ATB-346 induces apoptosis of human melanoma cells and inhibits melanoma development in vivo.

G. Ercolano[1], C. Armogida[1], E. Panza[1], P. De Cicco[1], G. Cirino[1], A. Ianaro[1].
[1]Dept. of Pharmacy, University of Naples Federico II, Naples, Italy.

Chronic inflammation is associated with an increased risk of malignant disease. The main enzyme involved in tumor promotion and development is cyclooxygenase-2 (COX-2), through the production of prostaglandins, which in turn act directly on cancer cells to inhibit apoptosis and enhance cell migration[1]. Numerous physiological and patho-physiological roles have been proposed for the gasotransmitter hydrogen sulfide (H_2S), along with a plethora of cellular and molecular targets [2]. H_2S is also involved in the regulation of cancer biological processes and both pro- and anti-cancer effects have been described for this molecule [3]. An interesting class of new compounds has been developed in the last few years combining traditional NSAIDs with a chemical moiety that donates hydrogen sulfide. To gain further insights into the role played by H_2S and COX-2 in human melanoma, we evaluated, by using in vitro and in vivo approaches, the effect of ATB-346, a new H_2S-releasing derivative of naproxen that inhibits COXs but also releases H_2S.
Toward this goal, we evaluated the effect of ATB-346, Naproxen (parent drug), and TBZ (the hydrogen sulfide-releasing moiety), on a panel of human melanoma cells. Only ATB-346 inhibited the growth of all cell lines tested in a time-dependent manner. In order to evaluate if the anti-proliferative effects of ATB-346 was due to apoptosis or necrosis, flow citometry analysis by double staining with Annexin V and propidium iodide (PI), was carried out on A375 cells with ATB-346, Naproxen and TBZ (100 μM for 24 and 48h). Only ATB-346 induced apoptosis of A375 cells in a time-dependent manner. This effect was accompanied by a time-dependent activation of caspase 3 and the cleavage of its substrate PARP. In melanoma the constitutive activation of NF-κB confers tumor survival capacity and apoptosis avoidance[4], thus Western blot analysis was carried out on the cytosolic extracts obtained from A375 cells treated with ATB-346 100 μM for 15, 30, and 60 min and showed an inhibition of IκBÎ± degradation at the earliest time points and a reduction in band intensity of the subunit p65. Moreover, the expression of the anti-apoptotic proteins, XIAP and Bcl-2, that is transcriptionally regulated by NF-κB, was greatly reduced.
The most frequently deregulated pathway in melanoma is PI3K/Akt[5], that plays an important role in melanoma development and progression and is involved in the mechanism of resistance to targeted therapy. Treatment of A375 cells with ATB-346 (100 μM) significantly reduced p-Akt band intensity, suggesting a specificity of effect on the activation of the Akt/p-Akt/NF-κB signaling pathway. Finally to define if the effects described above translate in an in vivo setting, we subcutaneously implanted B16-F10 murine melanoma cells in C57BL/6 mice. Tumor-bearing mice were treated twice daily with ATB-346, naproxen or TBZ, all drugs were administered orally at an equimolar dose of 43 μmol/kg. Mice receiving ATB-346 displayed a reduction of tumor size by 61% as compared to control mice 14 days after tumor implantation. Also tumor wet weight was significantly reduced by ATB-346 by 62% as compared to control mice. Among chemokines CXCL1, a member of the CXC chemokine subfamily, has been associated with metastatic melanoma [6]. Only ATB-346 induced a significant reduction of CXCL1 plasma levels by 67% as compared to control mice. In conclusion our results demonstrated that ATB-346 is a more effective agent that can decrease melanoma development, by targeting the COX and PI3K/Akt pathways, without causing major organ-related toxicity.

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