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Antitumoral activity of FAC-1 and FIH-2, two hydroalcoholic extracts from plants of the Peruvian Amazonia

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Plant-based molecules have provided a rich source of compounds playing an important role in treatment and prevention of many human diseases, including cancer. In this sense, Amazonian flora is one of the largest reserves of therapeutic resources. We have here studied the *in vitro* antitumoral activity of the hydroalcoholic extracts (code names: FIH-2, FAC-1, AAD-3 and LMC-4) obtained from four plant species (*A. chica*: cortex*; I. phyllomega*: rizhome*, M. cuspidatum*: cortex *and A. duckei*: leaves) collected in the Amazonian native community of Payorote (Loreto, Peru).

First, we evaluated the antiproliferative activity of these extracts in two tumor cell lines MDA-MB-231 (human breast adenocarcinoma) and SKOV-3 (human ovary adenocarcinoma), cultured according to previously described protocols (Walter-Yohrling *et al.*, Cancer Res 63: 8939-47, 2003). Significant differences were determined by one-way ANOVA followed by Bonferroni's *post-hoc* test. FAC-1 (300 µg/ml) inhibited proliferation in MDA-MB-231 after 48 h (relative number of treated cells: 0.37±0.34 *vs.* 0.86±0.01 control cells, $P< 0.05$, n=3) and 72 h (0.41±0.06 *vs.* 0.95±0.02, $P< 0.01$, n=3) in SKOV-3 after 72 h (0.47±0.03 *vs.* 0.70±0.03, $P< 0.01$, n=3). FIH-2 (300 µg/ml) significantly inhibited proliferation of MDA-MB-231 after 72 h (0.97±0.01 *vs.* 1.22±0.002, $P< 0.01$, n=3) although it did not significantly modify SKOV-3 proliferation. On the other hand, AAD-3 y LMC-4 (3-300 µg/ml) did not shown *in vitro* antiproliferative activity in any of the cell lines studied. The antiproliferative activity of FAC-1 (300 µg/ml) was confirmed by experiments of bromodeoxyuridine (BrdU) incorporation (which allows labelling of cells in S phase of the cell cycle). % of incorporated BrdU: 9.4±0.6 (24 h) and 5.2±0.9 (72 h) in treated cells *vs.* 23.9±2.9 (24 h) and 37.3±6.5 (72 h) in control cells ($P< 0.01$, n=3). We then evaluated the pro-apoptotic activity of FAC-1 by means of Hoechst-33258 solution (bisbenzimide, 50 µM), which binds adenine plus thymine rich DNA regions, and fluorescence microscopy. A 72 h incubation of cells with FAC-1 (300 µg/ml) significantly induces apoptotic activity in both MDA-MB-231 (% of apoptotic cells: 80.2±5.2 of treated cells *vs.* 4.1±0.9 of control cells, $P< 0.01$, n=3) and SKOV-3 cells (64.4±2.3 *vs.* 3.3±1.4, $P< 0.01$, n=3). In another set of experiments, we have investigated if FAC-1 alters the expression of several proteins implicated in cell cycle progression and apoptosis. Pre-treatment of MDA-MB-231 with FAC-1 (300 µg/ml) induces a time-dependent increase in the protein expression of p53, a transcription factor that acts as a tumor suppressor, although the expression of phopho-p53 was similar after 24 h or 72 h of pre-treatment. However, there was a lack of expression of the tumor suppressor p21 both in control or FAC-1 pretreated MDA-MB-231. Pre-treatment of SKOV-3 with FAC-1 (300 µg/ml) did not induce expression of p53, p21 or □-H2AX, a marker of DNA damage response (DDR).

In conclusion, FAC-1 possesses an *in vitro* antitumoral activity in MDA-MB-231 and SKOV-3 cell lines. It presents antiproliferative and pro-apoptotic activity and actives DDR. FIH-2 presents antiproliferative activity in MDA-MB-231 cells. Therefore, both extracts may provide a basis for subsequent isolation studies of active compounds or serve as a model for the development of new synthetic or semi-synthetic molecules with potential anticancer action.