showed (11) that these products consist of 4-hydroxyalkenals, almost entirely of 4-hydroxy trans 2,3 nonen-1-al (with minor amounts of 4-hydroxyoctenal, 4-hydroxydecenal and 4-hydroxyundecenal).

That a number of aldehydes originate from the peroxidation of unsaturated fatty acids has been known for a long time (12). Aldehydes have been shown to produce many biological effects (13). Recently it has been suggested that aldehydes are produced by inflammatory cells during phagocytosis (14). It seemed therefore of interest to investigate whether the above referred carbonyl compounds originating from the peroxidation of liver microsomal lipids exhibit an

inflammatory activity. Preliminary studies demonstrating that these carbonyl compounds are capable of inducing foot-edema in the rat, are reported in this communication.

The carbonyl compounds tested for their capacity to induce foot-edema were prepared as described in a previous paper (10). The carbonyl compounds [300 or 150 nmoles, determined as previously done (10)] were resuspended in 0.05 ml of sterile saline containing 0.0005% (w/v) Tween 80 and injected into the plantar surface of the hind paw of the rat (male Sprague-Dawley rats, 150-160 g, Nossan, Correzzana, Milan, Italy). The controlateral paw (left) was injected with the proper control sample [i.e. the sample obtained from the TLC plate to which the dialysate extract derived from the control flask (plus microsomes, minus NADPH) was applied (see ref. 3)]. Immediately before and after the injection (0 time), the volume of the foot was measured with a volume differential meter apparatus (U. Basile, Milan, Italy). The volume was then measured at various times after the injection.

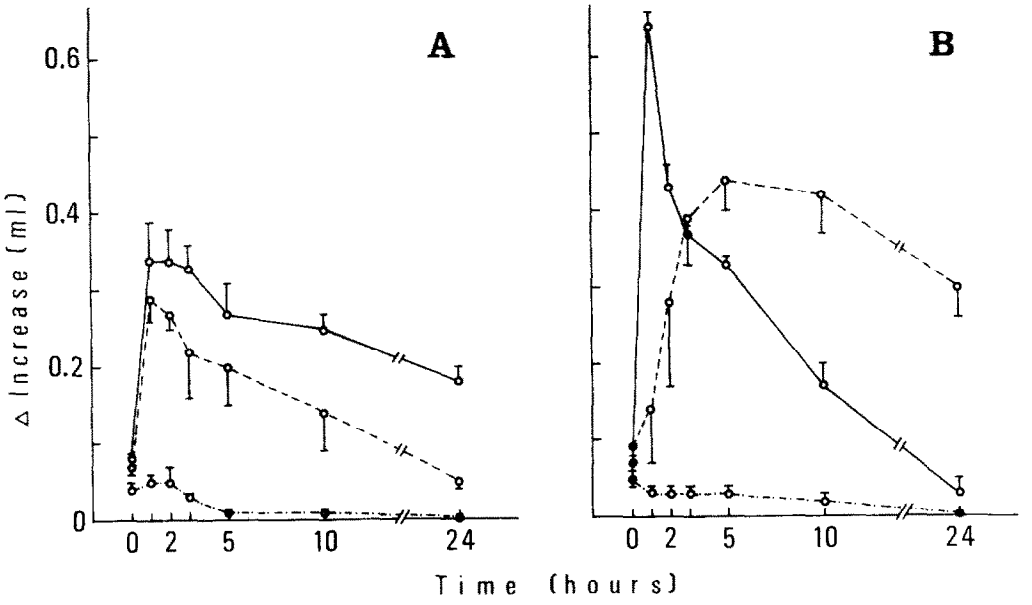

Fig. 1. A - Time-course of foot-edema induced by carbonyl compounds derived from the peroxidation of liver microsomal lipids. (O——O) 300 nmoles of carbonyl compounds; (O----O) 150 nmoles of carbonyl compounds; (O·-·-·O) control samples.

B - Time-course of foot-edema induced by serotonin or carregeenan. (O——O) Serotonin; (O----O) carrageenan; (O·-·-·O) saline without serotonin or carrageenan. The amounts of serotonin and carrageenan injected into the foot of the rat were 2.8 nmoles and 0.5 mg (in 0.05 ml of saline) respectively.

Fig. 1A shows the time-course increase in foot volume after the injection of either 300 or 150 nmoles of carbonyl compounds originating from the peroxidation of liver microsomal lipids.

In either case the foot-edema reached maximum in 1 h and decreased thereafter. When the higher level of carbonyl compounds was used, the increase in foot volume was still evident at 24 h. The small increase in the volume of the foot injected with the control sample, did not exceed the volume of the injected material (0.05 ml). Synthetic 4-hydroxynonenal(*) injected in equimolar amounts (300 nmoles) produced the same increase in foot volume as that produced by biogenic carbonyl compounds derived from lipid peroxidation.

The time-course increase in foot volume produced by the carbonyl compounds derived from lipid peroxidation resembled, for its rapid onset (peak at 1 h), that produced by the serotonin (Fig. 1B). However the foot-edema due to the carbonyl compounds (300 nmoles) was still evident at the later stages (10-24 h) as in the case of the foot-edema produced by carrageenan (Fig. 1B).

The increased permeability caused by the carbonyl compounds originating from the peroxidation of liver microsomal lipids might be related to the increase in the water content of the liver cell following carbon tetrachloride poisoning, that is, in a situation in which the peroxidation of membrane lipids of liver endoplasmic reticulum has been unequivocally demonstrated (15-17).

The fact that carbonyl compounds derived from the peroxidation of membrane lipids are active on vascular permeability could be of interest in studies concerned with the possible relationships between lipid peroxidation and the inflammatory process.

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(*) 4-hydroxynonenal was prepared by Prof. Esterbauer, Department of Biochemistry, University of Graz.

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