

DOXORUBICIN MEDIATED FREE IRON RELEASE FROM FERRITIN MAGNETOPARTICLES IS ENHANCED AT HIGHER TEMPERATURES: IMPLICATIONS FOR CANCER THERAPY

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INTRODUCTION: One of the principal forms in which iron is stored in living organisms are magnetic nanoparticles of hydrated iron oxide of the composition $5\text{Fe}_2\text{O}_3 \cdot 9\text{H}_2\text{O}$ bound by an apoferritin protein shell. These iron containing proteins, i.e. ferritins, play an important role in the regulation of free iron within the cell. Mobilization of iron from ferritin requires reduction of ferric Fe(III) iron to ferrous Fe(II) iron and several reductants have been identified. Among them are also some antitumor anthracycline drugs. The anthracycline antibiotic doxorubicin (adriamycin[®]) has been in use for more than 30 years for the treatment of a variety of malignancies such as those arising in the breast, bile ducts, endothelial tissue, the esophagus and liver, osteosarcomas, and soft-tissue sarcomas [1]. Despite the extensive and long-standing clinical utilization of this drug, its mechanism of action is not clear. A number of different mechanisms have been proposed, including intercalation into DNA, free radical formation with consequent DNA damage, DNA cross-linking and inhibition of topoisomerase II [2]. Tritton and Yee [3] reported that doxorubicin (DOX) coupled to insoluble agar beads that cannot traverse the membrane was more toxic than free drugs, and as an alternative hypothesis direct membrane-mediated effects were proposed for the DOX toxicity. Due to the fact that DOX was found to stimulate iron release from ferritin in the presence of P450 reductase and NADPH [4] and because reduced Fe^{2+} is required for oxidative damage of membrane lipids, we have in this paper investigated DOX mediated iron mobilization at various temperatures. We were stimulated by an experimental finding that DOX efficacy is enhanced at higher temperatures [5], explained by a possibly higher permeability of cells.

MATERIAL AND METHODS:

1. Chemicals

Horse spleen ferritin, ferrozine NADPH, TBA, and trichloric acid were obtained from Sigma (St. Louis, USA). NADPH-cytochrome P-450 reductase isolated from rat liver microsomes was kindly supplied by Dr. P. Sourivong. Other chemicals

were from commercial sources and were of the highest analytical grade.

2. Iron release assay

Iron concentration was determined by the spectrophotometric measurement of the iron-ferrozine complexes [7]. Reaction mixture containing ferritin (100 $\mu\text{g}/\text{ml}$), ferrozine (1.0 mM), NADPH-cytochrome P-450 reductase (0.2 U) and DOX (1 mM) was preincubated at various physiological temperatures (36-42 °C) and the reaction was initiated by the addition of NADPH (0.7 mM). The absorbance at 562 nm (the absorption coefficient of iron-ferrozine complex is $27\,900\text{ M}^{-1}\text{ cm}^{-1}$) was measured against a blank containing the same reaction mixture except NADPH using spectrophotometer Specol 210 (Carl Zeiss, Jena, Germany).

3. Lipid peroxidation measurement

The extent of lipid proxidation was determined by measuring TBA-reactive substances (TBARS) [8]. The reaction mixture described above was augmented with phosphatidylcholine liposomes (5 $\mu\text{mol}/\text{ml}$) and 30 min after NADPH addition 0.5 ml of it was added to 2 ml TBA reagent consisting 15 % trichloric acid 0.375 % TBA and 0.5 HCl and heated in 100 °C water bath for 15 min. After cooling in ice-cold water, the mixture was centrifuged at $1250 \times g$ for 10 min. The absorbance of the supernatant at 535 nm was read against a blank containing 2 ml TBA and 1 ml distilled water. The concentration of TBARS was calculated using the molar absorption coefficient $156000\text{ M}^{-1}\text{ cm}^{-1}$.

RESULTS AND DISCUSSION: Figure 1 shows that the presence of DOX actually release iron from horse spleen ferritin and that the release at 42 °C is almost 4 times higher than at 36 °C. We have further investigated lipid peroxidation which occurred when phospholipid liposomes were incubated with ferritin, DOX, and other reagents for 30 min. The formation of TBARS increased 8-fold at 42 °C as compared with 36 °C (Fig. 2).

The potential involvement of free radical generation in the cytotoxicity of the anthracyclines (both in terms of antitumor effects and cardiotoxicity) is complex and confusing. There is no question that under appropriate conditions the chemistry of the anthracyclines lends itself to the generation of reactive free radicals. The quinone structure permits DOX to act as electron acceptors in reactions mediated by oxoreductive enzymes including cytochrome P-450 reductase. The addition of the free electron converts the quinones to semiquinone free radicals, which may induce free radical injury to DNA as well as membranes. The problem is that the required DOX concentrations are substantially lower than clinically relevant concentrations.

The analysis of a new mechanism proposed in our study clearly demonstrated that the damage of membrane lipids due to their peroxidation may be a key element of the DOX cytotoxic effect by iron mobilized from ferritin (as has been found [6], ferritin levels in tumors are substantially higher than in the normal tissue) and moreover it offers a theoretical rationale for the combination of DOX chemotherapy with local hyperthermia for more effective treatment of tumors.

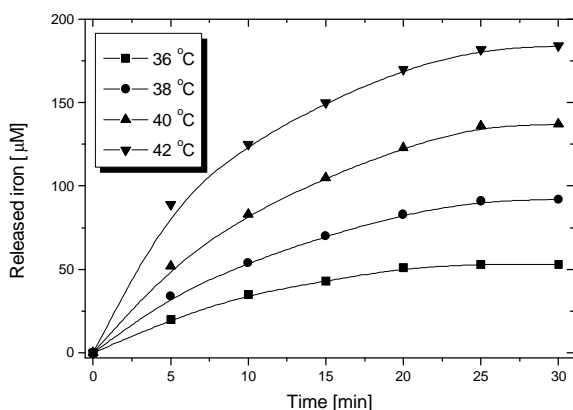


Fig. 1: Time course of iron release from ferritin by 1 mM doxorubicin at various temperatures.

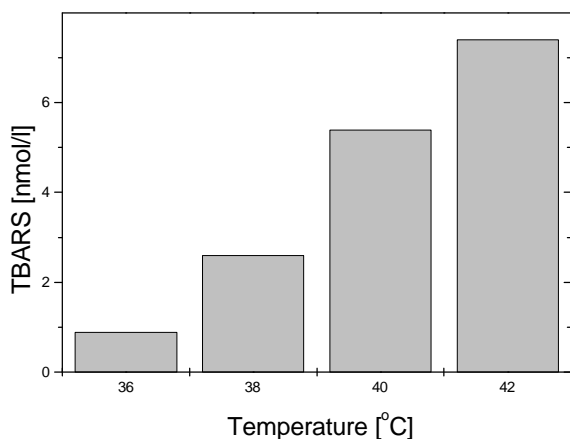


Fig. 2: Lipid peroxidation of phosphatidyl-choline liposomes induced by doxorubicin in the presence of ferritin at various temperatures.

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