On the cardiac safety of pixantrone in doxorubicin-treated patients: pharmacokinetic and metabolic characterizations in an ex vivo human myocardial strip model

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Doxorubicin (DOX) and other antitumor anthracyclines are life-saving but induce cardiotoxicity. The risk of DOX-induced cardiotoxicity correlates with pharmacokinetic and metabolic determinants like plasma C_{max}, cardiac accumulation, redox activation to reactive oxygen species (ROS) like superoxide anion ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2), reductive metabolisation to the long-lived secondary alcohol metabolite, doxorubicinol (DOXOL). With current protocols and dose reduction strategies, cardiotoxicity may remain subclinical until patients were exposed to second or third line therapies with other drugs that also formed ROS or interfered with the cardiac clearance of residual DOX or increased its metabolisation to DOXOL. The risk of cardiotoxicity is higher when salvage drugs share structural or functional similarities with DOX; for example, the prototypic anthracenedione, mitoxantrone (MITOX), precipitates serious cardiac events in anthracycline-pretreated patients. The novel anthracenedione, pixantrone (PIX) received conditional approval from the European Medicines Agency as monotherapy for the treatment of adult patients with aggressive B-cell non-Hodgkin lymphoma who progressed or relapsed after first line therapy with the DOX-containing CHOP protocol and failed second or third line therapies with other drugs. In the phase III registration study, PIX caused only asymptomatic reversible cardiotoxicity (Pettengel et al., 2012). We therefore characterized whether PIX and MITOX caused different effects on the cardiac levels of DOX, ROS, DOXOL. PIX and MITOX were probed at their clinically documented C_{max} values in a validated ex vivo human myocardial strip model (Salvatorelli et al., 2006). Myocardial strips were used DOX-naïve or were preliminarily subjected to DOX loading and multiple washouts that simulated pharmacokinetic events associated with DOX C_{max} and elimination. In DOX-naïve strips, neither PIX nor MITOX proved able to form ROS, measured by $O_2^{\cdot-}$-dependent inactivation of mitochondrial aconitase or H_2O_2-dependent peroxidase activation and oxidation of dichlorofluorescin to HPLC-detectable dichlorofluorescein (DCF). In DOX-pretreated strips, neither PIX nor MITOX altered the distribution and clearance of residual DOX; however, the two drugs caused different metabolic effects. MITOX synergized with DOX to form more $O_2^{\cdot-}$and H_2O_2. Increased formation of H_2O_2 was also shown by peroxidase activation and formation of peroxidatic MITOX metabolites that were characterized by liquid chromatography-tandem mass spectroscopy. In contrast, PIX lacked a redox synergism with DOX but decomposed spontaneously to an N-dealkylated metabolite that competed for the active site of cytoplasmic reductases and inhibited metabolisation of residual DOX to DOXOL (Salvatorelli et al., 2013). Redox inactivity and inhibition of DOXOL formation correlate with the cardiac safety of PIX in DOX-pretreated patients at risk for cardiac toxicity. Redox inactivity anticipates that PIX might prove acceptably safe also in chemotherapy naïve patients.

Pettengel et al. (2012). *Lancet Oncol*. 13, 696–706.
Salvatorelli et al. (2006). *J Biol Chem* 281, 10990-11001.
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Supported by research agreement with Cell Therapeutics Inc., Seattle, WA