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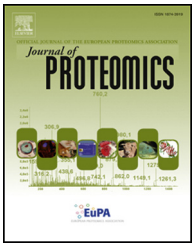

Activity of aaptamine and two derivatives, demethyloxyaaptamine and isoaaptamine, in cisplatin-resistant germ cell cancer

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ABSTRACT

We analyzed the effects of all three marine alkaloids aaptamine, demethyloxyaaptamine and isoaaptamine in NT2-R, a cisplatin-resistant subline of the human embryonal carcinoma cell line NT2. All aaptamines were found to be equally effective in both cell lines, excluding cross-resistance between aaptamines and cisplatin in vitro. At the inhibitory concentration (IC_{50}), aaptamine exerted an antiproliferative effect, whereas demethyloxyaaptamine and isoaaptamine were strong apoptosis inducers. We analyzed the changes in the proteome of NT2-R cells treated with these compounds. 16–22 proteins were found to be significantly altered, of which several were validated by Western blotting and two-dimensional Western blotting analysis. Changes in the proteome pattern frequently resulted from post-transcriptional protein modifications, i.e. phosphorylation or hypusination in the case of eIF5A. Although the lists of altered proteins were heterogeneous and compound-specific, gene ontology analyses identified rather similar profiles regarding the affected molecular functions. Ingenuity pathway analysis by IPA put the following factors in a central position of the hypothetical networks: myc and p53 for aaptamine; tumor necrosis factor (TNF) for demethyloxyaaptamine; and all three, myc, p53, and TNF for isoaaptamine. Our results represent an important step towards a better understanding of the molecular basis underlying the observed bioactivity of these promising marine compounds.

Abbreviations: 2-D WB, Mini-2D Western blotting analysis; Cisplatin, *Cis*-diamminedichloroplatinum (II); eIF5A, Eukaryotic initiation factor 5A-1; Hsp70, Heat shock protein 70 kDa; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

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Biological significance

We characterized the mode-of-action of three aaptamines, marine natural compound with anti-tumor activity, using a functional proteomics approach and cisplatin-resistant pluripotent human embryonal carcinoma cell line NT2-R. The manuscript is of particular scientific interest, because we could revile the similarities and differences of the modes of action as well as to identify several new targets of these promising compounds. We found hypusination promotion of eIF5A to be a prominent feature of aaptamine treatment, and was not observed upon treatment with demethyloxyaaptamine or isoaaptamine. Our results are a step towards unraveling the mode of action of these interesting compounds.

1. Introduction

Thirty years ago, Nakamura et al. reported the isolation of aaptamine, a new benzo[*de*] [1,6]-naphthyridine alkaloid with α-adrenoreceptor blocking activity, from the marine sponge *Aaptos aaptos* [1]. Later, other substances belonging to this class of heteroaromatic substances were isolated from marine sponges belonging to different families and orders. The most commonly occurring and therefore best characterized natural aaptamine alkaloids are aaptamine (**1**), demethyloxyaaptamine (**2**), and isoaaptamine (**3**) [2] (Fig. 1A).

Aaptamines have previously been shown to possess antimicrobial, antifungal, antiretroviral, cytotoxic, antifouling, enzyme inhibiting, antioxidant (for review, see [2]), as well as cancer-preventive activity [3]. At the same time, the molecular modes of action of aaptamine and its derivatives are largely unknown. In recent studies, it was shown that aaptamine intercalates into DNA with relatively weak binding affinity [4], inhibits proteasome activity in a manner unrelated to cytotoxicity [5], and possesses antiproliferative properties (stipulated by p21 up-regulation and induction of a G2/M cell cycle arrest) at low [6–8], and pro-apoptotic properties at high concentrations [7,9]. Molecular

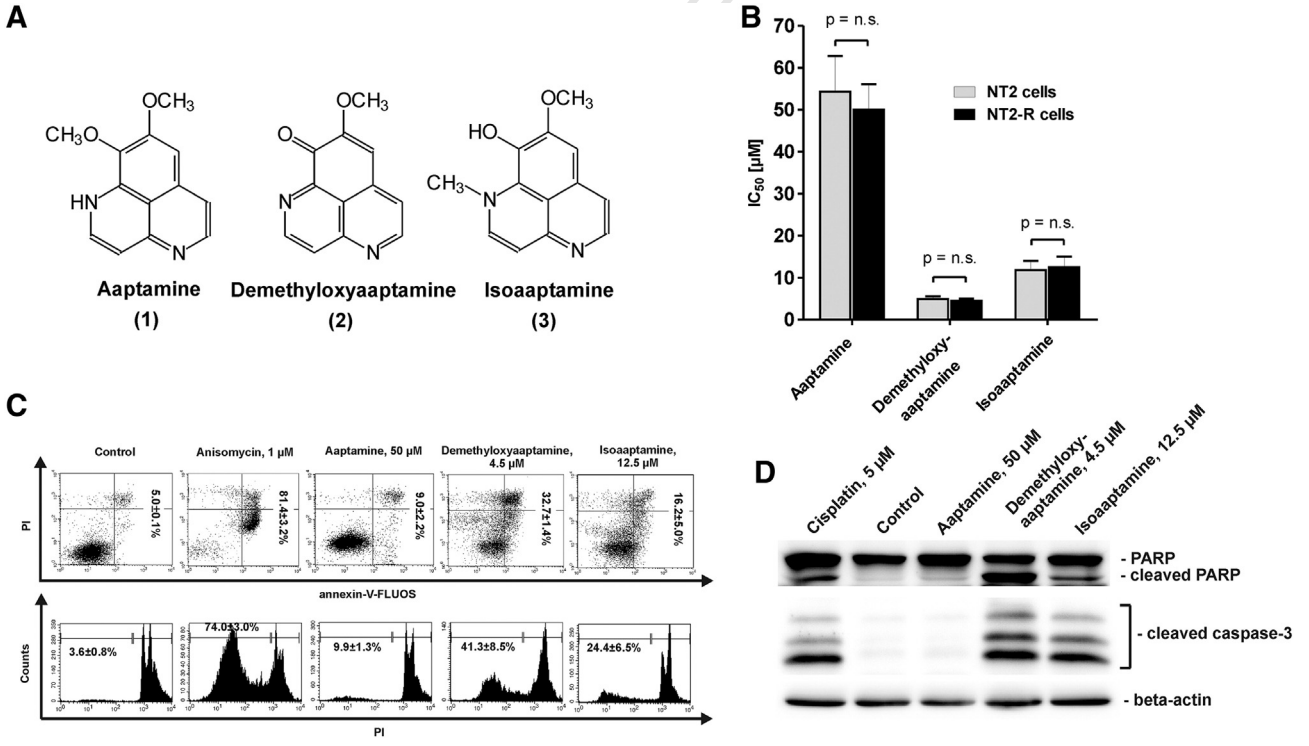

Fig. 1 – Effect of aaptamine, demethyloxyaaptamine, and isoaaptamine on cell proliferation and viability in NT2-R cells treated with aaptamine, demethyloxyaaptamine, and isoaaptamine f. (A) Structure of aaptamine (1), demethyloxyaaptamine (2), and isoaaptamine (3). (B) IC_{50} values of the substances 1, 2, and 3, (trypan blue assay). No difference in the values for NT2 and NT2-R cells were detected; n.s.: not significant. (C) Flow cytometry analysis of NT2-R cells treated with 1, 2, or 3: Annexin-V-FLUOS versus PI (double staining, upper row), and PI staining of DNA (lower row). Apoptotic cells appear in the right upper and right lower quadrants (upper row) or as a sub-G1 peak in the cell cycle analysis (lower row). NT2-R cells treated with anisomycin (1 µM for 48 h) represent the positive control (D) Western blotting analysis of protein extracts of NT2-R cells treated with 1, 2, or 3, analyzing cleavage of PARP and caspase-3 as hallmarks of apoptosis. NT2-R cells treated with cisplatin (5 µM for 48 h) represent the positive control.

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