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Yeast cytotoxic sensitivity to the antitumour agent β-lapachone depends mainly on oxidative stress and is largely independent of microtubule- or topoisomerase-mediated DNA damage

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ABSTRACT

β-Lapachone (β-lap) is a promising antitumour drug currently undergoing clinical trials. Although it is known that β-lap generates reactive oxygen species (ROS), its actual mechanism of action is still controversial. Especially important is to determine whether concomitant DNA or microtubule damage is the key target of its antitumour properties and whether DNA damage is mediated by topoisomerases as previously suggested. Here, we have searched for determinants of β-lap cytotoxicity in the model organism *Saccharomyces cerevisiae* through a mechanism-driven approach whereby several pathways of the DNA and microtubule integrity responses, as well as the antioxidant response, were downregulated and the outcome of β-lap treatment examined. We also included in the analysis several β-lap derivatives expected to modify drug bioavailability and activity. We found that neither topoisomerase II nor microtubules contributed to yeast sensitivity to β-lap and its equitoxic derivative 3-bromo-β-lapachone. Instead, we found that oxidative and related environmental stresses were primarily responsible for toxicity. Accordingly, Yap1, the central transcription factor in the antioxidant response in yeast, together with several components involved in stress tolerance (i.e., Snf1 and Hog1) and chromatin remodelling (i.e., the SWR1 and RSC complexes), played major roles in protection against β-lapachone. Critically, we show that dioxygen enhanced toxicity and that ROS scavengers protected cells from it. Furthermore, we show that both quinones resulted in cell death in a manner which cytologically resembled apoptosis/necrosis. We thus conclude that β-lap is toxic to yeast through massive ROS production that either directly kills the cells or else triggers programmed cell death.

1. Introduction

β-Lapachone (β-lap) is a naturally occurring β-cycled-pyran-1,2-naphthoquinone mainly obtained from the lapacho tree (*Tabebuia avellaneda*). The compound possesses significant antitumour activities and has entered phase I and II clinical trials against cancer, either alone or in combination with other treatments [1–5]. Besides this, β-lap has been tested against other human diseases with promising results in vitro or in animal models [6–13].

As for all quinones, β-lap cytotoxicity has been partly attributed to the chemistry and biochemistry of the quinone/

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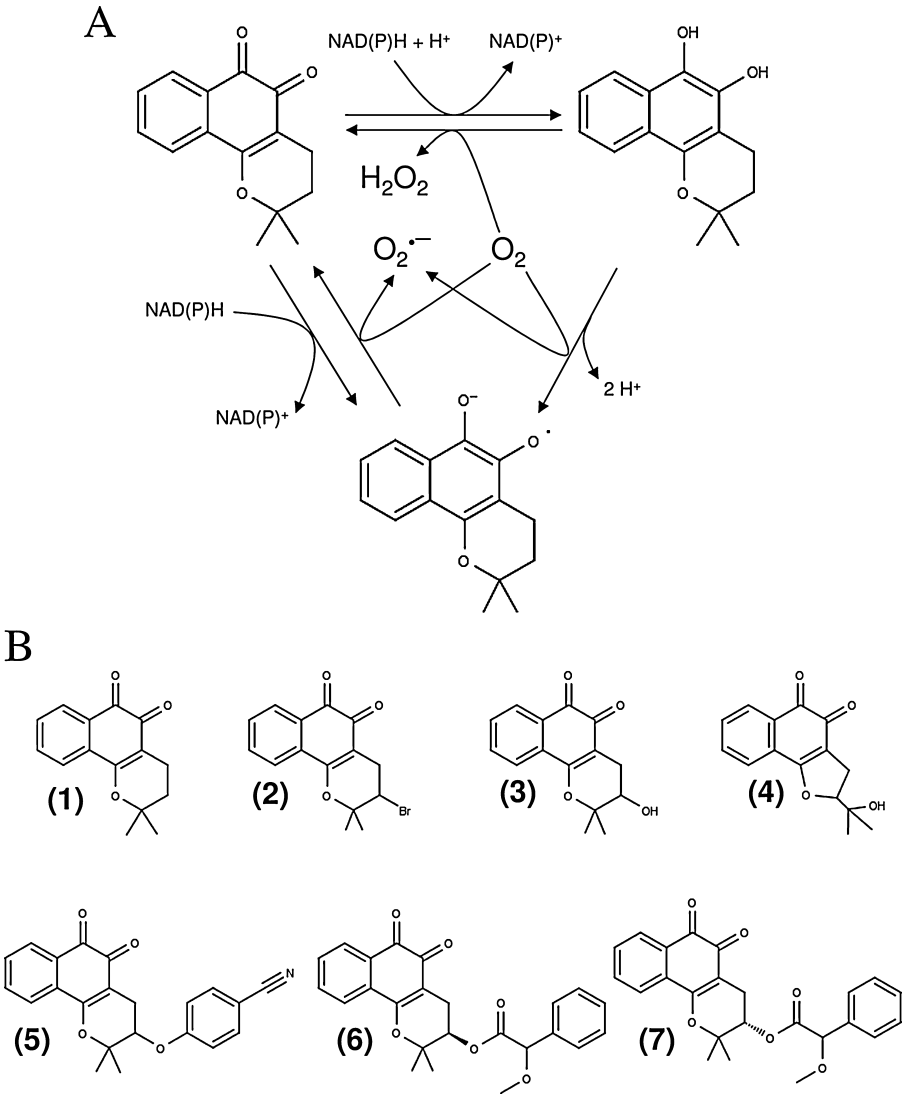

Fig. 1. (A) Schematic of the two futile redox cycles operating in the presence of β-lapachone and dioxygen. (B) Chemical structure of β-lapachone (**1**) and derivatives studied herein (**2–7**).

semiquinone-free-radical/hydroquinone triad ($Q/SQ^{\bullet-}/QH_2$)[1] (Fig. 1A). This triad generates reactive oxygen species (ROS) through the production of the superoxide radical ($O_2^{\bullet-}$) when $SQ^{\bullet-}$ transfers its unpaired electron to dioxygen [14–20]. The $SQ^{\bullet-}$ is formed when one-electron oxidoreductases, such as NADPH-cytochrome P450 reductases, reduce the Q form. Since Q is regenerated after the electron transfer to dioxygen, a futile redox cycle occurs which can lead to large amounts of $O_2^{\bullet-}$ from little initial Q. An important determinant of the enhanced toxicity of β-lap in comparison to the many other quinones is thought to be the presence of a second futile redox cycle that comprises the autooxidation of the QH_2 back to $SQ^{\bullet-}$ and Q [7,18,19,21]. These reactions also consume dioxygen and generate either further $O_2^{\bullet-}$ or directly produce hydrogen peroxide (H_2O_2) (Fig. 1A). Although there are different ways to get to QH_2 from Q, the most important within cells is the two-electron reduction of Q by the cytosolic enzyme NAD(P)H:quinone oxidoreductase 1 (NQO1, DT-diaphorase). NQO1 is generally a detoxifying enzyme for most quinones as it circumvents the one-electron reductions; however, all empirical evidence suggests that this is not the case for β-lap and, instead, the presence of NQO1 actually boosts β-lap cytotoxicity [4,18,21,22]. The consequence of this second futile cycle is not only an even larger production of ROS, but also the draining of both NADH and NADPH [21]. According to this model, massive ROS production, together with severe depletion of reducing power and energy, would trigger apoptosis and/or necrosis. Hence, the selectivity against cancer cells is likely to depend on the overexpression of NQO1 relative to normal surrounding tissue [23,24]. Interestingly though, β-lap is still highly toxic in tumour cell lines that completely lack NQO1 [25,26], pointing to other factors that also contribute to its cytotoxicity.

A second important determinant of quinone cytotoxicity is their ability to directly arylate nucleophiles, especially thiol groups,

[1] Abbreviations: Quinone (Q); semiquinone (SQ); hydroquinone (QH_2); reactive oxygen species (ROS); double-strand break (DSB); homologous recombination (HR); Dimethyl sulphoxide (DMSO); β-lapachone (β-lap); 3-bromo-β-lapachone (3-Br-β-lap); Menadione (MD); methyl methanesulphonate (MMS); reduced glutathione (GSH); oxidised glutathione (GSSG); 4′,6-diamidino-2-phenylindole (DAPI); optical density at 620 nm (OD_{620}); 50% growth inhibition (GI_{50}); relative cumulative growth (RCG); Mutant sensitivity (MS); three dimensional quantitative structure-activity relationship (3D-QSAR); non-homologous end joining (NHEJ); environmental stress response (ESR); postreplicative repair pathway (PRR); kinetoplast DNA (kDNA); spindle assembly checkpoint (SAC); spindle position checkpoint (SPC).