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SHORT COMMUNICATION

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Fetal bovine serum, but not human serum, inhibits the in vitro cytotoxicity of ET-743 (Yondelis, trabectedin)

An example of potential problems for extrapolation of active drug concentrations from in vitro studies

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The first studies to assess the biological activity of new anticancer drugs are usually done on tumor cells growing in vitro. The concentrations of the test compound that inhibit growth and cause cytotoxicity are calculated using a series of tumor cell lines [1]. The patterns of sensitivity of different cancer cell lines to the new drug compared to those of known anticancer drugs provide indirect information on the mode of action, as compounds with similar mechanisms appear to have similar patterns [8].

The active drug concentration is of great potential interest as it serves to estimate the concentration needed in plasma of patients to obtain an antitumor effect. The extrapolation, however, is not straightforward because the in vivo growth and survival of cancer cells is influenced by complex interactions with different normal cell populations, such as lymphocytes, macrophages, endothelial cells and stromal cells, that are not present in vitro. Furthermore, it is impossible to reproduce precisely the kinetic changes in the concentrations and exposure times of the drug and its metabolites that occur in vivo. Nevertheless, knowledge of the active in vitro concentrations is useful as a basis for establishing whether the doses achieved in the early clinical investigation with a new drug are potentially effective.

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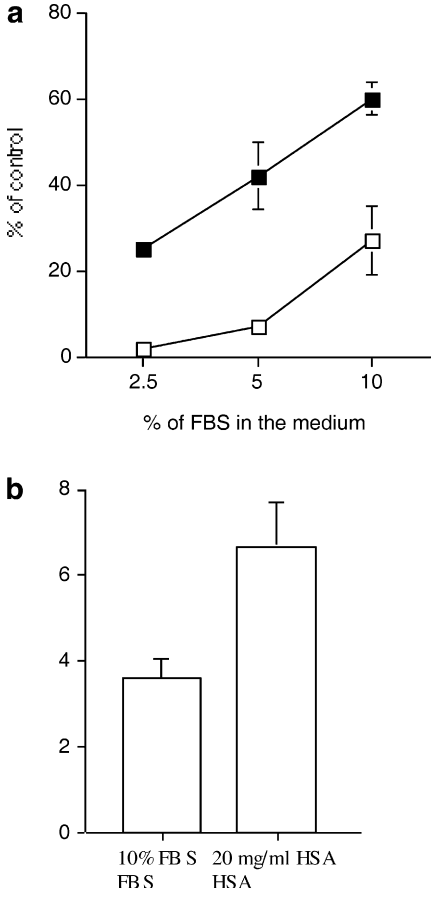

Fig. 1 **a** ET-743 cytotoxicity in Igrov-1 ovarian cancer cells with different added percentages of FBS. ET-743 was first diluted to a concentration of 1 μ*M*, either in medium plus 10% FBS (■) or in 200 mg/ml HSA (□), then diluted to a final concentration of 10 n*M* in medium at different FBS concentrations. Cells were counted 72 h after drug washout. **b** ET-743 intracellular concentrations of ET-743 in medium with 10% FBS or 20 mg/ml HSA. Igrov-1 cells were treated for 1 h with 10 n*M* ET-743 in the presence of 10% FBS or 20 mg/ml HSA and cellular uptake was evaluated by HPLC-MS [4]

When investigating the cytotoxicity of ET-743, a novel marine natural product that has shown antitumor activity in phase I and II clinical studies, particularly in patients with soft tissue sarcomas and ovarian cancer [2, 3, 5, 6, 7], we realized that a further obstacle in the evaluation of the active drug concentration involved the effect of fetal bovine serum (FBS), normally used in tissue culture. On increasing the concentration of FBS from 2.5% to 10% there was a significant loss of cytotoxicity. For example, a 1-h treatment with ET-743 at 10 n*M* caused approximately 75% cytotoxicity 72 h after drug washout against Igrov-1 ovarian cancer cells in the presence of 2.5% FBS, whereas only 40% cytotoxicity was observed in the presence of 10% FBS (Fig. 1a). In the absence of FBS, ET-743 tended to precipitate, and the experiment was no longer feasible, but it was possible to dissolve ET-743 in a 200 mg/ml human serum albumin (HSA) solution, obtaining much higher cytotoxicity (10 n*M* ET-743 caused 100% cytotoxicity). Interestingly, when ET-743 was first dissolved in HSA and then diluted in medium with different percentages of added FBS, there was still an inhibitory effect of serum, but it was less marked. This suggests that the binding of ET-743 to HSA protects the compound from inactivation or absorption (Fig. 1a). Since ET-743 remained stable under these conditions even in the presence of 10% serum, it seems likely that the drug's cytotoxicity is inhibited by FBS through a mechanism implying binding with some protein that partially prevents uptake by the cells.

Figure 1b shows that the intracellular concentration of ET-743 was actually lower in cells exposed to the drug in the presence of 10% FBS than in cells treated with the same concentration of ET-743 but with 20 mg/ml HSA. When the medium was supplemented with human serum, in which the albumin concentration (mean 40 mg/ml) is higher than in the FBS used (23.2 mg/ml), ET-743 cytotoxicity was not significantly reduced.

These findings suggest that any extrapolation from the in vitro condition should be viewed with caution, since the active concentrations determined in vitro can vary several fold depending on culture conditions.

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