Comparative *in vitro* antitumor activity of homoharringtonine and harringtonine against clonogenic human tumor cells

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**Summary**

Harringtonine and its derivative homoharringtonine are ester-containing anti-leukemic alkaloids isolated from the tree Cephalotaxus harringtonia. In order to compare their antitumor activity against solid tumors, *in vitro* culture of fresh tumor cells from 23 patients was carried out with a soft agar assay system. Tumor cells were exposed to 0.001-1.0 μg/ml of each agent for either 1 h prior to plating or by continuous exposure. Significant antitumor activity was noted for harringtonine in ovarian and endometrial carcinoma at the 0.01 μg/ml concentration. In the continuous exposure studies, homoharringtonine proved to be more potent than harringtonine. Significant antitumor activity of homoharringtonine was noted in sarcoma and breast cancer as well as in ovarian and endometrial carcinoma. In the continuous exposure studies the mean area under the survival-concentration curve was significantly less for homoharringtonine than for harringtonine (5.04 ± 3.87 and 6.15 ± 3.46, respectively (p < 0.005). The ratio of mean ID₅₀ (harringtonine/homoharringtonine) was 5.2. However, there was no significant difference between those two agents with a 1 h exposure. Our results suggest that homoharringtonine and harringtonine may be of use in selected solid tumors, and that homoharringtonine has a greater degree of colony inhibition with continuous exposure.

**Introduction**

Harringtonine is an alkaloid isolated from the Chinese evergreen tree Cephalotaxus harringtonia, and is active against murine leukemias, including the L1210, P388, L615, L7212 and 6MP-resistant line of L615 (1, 2). Harringtonine has been tested clinically in China in acute myeloblastic leukemia, acute monoblastic leukemia and erythroleukemia and appeared to have therapeutic value. One of the major limiting factors in the use of harringtonine was tachycardia (3). An analog, homoharringtonine has also been developed.

The recently developed human tumor stem cell assay of Hamburger and Salmon (4) provides a potentially useful tool for evaluation of activity of new compounds against human tumors. A correlation between activity of conventional cytotoxic agents in this system and clinical effectiveness against the respective human cancer has recently been reported from several centers (5, 6). The assay is also useful for evaluation of new antitumor agents and structural analogs in relation to the parent compound (7). In prior studies using this assay, we found that harringtonine had antitumor activity in sarcoma, melanoma, and mesothelioma as well as adenocarcinomas of unknown origin (8).

Homoharringtonine, a derivative of harringtonine that is isolated from the same tree, has demonstrated antitumor activity against the murine colon 38 tumor, CD8F1 mammary carci-

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noma, B16 melanocarcinoma and P388, L1210 leukemias. Defined doses in mice were determined to be: LD$_{10}$ 4.26 mg/kg and 1.94 mg/kg/day, LD$_{50}$ 5.99 mg/kg and 2.88 mg/kg/day on single and five daily dose schedules, respectively (9). Homoharringtonine is now approved to enter clinical trial under the sponsorship of the National Cancer Institute (U.S.A.). The structures of harringtonine and homoharringtonine are quite similar (Fig. 1). However, it is unknown whether the two drugs will have similar activity as the preparation may not be comparable (6). It was therefore of interest to compare the activity of these agents to assess their relative antineoplastic activity. In this study, we compared harringtonine and homoharringtonine directly in the human tumor stem cell assay.

Materials and methods

Harringtonine was obtained from the Institute of Chinese Materia Medica, Beijing, China. Homoharringtonine was kindly provided by Dr. John Venditti of the Drug Evaluation Branch, Division of Cancer Treatment, NCI. Both agents were water soluble and diluted with sterile water, stored in aliquots at $-80^\circ$C.

Solid tumor biopsies and malignant effusions from a variety of human tumors referred to the Human Tumor Stem Cell Assay Laboratory of the University of Arizona Cancer Center were utilized in these studies. Single cell suspensions were prepared promptly using mechanical techniques described previously (10).

The soft agar method of Hamburger and Salmon (4) was employed for the tumor stem cell assay. Tumor cells to be tested were suspended in 0.3% agar in enriched CMRL 1066 medium to yield a final concentration of $0.5 \times 10^6$ cells per ml. One milliliter of this mixture was pipetted into each of three 35mm petri dishes containing 1 ml 0.5% agar in enriched McCoy’s 5A medium.

For comparison of the activity of homoharringtonine and harringtonine, two schedules of exposure of tumor cells to three concentrations of both drugs were carried out with the same samples. Drugs to be evaluated for their antiproliferative capacity were either preincubated with the tumor cells in McCoy’s 5A medium with 10% heat-inactivated fetal calf serum for 1 h at $37^\circ$C (1 h exposure) or incorporated into the upper layer of the culture system for the duration of culture (continuous exposure). In the latter studies, twice the final concentration of the drug was added to the upper layer, as it rapidly equilibrates in both agar layers.

Colonies (> 60 micron in diameter) were counted 5 - 30 days after plating (average: 24 days) with an automated image analysis system (Bausch and Lomb Omnicon FAS II). At least 30 tumor colonies per control plate were required for a drug experiment to be considered for measurement of drug effect. In this study, the mean number of colonies was 94 per control plate.

In vitro sensitivity was arbitrarily defined as a reduction in survival of tumor colony forming units (TCFU) to 30% of the control at relatively low drug doses (0.01 µg/ml for continuous exposure, 0.1 µg/ml for 1 h). The area under survival-concentration curves was calculated with the trapezoid rule to compare the activity of both agents, with a smaller area considered to represent greater sensitivity. The dosage of each agent which inhibited colony growth to 50% of control (ID$_{50}$) was also calculated, and the ratio of ID$_{50}$ values for the two drugs determined.