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Cytotoxicity of quinone drugs on highly proliferative human leukemia T cells: Reactive oxygen species generation and inactive shortened SOD1 isoform implications

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ABSTRACT

Drugs containing the quinone group were tested on hyperproliferative leukemia T cells (HLTC: Jhp and Jws) and parental Jurkat cells. Doxorubicin, menadione and adaphostin produced different effects on these cell lines. Rapid doxorubicin-induced cell death in Jurkat cells was mediated by caspase activation. Doxorubicin-induced cell death of HLTCs was delayed due to the absence of caspase-3 and -8 expression. Delayed HLTC cell death was mediated and triggered by the generation of reactive oxygen species (ROS). Other drugs containing quinone groups, such as menadione and adaphostin, were also tested on HLTC and both were toxic by a caspase-independent mechanism. The toxicity of these drugs correlated with the generation of the superoxide anion, which increased and was more effective in HLTCs than in parental Jurkat cells. Accordingly, SOD1 activity was much lower in HLTCs than in Jurkat cells. This lower SOD1 activity in HLTCs was associated not only with the absence of the wild-type (16 kDa) SOD1 monomer but also with the presence of a shortened (14 kDa) SOD1 monomer isoform. Moreover, the cytotoxicity of drugs containing the quinone group was prevented by incubation with manganese(III) tetrakis (4-benzoic acid) porphyrin (MnTBAP), a cell-permeable superoxide dismutase mimetic and a potent inhibitor of oxidation. These findings could explain the sensitivity of HLTCs to drugs containing the quinone group using a mechanism dependent on oxidative stress. These observations can also be useful to target hyperproliferative leukemias that are resistant to the classical caspase-dependent apoptotic pathway.

1. Introduction

Hyperproliferative leukemia T cells (HLTC) were selected from Jurkat cells cultured in serum free medium (Jws, Jurkat without serum) or in exhausted media (Jhp, Jurkat highly proliferative) [1]. These cells proliferated at a much higher rate and exhibited a higher saturation density than parental Jurkat cells. Interestingly, these HLTCs lost Fas surface and caspase-3 intracellular expression, were resistant to Fas- and doxorubicin (Dox)-induced apoptosis [1] and contained large multilamellar bodies with lysosomal features [2]. Dox is a DNA-damaging drug used in the clinical treatment of leukemia and other cancers [3–5]. Dox-induced apoptosis through the so-called "intrinsic pathway" is characterized by the disruption of the mitochondrial membrane potential ($\Delta\Psi m$) and subsequent activation of the caspase cascade. Recently, a model for Dox-induced apoptosis in Jurkat cells has been proposed in which caspases are dispensable for the induction phase of apoptosis but are needed to accelerate the execution phase [6]. Other studies have also indicated that caspases are not strictly necessary for the induction of apoptosis by DNA-damaging drugs [7].

Dox and menadione (vitamin K3) both contain a quinone group, while adaphostin (NSC680410) is a dihydroquinone derivative. These compounds may exhibit anti-cancer activities. Menadione is chemically synthesized and is toxic against various cancers including breast and bladder tumors [8]. Metabolism of menadione in the presence of metal ions has been reported to generate reactive oxygen species (ROS) such as the highly deleterious OH^- radical [9,10]. Furthermore, ROS generation induced by menadione metabolism promoted the depletion of cellular thiol levels, DNA damage and cytotoxic effects in glioma cells [11]. On the other hand, adaphostin is undergoing preclinical testing as a potential anti-leukemic drug. Previous studies have suggested that the generation of ROS

Abbreviations: Dox, doxorubicin; HLTC, hyperproliferative leukemia T cells; PS, phosphatidylserine; $\Delta\Psi m$, mitochondrial membrane potential; ROS, reactive oxygen species; Z-VAD-fmk, *N*-benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone.

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plays a critical role in the cytotoxicity of this agent [12,13]. More recently, it has been reported that mitochondrial respiration rather than direct redox cycling of the hydroquinone moiety is responsible for adaphostin-induced ROS, and mitochondrial complex III was identified as a potential target for antineoplastic agents [14].

Despite the harmful effects of ROS, their levels appear to be increased in cancer cells [15] and this increase seems to be important in tumor initiation and growth [16]. ROS are able to induce DNA mutations and genomic instability [17], important events in carcinogenesis that may promote oncogene alterations [18]. Moreover, it has also been suggested that ROS may act as messengers of cellular signal transduction [19,20]. Mitochondria seem to be a major organelle leading to ROS production in cancer cells. Indeed, mtDNA mutations are frequently detected in tumor cells. Damage of the mtDNA normally results in a less effective respiratory chain resulting in increased superoxide generation [21]. Although the higher ROS levels intrinsically generated by tumor cells seem to promote carcinogenesis, these cells could also be nearer than normal cells to the ROS toxicity threshold [22], making them more sensitive to ROS-generating drugs. Thus, the unique behavior of cancer cells in relation with ROS has emerged as a promising approach to the development of new strategies in the design of chemotherapeutic drugs. Cells maintain control over physiological ROS levels through antioxidant protective mechanisms. Proteins in the superoxide dismutase (SOD) family scavenge superoxide radicals by dismutation into hydrogen peroxide and molecular oxygen. There are three isoforms of superoxide dismutase in animal cells: Cu, ZnSOD (SOD1), MnSOD (SOD2), and EC-SOD (SOD3). SOD2 is located inside the mitochondrial matrix and is considered the major protective barrier against the superoxide produced during mitochondrial respiration. SOD1 has typically been considered a cytosolic enzyme, but its presence has also been confirmed in the mitochondrial intermembrane space [23].

This study compares the sensitivity to ROS toxicity between the parental leukemic Jurkat cell line and the hyperproliferative and more aggressive derivatives Jhp and Jws. Several chemotherapeutic agents are tested that include a quinone group and generate ROS, with the results demonstrating greater sensitivity in Jhp and Jws cells. We also observed that these cells express an inactive SOD1 isoform. Finally, this study demonstrates that chemotherapeutic drugs can use different mechanisms of action even in closely related tumor cells.

2. Materials and methods

2.1. Cell culture

The human T-cell leukemia Jurkat, clone E6.1 (ATCC, Rockville Pike, USA) and the Jhp cell subline were cultured in RPMI 1640 medium supplemented with 5% fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin (complete

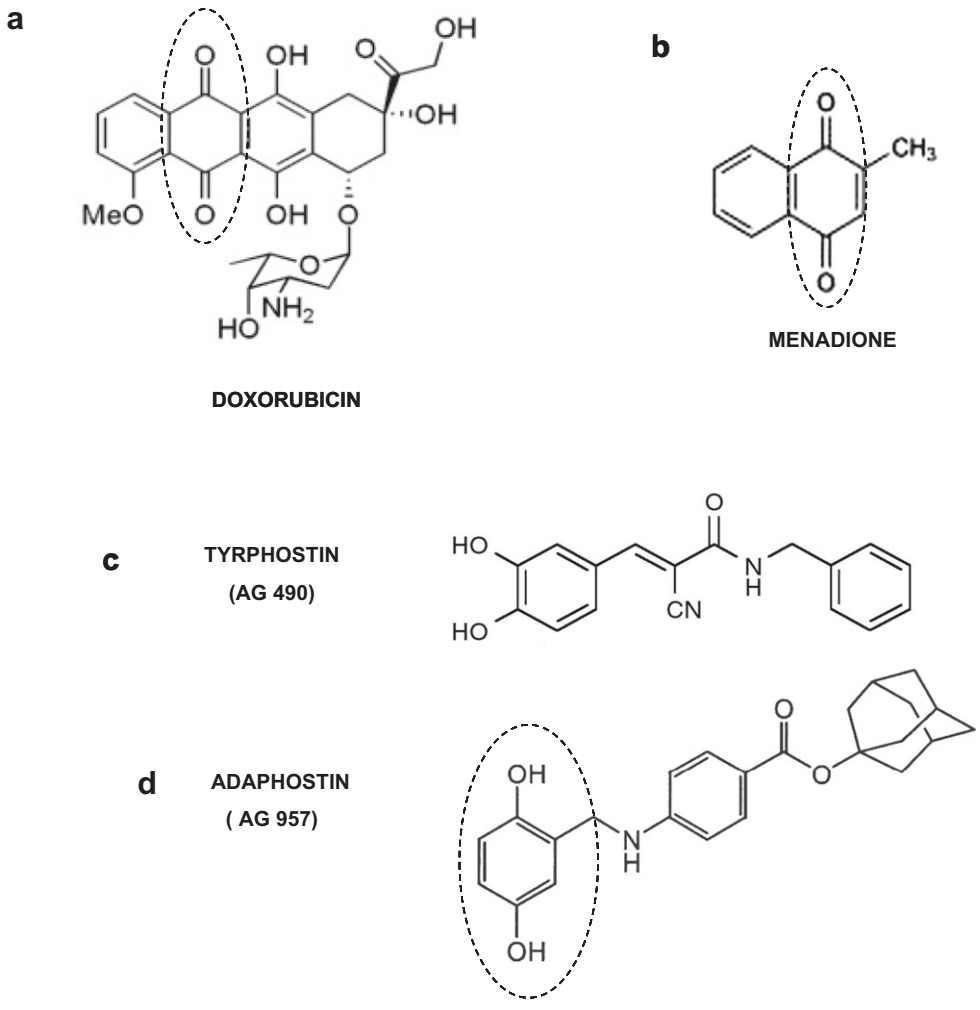

Fig. 1. Chemical structures of the drugs studied herein with the quinone group highlighted. (a) Doxorubicin, (b) menadione, (c) tyrphostin AG 490 and (d) tyrphostin AG 957 (adamantyl ester or adaphostin).