Nimesulide acts synergistically with ionizing radiation against A549 human lung cancer cells through the activation of caspase-8 and caspase-3

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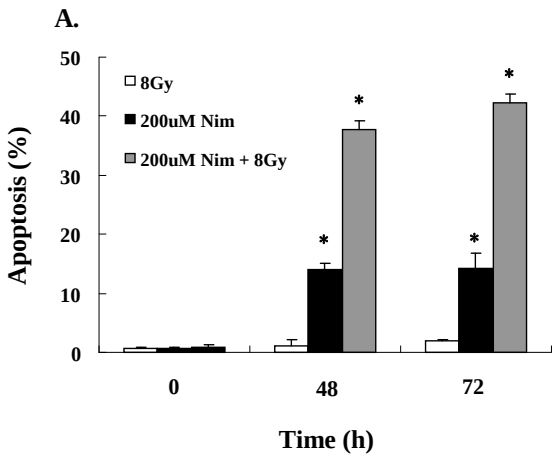
1. Introduction

Radiotherapy is important in the treatment of non-small cell lung cancer, but very few malignancies have been cured using single modalities of radiotherapy. Therefore, molecules that can target specific pathophysiological or molecular pathways have been investigated for use as radiation sensitizers. Cyclooxygenase (COX)-2 inhibitors have been shown to enhance the radioresponse of cultured human cancer cell lines and immunodeficient mice . However, little is known about the molecular and biochemical mechanisms by which COX-2-selective non-steroidal anti-inflammatory drugs (NSAIDs) enhance the radioresponse of tumor cells. In some types of cancer, radiation is thought to work by inducing apoptosis, and effective anticancer radiotherapy is frequently associated with increased levels of apoptosis markers *in vitro* and *in vivo*.

2. Methods and Results

1. ***Combined nimesulide–radiation treatment enhances the radiation response of A549 lung cancer cells***



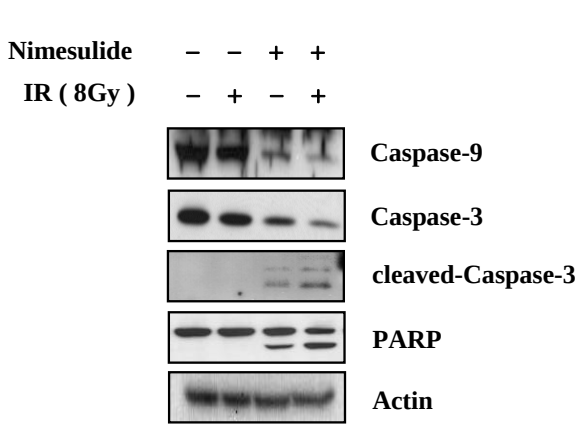


Figure 1 Pro-apoptotic effect of combined nimesulide–radiation treatment in A549 cells. At $t = 0$, cells were pretreated with nimesulide (200 μM) for 24 h before treatment with radiation (8 Gy), and then cultured for an additional 24 h or 48 h ($t = 48$ h or 72 h, respectively). (A) To measure apoptosis, cells were stained with propidium iodide and analyzed by flow cytometry to detect the sub-G1 cellular DNA fraction. Error bars represent the S.E.M. of three independent experiments (*$P<0.05$; ANOVA/Dunnett test). (B) Protein levels of caspase-9, caspase-3, and PARP were analyzed by Western blotting at $t = 48$ h. Blots shown are representative of three experiments.

2. Activation of the caspase-8/Bid pathway by combined nimesulide–radiation treatment

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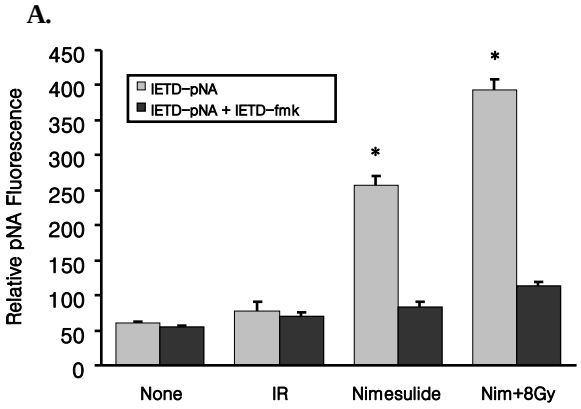

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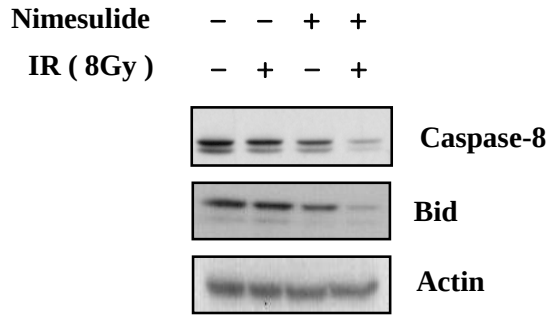

Figure 2. Activation of the caspase-8/Bid pathway in A549 cells by combined nimesulide–radiation treatment. (A) Caspase-8 activity was monitored *via* the detection of pNA liberated from the IETD-pNA substrate. The increase in activity was calculated by comparing treated samples to untreated controls. All samples were measured in triplicate. Data points represent the mean ± S.E.M. of three independent experiments (*P<0.05; ANOVA/Dunnett test). (B) Western blotting was used to analyze the expression of caspase-8 and Bid. Blots shown are representative of three separate experiments.

3. Prolonged inhibition of tumor growth in a lung carcinoma xenograft model by combined nimesulide–radiation treatment

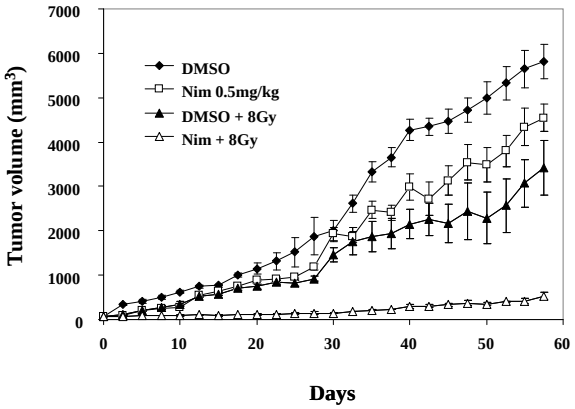

Figure 3. Effect of combined nimesulide–radiation treatment on the growth of A549 lung cancer xenografts in nude mice. Nude mice (7 weeks old) bearing subcutaneous A549 lung carcinoma xenografts (70–80 mm^3) were pretreated with vehicle (10% DMSO in PBS, pH 7.4) or nimesulide (0.5 mg/kg) on day 0. After 24 h, 8 Gy of radiation was delivered locally to the mouse xenografts using a ^{60}Co irradiator. Data shown represent the mean ± S.E.M. of two independent experiments using five mice per group in each experiment.

3. Conclusion

The combined nimesulide–radiation treatment increased apoptosis, induced the cleavage of caspase-3, caspase-9, and poly(ADP-ribose) polymerase (PARP), activated caspase-8, and induced cleavage of Bid. A pan-caspase inhibitor, z-VAD-fmk, suppressed this increase in apoptosis and also suppressed the cleavage of caspase-8, caspase-3, and PARP, suggesting a caspase-dependent mechanism. In addition, z-IETD-fmk, a selective caspase-8 inhibitor, suppressed the nimesulide- and radiation-induced cleavage activation of caspase-9, caspase-3, caspase-8, and Bid, and suppressed the concomitant apoptosis, indicating that the nimesulide-induced increase in radiosensitivity was initiated by caspase-8. However, the caspase-3 inhibitor z-DEVD-fmk failed to suppress activation of the caspase-8/Bid pathway, indicating that caspase-3 activation occurred downstream of caspase-8 activation in our experiments. Marked antitumor effects, which were evaluated by measuring protracted tumor regression, were observed when nude mice were treated with a combination of nimesulide at a clinically achievable dose (0.5 mg/kg) and radiation therapy. Our results, demonstrating the radiosensitivity-increasing and tumor growth-inhibiting effects of nimesulide, suggest that nimesulide may be suitable as an adjuvant to enhance the efficacy and selectivity of radiotherapy.

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