Effects of Sulfasalazine on Cell Death Induced by Cold AtmosphericHelium Plasma and X-irradiation in Molt-4 Cells

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研究分野 Research area

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研究内容 Research content

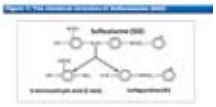
Sulfasalazine (SSZ) is an FDA- approved a non-toxic anti-inflammatory drug. It also acts as an inhibitor of tumor growth and induces apoptotic cell death by suppression of glutamate transporter xCT, which mediates to maintain the synthesis of intracellular glutathione (GSH) and redox balance. However, the combination of SSZ with other physical modalities remains elusive. Therefore, the present study aimed to clarify the efficacy of SSZ in combination with helium cold atmospheric plasma (He-CAP) or X-irradiation (IR)-induced cell death in human T lymphoblast; acute lymphoblastic leukemia Molt-4 cells. Molt−4 cells were treated with He−CAP or IR in present or absence of SSZ and apoptosis was measured by DNA fragmentation assay and Annexin V-FITC/PI, double staining flow cytometrically. Detection of intracellular reactive oxygen species (ROS), intracellular GSH, and mitochondrial membrane potential (MMP) was performed by using flow cytometry. Furthermore, western blot analysis was employed to determine the expression of caspase-3 & 8, ER-stress related proteins (Chop & Bip) and Bcl-2 family proteins. The results showed that the combined treatment of SSZ with He-CAP and IR can enhance the apoptotic cell death. Markedly increased in the reactive oxygen species (ROS) production and decreased intracellular GSH was observed following combined treatment. These findings suggest the potential of SSZ in combination with He-CAP or IR and might be helpful for the future therapeutic application.

研究のポイント Research point

The present studies clarify that SSZ can potentiate the He-CAP and IR-induces apoptotic cell death in Molt-4 cells by reducing intracellular GSH while increasing reactive oxygen species (ROS) and subsequent activation of the cell death related signaling. As a result, co-treated Molt-4 cells showed significantly enhanced of apoptosis cell death compared with treatment alone. SSZ enhanced the He-CAP-induced apoptosis via a modulation of ROS-mediated mitochondrial-caspase and Ca2+ dependent pathways. The molecular mechanisms of enhancement of IR-induced apoptosis by SSZ are associated with extrinsic and intrinsic apoptotic pathways. Furthermore, ROS-mediated ER stress induction may also a role in the enhancement of He-CAP and IR-induced apoptosis by SSZ. In addition, we found that SSZ does not show the crucial cytotoxic effect in human lymphoma Molt-4 cells. Based on these results we proposed that only SSZ might be an effective sensitizer for He-CAP and radiotherapy. Considering the low toxic and clinically evaluated SSZ, in combination with He-CAP or IR may appear a good lead for future clinical studies to establish promising therapeutic strategy against lymphoma cancer. However, for any progression towards a patient therapy, more studies need to be performed regarding the mechanism of action.

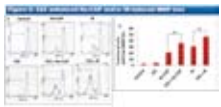
研究への取組、今後の展望

研 究 REPORT

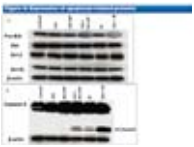


The chemical diagram of Sulfasalazine (SSZ) and its bio-activation components

SSZ increased He-CAP and IR-induced cell death in Molt-4 cells. The cells were pre-treated with SSZ for 90 min and then exposed to He-CAP and IR and then incubated at 370C. (A) Cell survival analysis was carried out by cell counting kit-8 after 6 h. (B) The viability of cells in the presence or absence of SSZ was examined by using trypan blue exclusion dye at 6, 12, and 24 hrs after incubation. The results are presented as mean ± SD (n=3).*P<0.05, **P<0.01 as compared with He-CAP or IR treatment alone.



SSZ increased He-CAP and IR-induced MMP loss in Molt-4 cells. Cells were treated with He-CAP and IR in the presence or absence of SSZ and then incubated at 370C for 6 h. (A) Flow cytometric histograms of MMP loss. (B) Increased loss of MMP was detected by flow cytometry by using TMRM staining, 6 h after He-CAP and IR exposure. The results are presented as mean ± SD (n=3).*P<0.05, **P<0.01 as compared with He-CAP or IR treatment alone

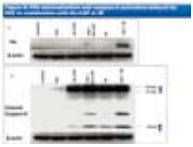


Assessment of apoptosis related proteins. Molt-4 cells were treated with He-CAP and IR with or without SSZ. Protein was extracted from the cells 6 h after treatment and western blot analysis was performed to observe the changes in the expression of apoptosis related proteins. (A) Changes in the expression of Bcl-2 family proteins and (B) caspase-3 were detected by western blot.

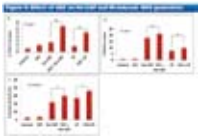
SSZ enhanced He-CAP and IR-induced apoptosis in Molt-4 cells. The cells were treated with He-CAP (1min) and IR (2 Gy) with or without pre-treated with SSZ (0.25mM) for 90 min and incubated at 370C. Apoptotic features of Molt-4 cells were analyzed 6 h after He-CAP and IR treatment. (A) Percentages of early apoptosis and secondary necrosis were measured by flow cytometry after stained the cells with Annexin V-FITC and PI. (B) Morphological feature of apoptosis in Molt-4 cells in response to the combined treatment with He-CAP and/or IR and SSZ. Signs of apoptosis were detected by Giemsa staining and then examined under a microscope at x400 magnification. One representative photomicrograph from three independent experiments was shown here. (C) DNA fragmentation assay was carried out by using flow cytometry. (D) Molt-4 cells were pre-treated with 5 mM NAC for 1 h prior to the treatment of SSZ. Cells were harvested 6 h after He-CAP and IR treatment and measurement of apoptosis by annexin V-FITC/PI staining. The results are presented as mean ± SD (n=3).*P<0.05, **P<0.01 as compared with He-CAP or IR treatment alone.

SSZ increased GSH levels in combination with He-CAP or IR by inhibiting xCT. Molt-4 cells were pre-treated with SSZ for 90 min prior to exposing He-CAP and IR and then incubated at 370C for 6 h. (A) Changes in the expression level of xCT were detected by western blot analysis. (B) Low intracellular reduced GSH was measured by using an intracellular GSH kit. The results are presented as mean ± SD (n=3).*P<0.05, **P<0.01 as compared with He-CAP or IR treatment alone.

SSZ enhanced He-CAP and IR-induced [Ca2+]i levels via ER-stress mediated pathway. Molt-4 cells were pre-treated with SSZ for 90 min and then exposed to He-CAP and IR and then harvested 6 h after treatment. (A) ER-stress related proteins Chop and Bip expression were detected by western blot analysis. (B) Cells were loaded with 5 μM calcium probe Fluo-3/Am for 30 min and fluorescence intensity was detected by flow cytometry. The results are presented as mean ± SD (n=3). *P<0.05, **P<0.01 as compared with He-CAP or IR treatment alone.



Effect of SSZ on He-CAP and IR-induced FAS externalization and caspase-8 activation. Molt-4 cells were pre-treated with SSZ for 90 min and then exposed to He-CAP and IR. Western blot analysis was performed to detect the expression of diverse proteins 6 h after He-CAP and IR treatment. (A) The externalization of Fas and (B) The expression of caspase-3 were evaluated by western blot analysis.



Effect of SSZ on He-CAP and IR-induced ROS generation. Cells were pre-treated with SSZ for 90 min and then treated with He-CAP and/or IR. The percentage of cells with an elevated level of ROS species were detected by flow cytometry at the indicated time after He-CAP and IR treatment. (A) DCFH stains, (B) HPF staining. Fluorescence intensity was detected immediately after He-CAP and IR treatment. (C) HE staining. cells were harvested 6 h after He-CAP and IR treatment. The results are presented as mean ± SD (n=3).*P<0.05, **P<0.01 as compared with He-CAP or IR treatment alone.

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