Activity of Novel Plant Extracts Against Medullary Thyroid Carcinoma Cells

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Abstract. Background: Medullary thyroid carcinoma (MTC) is a rare calcitonin-producing tumor, derived from the parafollicular C-cells of the thyroid. MTC is known to be relatively insensitive to conventional chemotherapy. Materials and Methods: Eight cell lines were established from MTCs; each showed an up-regulation of Bcl-2. We investigated ten agents from plants of the genera Stemona (Stemonaceae), Aglaia (Meliaceae) and Artemisia (Asteraceae) for their effects on proliferation and apoptotic rates. Extracts have been used in traditional Chinese medicine; however, no experience on their effects on medullary thyroid carcinomas has been reported so far. Growth kinetics and viability were examined using the Casy-1-Cell Counter & Analyzer and the WST-1 -based cytotoxicity assay. Apoptosis was studied by DAPI staining, by measurement of caspase-3 activity and Bcl-2 expression. Results: A strong antiproliferative effect was recognized in each Aglaia species and with Artesunate, whereas an enhancement of apoptosis was provoked particularly by Stemona tuberosa Lour. Conclusion: The activity of the novel plant extracts possibly offers a new approach towards successful chemotherapy of the so far chemo-resistant medullary thyroid carcinoma.

Medullary thyroid carcinoma (MTC) is a rare calcitonin-producing tumor, derived from the parafollicular C-cells of the thyroid. MTC can be sporadic, familial (FMTC), or part of the inherited cancer syndromes Multiple Endocrine Neoplasia type 2A (MEN2A) and type 2B (MEN2B). (1) MTC is inherited in 25-30% of all cases due to mutation in the RET-proto-oncogene, located on chromosome 10 (10q11.2). Presently the only treatment option is surgery, as MTC is known to be relatively insensitive to chemo- or radiation therapy.

Increased expression of the anti-apoptotic protein Bcl-2 is involved in the development and progression of many tumors (2). Bcl-2 localizes to cellular membranes, particularly in mitochondria, where it stabilizes the transmembrane potential and reduces membrane permeability. Bcl-2 seems to contribute to tumor cell survival by enhancing the rate of cell proliferation and by allowing tumor cells to escape destruction by effector cells of the immune system.

Caspase-3 is a key protease that is activated during the early stages of apoptosis and, like other members of the caspase family, is synthesized as an inactive proenzyme that is processed in cells undergoing apoptosis by self-proteolysis and/or cleavage by another protease.

The aim of this study was to investigate new approaches using plant extracts against chemo-resistant medullary thyroid carcinoma.

Materials and Methods

Cell culture. Eight continuous cell lines had been previously established and characterized from biochemically and histologically confirmed medullary thyroid carcinomas: MTC-SK (3), SINJ (4), GRS-IV, GRS-V, BOJO, SHER-1, RARE and GSJO (5). The cells were cultured in Ham's F 12 medium (Biowhittaker, Belgium) containing 10% fetal bovine serum (PAA Laboratories, Exton, PA, USA), at a cell density of 2 x 10⁵ cells/ml and incubated at 37°C in a 5% CO₂ and 90% humidity incubator. Control cells were normal human skin fibroblasts.

Tested substances. Crude extracts of seven Aglaia species, of two Stemona species and one semisynthetic substance (Artesunate) were tested. Each substance was screened in concentrations between 0.1 and 135 µg/ml in order to define the LC₅₀.
a) Aglaia sp: The genus Aglaia (Meliaceae) is characterized by the presence of different types of flavaglines, comprising cyclopenta[b]tetrahydrobenzofurans (roc-agonamides), cyclopenta-[bc]benzopyrazines (aglain, aglaforbesins, thapsakins) and benzo [b]pyrroloines (forbaglines, thapoxepines) (6,7). Together with typical bisamides, flavaglines in particular represent a distinct phytochemistry restricted to the genus Aglaia (8). Extracts from seven Aglaia species were tested: A. odorata Lour, A. basiphylla A. Gray, A. gracilis A.C. Smith, A. edulis (Roxb.) Wall, A. tenuicaulis Hiern, A. coriacea Miq. and A. eaeagnoidae (A. Juss) Benth. 
b) Stemona sp: The roots of Stemona have long been recommended in Chinese and Japanese traditional medicine for the treatment of respiratory diseases, as well as against enteric helminths and ectoparasites on humans and cattle and insect pests. The biological activity of alkaloids, stilbenoids and tocopherols is gaining particular interest. Rare pyrrolo(1,2-a)azepin-alkaloids, so far verified solely in Stemonaaceae, are responsible for the insecticidal effects (9, 10). Extracts from Stemona tuberosa Lour and Stemona collinsae Craib were tested. 
c) Artesunate: Artesunate is a semisynthetic derivative of artesunate, the active compound of the Chinese herb Artemisia annua L. It shows remarkable activity against Plasmodium falciparum and P. vivax; it is highly effective in the treatment of severe malaria and shows antiviral properties. Artesunate is also active against cancer (11-13), but has never been tested against medullary thyroid carcinoma.

**Results**

The LC₅₀ was determined for each substance. Figures 2 and 3 demonstrate the effects of crude extracts of Stemona tuberosa Lour on proliferation and caspase-3 activity on the MTC cell line GSJO. 

Aglaia edulis (Roxb.) Wall, A. gracilis AC Smith, A. coriacea Miq., A. eaeagnoidae (A. Juss) Benth, A. odorata Lour, LC₅₀ for each extract was 0.5 µg/ml. The decrease in cell counts was dose-dependent. The accumulation of cells was not influenced. DAPI-staining and Bcl-2-anaalyses showed no apoptotic cells. The highest caspase-3 staining was observed after 48 h and did not exceed 15%. 

Aglaia tenuicaulis Hiern. LC₅₀ was 30 µg/ml. Antiproliferative effects but no apoptotic signals were observed. A. basiphylla A. Gray. LC₅₀ was 5 µg/ml. Antiproliferative effects but no apoptotic signals were observed.

Stemona tuberosa Lour. LC₅₀ was 50 µg/ml. Originally aggregating cells altered their morphology and separated (Figures 1a and b). The numbers and sizes of cells decreased with increasing drug concentration (Figures 2a, b, c). St. tuberosa Lour caused apoptotic effects: in contrast to normal cells, the nuclei of apoptotic cells had highly condensed chromatin that was uniformly stained by DAPI. Crescents appeared around the periphery of the nucleus, or the entire nucleus appeared to be one bead, or a group of featureless, bright spherical beads. After 12h, flow cytometric analyses showed no effects on Bcl-2 expression; however, one-third of the treated cells were positive for active caspase-3 staining (Figures 3a, b, c).

Stemona collinsae Craib. LC₅₀ was 40 µg/ml. Originally aggregating cells altered their morphology and separated. The numbers and sizes of cells decreased with increasing drug concentration. After 48h, flow cytometric analyses displayed only 14% of treated cells positive for active caspase-3 staining. DAPI-staining showed no apoptotic cells. Artesenate: LC₅₀ is 0.04 µmol. Antiproliferative effects but no apoptotic signals were observed.

**Discussion**

A previous study (Strasser *et al.*, [2]) supports the hypothesis that high-level expression of Bcl-2 confers a chemo-resistant phenotype on tumor cells. We studied the use of plant extracts at room temperature in the dark. After incubation, the cells were washed in Perm/Wash™ Buffer twice and analysed by flow cytometry (Partec Pas III).
Figure 1. a) GSJO cell line, growing as a suspension of multicellular aggregates, untreated control. b) Treatment with extracts from *S. tuberosa* Lour (LC$_{50}$) for 24 h. The multicellular aggregates dissociated.
Figure 2. a) GSJO cell line, cell counts in untreated control. Low percentage of dead cells and cell debris (left peak). b) Cell counts after treatment with S. tuberosa Lour extract (LC₅₀) for 24 h. High percentage of dead cells and cell debris (left peak). c) Cell counts after treatment with S. tuberosa Lour extract (LC₅₀) for 48 h. High percentage of dead cells and cell debris (left peak).
to inhibit proliferation and to induce apoptosis in medullary thyroid cancer cells. Efferth et al. (11 - 13) tested Artesunate on 55 human tumor cell lines (leukemia, melanoma, non-small cell lung cancer, colon cancer, renal cancer, ovarian carcinoma, tumors of the central nervous system, prostate carcinoma and breast cancer). All tumor cell lines were sensitive towards the drug. In our study, the MTC cell lines became necrotic after Artesunate treatment but apoptosis was not found. Extracts of Aglaia sp. had a similar effect; cell death could be quickly achieved, but it was not possible to observe an increase in caspase-3 or positive DAPI-staining.

However, extracts from different Stemonaceae caused different effects: Stemona collinsae Craib had a moderately enhancing effect on apoptosis, while Stemona tuberosa Lour enhanced apoptosis considerably. Both extracts from S. tuberosa Lour and S. collinsae Craib altered the phenotype of the MTC cells from originally aggregating cells towards single-cell suspensions. We assume that the loss of cellular aggregation actually contributes to the effects of the tested substances.

Compared with the effects of the other extracts from Aglaia sp. and Artesunate, Stemona extracts had the strongest apoptotic effects on MTC cells. Stemona tuberosa Lour in particular showed the most promising results in MTC cells. Normal cells were less impaired.

In summary, our findings demonstrate that the proliferation of medullary thyroid carcinoma cells is influenced by different plant extracts, and that Stemona does induce apoptosis. Stemonaceae had not been tested before on MTC cells, but the activity of the lipophilic plant extracts possibly offers a new approach towards successful chemotherapy of the so far chemo-resistant medullary thyroid carcinoma.

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References


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