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PRELIMINARY COMMUNICATIONS

ADRIAMYCIN STIMULATES ONLY THE IRON ION-INDUCED, NADPH-DEPENDENT MICROSOMAL ALKANE FORMATION

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Anthracyclines like adriamycin are used as anticancer drugs. However, the chemotherapy with adriamycin is limited by its cardiotoxicity (for review see 1). The congestive heart failure observed is presumably associated with the adriamycin-induced redox cycling which forms high amounts of superoxide anion ($O_2^{\cdot}$) and hydrogen peroxide (H_2O_2) (1). It has been assumed that other reactive oxygen species like hydroxy radicals ($^{\cdot}OH$) also occur which would initiate the peroxidation of lipid membranes (1). Lipid peroxidation has been proposed as a molecular mechanism of adriamycin-induced cardiotoxicity (2). But in vivo lipid peroxidation as measured by ethane expiration of rats was not increased after high acute doses of adriamycin (3, 4). Our results were confirmed by experiments with isolated rat hepatocytes where lipid peroxidation measured by increased conjugated dienes of lipids was not stimulated by adriamycin alone (5). Nevertheless, Mimnaugh et al. (6, 7) found with adriamycin an increased malondialdehyde formation in hepatic microsomes. They concluded that during the redox cycle of adriamycin which is catalyzed by microsomal enzymes highly reactive oxygen species are formed which initiate lipid peroxidation. Therefore, we were interested to confirm these results using a different method for the measurement of microsomal lipid peroxidation. We determined the formation of alkanes, e.g. ethane and n-pentane, in rat hepatic microsomes. This method has recently been shown to present reliable and reproducible results on microsomal lipid peroxidation (8). Furthermore, we were interested in whether the presence of iron ions as microsomal contaminants could be responsible for the already suggested adriamycin-induced increase in lipid peroxidation (4, 9, 10).

Methods

Microsomes were prepared from the livers of male Wistar rats of 180 - 200 g body weight according to standard procedures (11). The animals received food (Altromin^R) and tap water ad libitum. To remove completely blood contaminants, the livers were perfused with 0.9 % NaCl in H_2O before homogenization. Incubations were carried out in special gas-tight flasks which have recently been described (8). The 5 ml incubation mixture in Tris-KCl-buffer (11) contained 1 mg microsomal protein/ml, 8 mM Na-isocitrate, 1 mM NADP and 0.05 mg/ml isocitrate dehydrogenase (12). Adriamycin (doxorubicin·HCl) which was from Farmitalia (3) was added before the pre-incubation period (3 min) at 37° C. Afterwards the microsomal enzyme reactions were initiated by NADP. $FeCl_2 \cdot 4H_2O$ (2.4×10^{-5} M) was added simultaneously with NADP (12). The gaseous phase above the incubation mixture which was shaken at 37° C was alkane-free synthetic air (80 % N_2 / 20 % O_2)

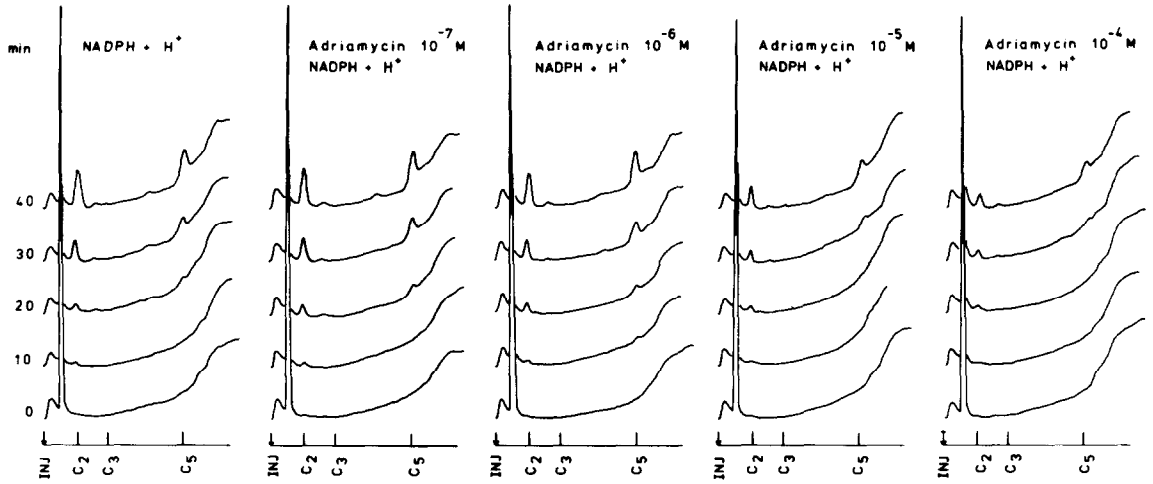

Figure 1: Effect of adriamycin on microsomal alkane formation in the presence of the NADPH-regenerating system (without $FeCl_2$). Representative original gas chromatograms (redrawn) of the air samples after 0, 10, 20, 30 and 40 min incubation are shown (4 experiments). INJ = injection, C_2 = ethane, C_3 = propane, C_5 = n-pentane. The peaks obtained after 70 min without adriamycin (left) correspond to 0.01 (ethane) and 0.02 (n-pentane) nmol/mg microsomal protein (for comparison see also (8)).

as already described (8). At various intervals gas samples of the head space of the incubation flasks were analyzed by gas chromatography for the alkanes formed (8). During each gas sampling 8 ml of the total gaseous phase (19 ml) of the flasks were replaced by synthetic air (8).

Results

Figure 1 shows the chromatograms (redrawn) of the gaseous phases above the microsomal incubations. The drawings demonstrate that only very small amounts of alkanes were formed during 40 min incubation even in the presence of the NADPH-regenerating system. This is in agreement with our previous studies (8). Adriamycin in concentrations of 1×10^{-7} M and 1×10^{-6} M did not influence this minor alkane formation, whereas 1×10^{-5} M and 1×10^{-4} M adriamycin were inhibitory (Figure 1). A stimulation of microsomal alkane formation by adriamycin was not detectable (Figure 1).

Figure 2 (left) demonstrates that 2×10^{-5} M $FeCl_2$ stimulated the NADPH-dependent formation of ethane and n-pentane considerably when compared to the controls without $FeCl_2$ (Figures 1 and 2). In controls without the NADPH-regenerating system alkane peaks were not detectable (data not shown, see also (8)). When 1×10^{-4} M adriamycin was present during incubation the $FeCl_2$-induced, NADPH-dependent alkane formation increased 2 - 3 fold (Figure 2, right). This stimulatory effect could also be seen using 1×10^{-5} M adriamycin and $FeCl_2$ (2.4×10^{-5} M); but it was less pronounced at 15 min (data not shown).

Discussion

In a previous report we have shown that under the conditions applied here an adriamycin-mediated enzymatic redox cycle occurs as measured by oxygen- and NADPH-consumption and by the formation of adriamycin metabolites (13). Therefore, the present data indicate that the liver microsomal adriamycin-mediated redox cycle is not necessarily associated with lipid peroxidation, although $O_2^{\cdot}$ and H_2O_2 are formed. This suggests that other highly reactive oxygen species must be responsible for the reported stimulation of microsomal lipid peroxida-

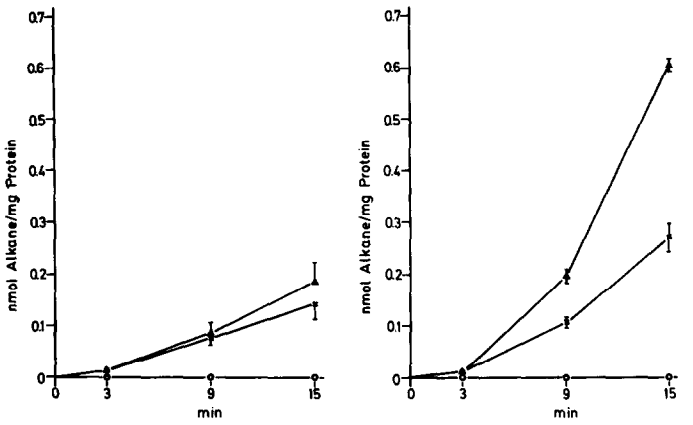

Figure 2: Microsomal ethane (x——x) and n-pentane (Δ——Δ) formation induced by 2.4 x 10^{-5} M $FeCl_2$ and the NADPH-regenerating system.
Left part: Without adriamycin.
Right part: With 1 x 10^{-4} M adriamycin.
Ethane- or n-pentane-controls (o——o): Only $FeCl_2$ was omitted. Mean values ± SD (n = 4) are shown.

tion by adriamycin (6, 7). But our results also show that ferrous ion-induced microsomal lipid peroxidation can be increased by adriamycin. These data are in agreement with experiments using isolated NADPH-cytochrome P-450 reductase, liposomes, adriamycin, NADPH, ADP and iron ions which have demonstrated that adriamycin stimulates lipid peroxidation induced by iron-ADP-complexes (9).

Therefore, the stimulatory effect of adriamycin on liver microsomal lipid peroxidation observed by others (6, 7) might be due to an increase in the iron ion-induced lipid peroxidation. Due to the isolation procedure of microsomes and the iron-free agents used the spontaneous lipid peroxidation in the presence of the NADPH-regenerating system is very low in our system (8, 12) as claimed by Kornbrust and Mavis (14). In contrast, Mimnaugh et al. (6) reported a control value of about 30 nmol malondialdehyde/mg microsomal protein/60 min which is about one third of the total peroxidizable microsomal lipid normally present (14).

Several mechanisms could explain the observed stimulation of ferrous ion-induced microsomal lipid peroxidation:

1. It has been suggested that iron ions are a prerequisite for the formation of $^{\bullet}OH$ from $O_2^{\bullet}$. The hydroxy radical formed could then initiate lipid peroxidation.
2. It has recently been demonstrated that an iron-adriamycin-complex is able to destroy erythrocyte membranes (10). Such a complex might bind O_2 and could be recycled by microsomal NADPH-cytochrome P-450 reductase producing highly reactive oxygen species as in the case of the iron-bleomycin-complex (15).
3. Recent results (14, 16) favor a direct initiation of microsomal lipid peroxidation by oxygen and ferrous ions or ferrous ion-chelation-complexes respectively, the reduction of the ferric ions formed being the rate limiting step. On the other hand, $O_2^{\bullet}$ is able to reduce ferric ions. Therefore, the excess $O_2^{\bullet}$ formation which occurs during the NADPH-dependent microsomal redox cycling of adriamycin would accelerate the reduction of ferric to ferrous ions, thereby enhancing the initiation step of lipid peroxidation.

Regardless of the underlying mechanism we suggest from our results that the adriamycin redox cycling itself is not responsible for the in vitro lipid peroxidation observed, but that iron-induced lipid peroxidation is stimulated by adriamycin. However, the question remains whether in vivo circumstances exist where this could become relevant.

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