H_2S-donors induce apoptosis of human melanoma cells

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Cutaneous malignant melanoma is the most aggressive form of skin cancer, with a high mortality rate. Various treatments for malignant melanoma are available, but due to the development of multi-drug resistance, current or emerging chemotherapies have a relatively low success rates. This emphasizes the importance of discovering new compounds that are both safe and effective against melanoma (Tawona N. et al., 2014). In the last few years, numerous physiological and pathophysiological roles have been proposed for the gasotransmitter hydrogen sulphide (H_2S) along with a plethora of cellular and molecular targets. H_2S is endogenously produced by the action of three enzymes CBS, CSE and the newly discovered 3-MST (Wang R. 2012). While H_2S is cytoprotective at physiological concentrations, it seems to have pro-apoptotic actions in cancer cells (Predmore BL. et al., 2012). However, to date there are not definitive reports on the role played by H_2S in cancer development.To gain further insights into the role played by H_2S in human melanoma, we evaluated the effect of several H_2S-donors (NaHS, DATS, GYY4137, Thioglicyne and Thiovaline) on A375 melanoma cell proliferation. All the compounds, but NaHS, inhibited the growth of A375 cells in a concentration-dependent manner. Among all the H_2S-donors tested we focused our attention on the potential antitumor effect of synthetic H_2S-donor GYY4137. The effects of this compound was also evaluated on other three different human melanoma cell lines: SK-Mel-5, SK-Mel-28 and PES43. We found that GYY4137 decreased in a concentration-dependent manner the proliferation rate of all melanoma cells used. Normal human epidermal melanocytes (NHEM) were used as control.In order to evaluate if the anti-proliferative effects of GYY4137 was due to apoptosis or necrosis, flow cytometry analysis by double staining with Annexin V and propidium iodide (PI) was carried out. This dual staining distinguishes between unaffected cells early apoptotic cells late apoptotic cells and necrotic. Treatment of A375 cells for 24, 48 and 72h with GYY4137 (1000μM) resulted in a time-dependent induction of apoptosis. In particular, at 72h almost all cells (78%) exhibited markers of late apoptosis. This effect was accompanied by a time-dependent activation of caspase 3 and the cleavage of its substrate poly(adenosine diphosphate-ribose) polymerase (PARP).The main transcription factor involved in the regulation of apoptosis is NF-κB, thus we hypothesized that the H_2S donors induction of apoptosis was associated with suppression of NF-κB activation. Western blot analysis carried out on the nuclear extracts of A375 cells incubated with GYY4137 for 3-6-24h resulted in a time-dependent reduction of p65 nuclear translocation and activation. Moreover, the expression of the anti-apoptotic proteins c-FLIP, XIAP and Bcl-2, that is transcriptionally regulated by NF-κB (Ben-Neriah and Karin, 2011), was greatly reduced following treatment of cells with GYY4137 1000 μM for 3-6 and 24h.Two of the most frequently deregulated pathways in melanoma are mitogen-activated protein kinase (MAPK)/ERK and phosphoinositide 3-kinase (PI3K)/AKT (Hodis et al., 2012). These two pathways play an important role in melanoma development and progression and are involved in the mechanism of resistance to targeted therapy (Flaherty et al., 2012). Western blot analysis revealed that treatment of A375 cells with GYY4137 1000μM inhibited the phosphorylation and activation of both AKT and ERK at all time considered (3-6-24h). In conclusion we have demonstrated that both natural and synthetic H_2S-donors inhibits human melanoma cells proliferation by inhibiting pro-survival pathways associated to NF-κB activation. Our results establish H_2S-donors as new potential agents in the treatment of human metastatic melanoma and represent a very promising strategy to improve the fight against cancer.