SUPEROXIDE RADICALS MEDIATE HEPTATOXICITY INDUCED BY THE HEAT SHOCK PROTEIN 90 INHIBITORS BENZOQUINONE ANSAMYCINS

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Geldanamycin (GM), a benzoquinone ansamycin antibiotic, is a natural product inhibitor of the heat shock protein 90 (Hsp90) with potent and broad anticancer properties. However, its progression to clinical trials was halted due to unacceptable levels of hepatotoxicity. Consequently, numerous less toxic analogs differing only in their 17-substituent have been synthesized including 17-AAG and the water soluble 17-DMAG (Alvespimycin), which have recently entered clinical trials. The different hepatotoxicity induced by GM and its analogs may reflect the redox active properties of the quinone moiety (Q) and possibly the extent of superoxide radical formation, which may stimulate cellular oxidative injury.

$$Q^{\cdot-} + O_2 \rightleftharpoons O_2^{\cdot-} + Q \qquad (1)$$

Eq. 1 is established rapidly, and its actual position is governed by $E_7(Q/Q^{\cdot-})$ and $E_7(O_2/O_2^{\cdot-})$ and the relative concentrations of Q and O_2. Using pulse radiolysis, $E_7(Q/Q^{\cdot-})$ for 17-DMAG has been determined *vs.* O_2, 1,4-naphthoquinone or menadione to be -194 ± 6 mV, which is somewhat lower than $E_7(O_2/O_2^{\cdot-})$ = -180 mV (1 M O_2). Eq. 1 is well to the left in the case of 1,4-benzoquinone and substitution into the ring by electron-donating or -withdrawing groups reduces or increases, respectively, $E_7(Q/Q^{\cdot-})$ in a predictable manner, e.g. linearly related to the Hammett sigma value of the substituents. Hence, $E_7(Q/Q^{\cdot-})$ should follow the order GM < 17-AAG < 17-DMAG implying that O_2 is more readily reduced to $O_2^{\cdot-}$ by GM.

It is demonstrated that $O_2^{\cdot-}$ can be efficiently trapped by Tempol during the reduction of GM, 17-AAG and 17-DMAG by NADPH catalyzed by NADPH-cytochrome P450 reductase, and that $O_2^{\cdot-}$ formation rate, which reflects the rate of NADPH oxidation, follows the order 17-DMAG > GM > 17-AAG. In the absence of $O_2^{\cdot-}$ scavengers, the rate of NADPH oxidation follows the order 17-DMAG > 17-AAG > GM. The order of the drug cytotoxicity toward rat primary hepatocytes, as determined by their effect on cell viability, follows the order GM > 17-AAG > 17-DMAG. The apparent discrepancy between the order of toxicity and the rate of $O_2^{\cdot-}$ formation is discussed.