Pixantrone induces cell death through mitotic perturbations and subsequent aberrant cell divisions in solid tumor lines

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Abstract

Background and Aim: Pixantrone (Pix), an aza-anthracenedione which has demonstrable activity in patients with non-Hodgkin’s lymphoma, has both alkylating and intercalating activity but the mechanism of cell killing is unclear. Here we sought to elucidate the mechanisms by examining the effects of Pix on a number of cancer cell lines. Specifically, we assessed the impact of Pix on cell cycle, DNA damage response and their relationships to cell killing.

Methods: Cell lines derived from ovarian, colon, colorectal, pancreatic and breast cancers were tested in response to Pix, with the use of a combination of flow cytometry, microscopy, and immunofluorescence. Cytograms were interpreted in terms of clonogenic survival. We determined effects on cell death, which form (MTS) and long term (clonogenic assay) measures of cell killing. We used a combination of staining with cell cycle markers and immunofluorescence to examine the presence of cell cycle aberrations and the presence of DNA damage foci.

Results: We found that concentrations of Pix that were sufficient to induce cell death in clonogenic assays did not perturb cell cycle progression. We showed that sensitivity to Pix did not correlate with levels of topoisomerase II protein, arguing that cell death was not due to inhibition of topoisomerase II. Immunofluorescence staining showed that cells treated with killing doses of Pix lacked detectable phospho-H2AX foci, consistent with the absence of DNA double strand breaks. Instead, we observed discrete foci of pATM, pChk1, and 53BP1. How this relates to the standard damage response and their relationships to cell killing.

Methods:

Results:

1. Lack of cell cycle perturbation at killing concentrations of pixantrone

2. Lack of DNA damage induced by pixantrone

3. Lack of DNA damage induced by pixantrone

4. Pix induced aberrant DNA damage foci

5. Pixantrone resulted in abnormal mitoses and the generation of chromosome bridges and multinucleated cells

6. Cell death by pixantrone as a result of multiple aberrant cell divisions

7. Cells lacking p53 were less sensitive to Pix at 3 days, but not after 6 days of treatment.

8. Pixantrone-mediated cell death could be enhanced by Chk1 inhibition.

9. Summary

- Under the conditions tested, the effects of Pix on DNA damage induction and cell death appeared to be distinct from the effects of doxorubicin.
- Pix impaired mitotic fidelity, resulting in multiple rounds of aberrant mitosis.
- p53 status may play a role in the efficiency and mechanism by which Pix induced cell death.
- Chk1 inhibitors enhanced sensitivity to Pix, and this warrants testing in clinical trials.

10. Acknowledgements

We wish to thank: Imaging Facility (FCCC); Cell Therapeutics, Inc., for pixantrone and pilot funds; and FCCC Board of Associates Plain and Fancy Award (NB).

1 Pixantrone-induced cell death occurred >3 days after treatment

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