CORRESPONDENCE

Re: Enhanced Cancer Growth in Mice Administered Daily Human-Equivalent Doses of Some H₁-Antihistamines: Predictive In Vitro Correlates

Brandes et al. (1) published a report in the Journal in which they claimed that H₁-antihistamines, such as loratadine, astemizole, and hydroxyzine, enhanced murine tumor growth. As a formal opinion regarding this issue, Health Canada¹ and the Food and Drug Administration (FDA)² declared the necessity of confirmation of the results of Brandes et al. (1). As a result, we reassessed the effects of astemizole on the cell proliferation of murine tumors (B16F10 melanoma cells) to confirm the reproducibility of their results. However, we were not able to reproduce them (2).

As a final approach, we assessed the effects of astemizole on the proliferation of two human-derived tumor cells, HMV-1 melanoma (3-5) and HT1080 fibrosarcoma (6), both in vitro and in vivo.

In the in vitro studies, the number of colonies (≥1 mm diameter) and bromodeoxyuridine (BrdU) uptake (7,8) were measured after a 24-hour treatment with astemizole (10⁻¹²-10⁻³ M). For in vivo studies, 7-week-old female CB-17 scid/scid (SCID) mice (9,10) under anesthesia were given subcutaneous inoculations of cells from each solid tumor (2×2×2 mm). Tumor-bearing mice (each tumor approximately 100 mm³) were randomly assigned to each of four groups and then treated orally with astemizole (6.0, 20.0, or 60.0 mg/m² per day) or the vehicle control for 10 days. On the day after the final dosing, the tumors were excised and weighed.

In in vitro studies, the number of colonies and the amount of BrdU uptake in astemizole-treated groups were not significantly different from those in the control group. Also, in in vivo studies, astemizole did not promote the cell growth of human HMV-1 melanoma or HT1080 fibrosarcoma (Fig.1).

Thus, both our earlier results on B16F10 melanoma conducted in vitro...
and in vivo according to the methods of Brandes et al. (1) and the results of our current study fail to confirm what had been reported previously by Brandes et al. (1). In conducting Brandes’ in vivo test in our laboratory, we found some extreme variation in individual tumor weights in female C57BL/6 mice given subcutaneous transplants of B16F10 melanoma cells, 2) a time-dependent increase in the weight of this tumor, and 3) extreme growth of the tumor at 18-21 days after its subcutaneous transplantation. Generally, tumor transplantation tests require at least that the treatment period must be constant in individual animals. Unfortunately, this was not done in the study by Brandes et al. (1).

In addition, as mentioned above, our results demonstrate that astemizole does not promote the cell growth of human HMV-1 melanoma or HT1080 fibrosarcoma either in vitro or in vivo.

In conclusion, we could not reproduce the results published by Brandes et al. (1). Moreover, astemizole showed no evidence of a carcinogenic response in mouse and rat carcinogenicity studies.

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References


Notes

3) Janssen Pharmaceutica, Beersse, Belgium: personal communication.

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How Does the MRP/GS-X Pump Export Doxorubicin?

The multidrug resistance-associated protein (MRP) gene encodes a human GS-X pump (1,2) that plays a physiologically important role in oxidative inflammation, xenobiotic metabolism, and cancer drug resistance (3). The GS-X pump in mammals is expressed in different organs and cell types, and its function has been characterized by adenosine triphosphate (ATP)-dependent, primary active transport of the following: glutathione disulfide, glutathione S-conjugates (GS-conjugates), cysteinyl leukotrienes, glucuronide conjugates, and certain organic anions, such as methotrexate. In addition, we have recently shown that the human MRP/GS-X pump transports the reduced glutathione (GH)-platinum complex, suggesting a role in the excretion of GSH-heavy metal complexes from cells (4). such a role is consistent with the recent finding that the yeast cadmium factor (YCF1), which has extensive structural homology with the MRP gene (5), encodes a vacuolar GS-X pump in yeast cells (Li Z-S, Szczypka M, Lu Y-P, Thiele DJ, Rea PA: personal communication).

The human MRP complementary DNA (cdNA) was originally cloned and characterized by Cole et al. (6) in doxorubicin-resistant, small-cell lung cancer cells, and, subsequently, expression of the MRP gene was detected in other cell lines resistant to doxorubicin. At present, however, there is still no direct evidence for the ATP-dependent transport of doxorubicin by the MRP/GS-X pump. In our study with a membrane vesicle system, doxorubicin per se was not a direct substrate for the MRP/GS-X pump (Ishikawa T: unpublished results). ATP-dependent transport of GS-conjugates and leukotriene C4 in plasma membrane vesicles prepared from MRP cdNA-transfected cells were reportedly not inhibited by doxorubicin (1). Moreover, the 190-kd MRP/GS-X pump protein in MRP-overexpressing H69AR cells was not labeled with a photoaffinity analogue of doxorubicin (7). These observations suggest that the MRP/GS-X pump may transport metabolic derivatives of doxorubicin but not the original drug.

Zaman et al. (8) recently provided important evidence that cellular GSH is a critical factor for the export of daunorubicin by the MRP/GS-X pump. It has also been reported that non-P-glycoprotein-mediated resistance to doxorubicin is related to cellular GSH levels and GSH-metabolizing enzyme systems (9). Thus, identification of the actual metabolites of doxorubicin and daunorubicin that are substrates for the MRP/GS-X pump is crucial for gaining insight into the role of this export pump in anthracycline resistance.

We propose here a putative metabolic pathway that yields the GS-conjugates of doxorubicin (Fig. 1). Bird et al. (10)