Studies on lipid peroxidation using whole liver cells: Influence of damaged cells on the prooxidant effect of ADP-Fe^{3+} and CCl_4

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Summary. An increase in the number of damaged cells produces an enhancement of lipid peroxidation induced by ADP-Fe^{3+} or CCl_4 'in vitro' on hepatocytes in single cell suspension.

Until now the mechanisms of lipid peroxide formation have been studied mainly using isolated biological membranes, treated with ADP-iron complex or CCl_4 as free radical generating systems[2-4]. Since isolated organelles cannot take into account the potential effect of intracellular naturally-occurring antioxidants, it should be worthwhile to extend such a study using whole intact cells. Recent experiments from our laboratory have shown the possibility of inducing a reproducible stimulation of lipid peroxidation in isolated rat hepatocytes, not only with ADP-iron, as other authors have shown[5], but also with CCl_4[6]. The present communication reports studies carried out to evaluate whether variations in the number of irreversibly damaged cells in hepatocyte suspensions are able to affect the stimulation of lipid peroxidation induced by ADP-Fe^{3+} or CCl_4.

Materials and methods. Unstarved adult male rats of the Wistar strain were used. The rats were anesthetized with sodium pentobarbital (50 mg/kg) and given sodium heparin (2000 USP units per rat) to prevent coagulation of blood during liver cannulation. Both these agents were injected i.p. The hepatocyte isolation procedure was performed as previously described[6]. After hepatocyte isolation, cell viability was routinely assessed by the Trypan Blue exclusion test[7]. Hepatocyte preparations with a percentage of Trypan Blue stained cells higher than ten were commonly discarded. In experiments in which different numbers of damaged cells in the suspension were used, the initial hepatocyte preparation was suitably mixed with cells made permeable to Trypan Blue, by using a Potter-Elvehjem apparatus, fitted with a Teflon pestle (30 complete vertical movements at 1500 rev/min). The cell treatment with ADP-Fe^{3+} (2.5 mM and 100 μM respectively) or CCl_4 (129 μM) was carried out as described in preceding papers[6,8]. Lipid peroxide formation by liver cells after 30 or 60 min incubation at 37 °C with the drugs mentioned was monitored by determining the thiobarbituric acid (TBA)-reacting compounds in the cell suspension[6].

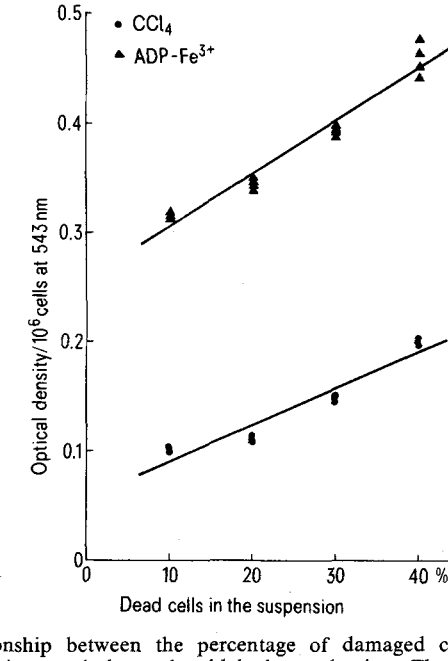
Results and discussion. Hepatocyte preparations with about 10% and 100% Trypan Blue stained cells respectively, were simultaneously treated with ADP-Fe^{3+} (2.5 mM-100 μM) or CCl_4 (129 μM) and the lipid peroxidation so induced was monitored as TBA-reacting compounds (see table). The lipid peroxidation levels obtained with the commonly-used cell suspensions (i.e. with no more than 10% damaged cells) after ADP-Fe^{3+} or CCl_4 treatment were respectively 6.5 and 1.7 times higher than those found in the untreated group. Moreover, lipid peroxidation in cell preparations containing only damaged cells was at least 2 times higher than that recovered in the corresponding groups with 10% damaged cells. The latter result shows the loss of cell viability in relation to an enhancement of lipid peroxidation either endogenous (in the case of untreated cells) or enzymatically induced (in the case of ADP-Fe^{3+} and CCl_4 treated cells).

On the one hand, the structural changes in the lipid and lipoprotein environment resulting from the loss of cell integrity could explain, at least in part, the increased endogenous lipid peroxidation in the untreated cell suspensions only containing damaged cells. On the other hand, the increased membrane permeability of damaged hepatocytes allows that higher concentrations of the drugs used are reached within the cells. Furthermore, since in the 100% damaged cell suspensions there is likely to be more debris than in the standard cell suspensions, the stimulation of lipid peroxidation induced by ADP-Fe^{3+} and CCl_4 on the 1st type of cell suspensions could be triggered in part outside the cells by fragments of endoplasmic reticulum.

TBA-reacting compounds produced by standard hepatocyte suspensions and 100% damaged cell suspensions after 30 min incubation at 37 °C in the presence of ADP-Fe^{3+} (2.5 mM-100 μM), CCl^4 (129 μM) or water

Experimental groups	TBA reacting compounds (OD at 543 nm/10^6 cells)
Control (10% damaged cells)	0.037 ± 0.005
ADP-Fe^{3+} (10% damaged cells)	0.244 ± 0.017*
CCl_4 (10% damaged cells)	0.063 ± 0.010*
Control (100% damaged cells)	0.101 ± 0.012* (273%)**
ADP-Fe^{3+} (100% damaged cells)	0.620 ± 0.036* (254%)**
CCl_4 (100% damaged cells)	0.149 ± 0.009* (236%)**

* Values represent means ± SD of 2 duplicated experiments.
** Values in parentheses are percentage stimulation with respect to the corresponding group with 10% damaged cells, taken as 100% value.



Relationship between the percentage of damaged cells in the suspensions and the malonaldehyde production. The cells were incubated at 37 °C for 60 min in the presence of ADP-Fe^{3+} (2.5 mM-100 μM) or CCl_4 (129 μM). The regression lines are shown; correlation coefficient for CCl_4 $r = 0.96$, $p \ll 0.001$; and for ADP-Fe^{3+} $r = 0.98$, $p \ll 0.001$.

In order to evaluate the actual modifying effect of different amounts of damaged cells on the ADP-Fe^{3+} or CCl_4 induced lipid peroxidation, simultaneous monitoring of the production of TBA-reacting compounds was carried out in cell populations containing 10-20-30-40% damaged cells. The cells were incubated for 60 min in the presence of ADP-Fe^{3+} (2.5 mM-100 μM) or CCl_4 (129 μM). As shown in the figure, the relationship between the stimulation of lipid peroxidation due to ADP-iron and CCl_4 and the amount of damaged cells present in the suspension is linear.

In cell suspensions containing 40% damaged cells, the lipid peroxidation induced by ADP-iron and CCl_4 was respectively 1.7 and 3.3 times higher than that theoretically obtainable with 100% viable cells (see figure). In other words, with an increase by 10% in the damaged cell content of hepatocyte preparations, an enhancement of ADP-Fe^{3+} and CCl_4 induced lipid peroxidation by 18% and 56% followed. In conclusion, the data reported here emphasize the critical role exerted by the quality of liver cell preparations on 'in vitro' lipid peroxidation studies employing hepatocytes in single cell suspension.

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Further studies on the sensitivity of plant pathogenic microorganisms towards some naturally occurring chalcones and flavanones

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Summary. The antifungal and antibacterial activities of some quinochalcones, chalcones and flavanones isolated from *Didymocarpus pedicellata* have been assayed.

A survey of the literature revealed that certain simple quinones and their derivatives possess marked fungicidal properties[2]. In view of the fact that the leaves of *Didymocarpus pedicellata* (Gesneriaceae) keep well on storage and are not attacked by fungi in a humid atmosphere[3], we thought it worthwhile to undertake biological investigations on the compounds isolated from different extracts of the dried leaves of this plant. Interestingly, we were able to isolate 2 quinochalcones, chalcones and flavanones (figure, **I**) from the leaves of *D. pedicellata*[4-9]. We have previously described the antifungal activities[10-12] of a number of furocoumarins from *Selinum tenuifolium* (Umbelliferae) and pterocarpans, coumestans and chalcones from *Flemingia chappar* (Leguminosae). This communication reports the antifungal and antibacterial activities of the compounds isolated from the leaves of *D. pedicellata*.

Materials and methods. A number of plant pathogenic sporeforming fungi viz., *Helminthosporium oryzae* Breda de Haan, *Fusarium oxysporum* f. spp. *ciceri* (Padwick) Synd. & Hans and *Rhizopus artocarpii;* 3 sclerotial pathogens, *Sclerotium rolfsii* Sacc., *Thanatephorus cucumeris* (Frank) Donk and *Rhizoctonia oryzae sativae* (Saw) Mordue, and 1 plant pathogenic bacterium, *Xanthomonas campestris* (Pammel) Dowson, were used as test organisms. Solutions of 8 test compounds **I-VIII** at 4 concentrations, viz., 500, 250, 100 and 50 ppm were prepared by dissolving them first in 1-2 ml of ethyl alcohol and making up the rest of the volume with distilled water. 3-day-old slant cultures (PDA) of *R. artocarpii* and 7-day-old cultures of *H. oryzae* and *F. O. ciceri* were used for preparation of spore suspensions with sterilized distilled water; these were filtered through a double layer of sterilized muslin cloth, maintaining a final strength of 0.25 million spores per ml of suspension (counted with a haemacytometer). For sclerotial germination, sclerotia from 7-day-old PDA cultures of *S. rolfsii, R. oryzae sativae* and *T. cucumeris* were taken. Sensitivity of fungi and bacteria to the compounds I+VIII were tested following standard methods of spore germina-

Table 1. Sensitivity of some plant pathogenic spore forming fungi towards naturally occurring chalcones and flavanones (average of 5 replicates after 24 h)

Compounds	pH	% of inhibition of spore germination at concentration (ppm):									
		H. oryzae			*R. artocarpii*				*F. o. ciceri*		
		100	200	500	50	100	250	500	100	250	500
Methyl pedicinin (**I**)	6.0	0	0	0	45	55	65	85	3	6	8
Isomethyl pedicinin (**II**)	6.0	0	0	0	50	53	75	90	15	45	85
Pedicinin (**III**)	8.0	0	0	0	48	55	60	85	3	6	8
Pedicellin (**IV**)	7.0	0	0	0	25	35	45	55	5	6	11
Isodidymocarpin (**V**)	7.0	0	0	0	60	75	85	92	7	9	15
Didymocarpin-A (**VI**)	7.0	56	60	72	53	69	76	81	54	69	73
Despedicellin (**VIII**)	7.0	42	47	50	35	57	61	69	25	50	62
Control	7.0	0	0	0	0	0	0	0	0	0	0