Transactions of the Korean Nuclear Society Autumn Meeting
Gyeongju, Korea, November 2-3, 2006

Nimesulide has a role of radio-sensitizer against lung carcinoma A549 cells

Joo –Yoon Won, Jong Kuk Park and Sung Hee Hong
Laboratory of Radiation Tumor Physiology, Korea Institute of Radiological and Medical Sciences, 215-4, Gongneung-Dong, Nowon-Gu, Seoul, 139-706, Korea

1. Introduction

Cyclooxygenases (COX) are key enzymes in the prostaglandin synthesis. There are two isoforms of the COX enzyme, COX-1 and COX-2. COX-2 expression is associated with carcinogenesis in variety of cancers and to render cells resistant to apoptotic stimuli (1, 2). Increased expression of COX-2 is shown in non-small-cell lung cancer (NSCLC), specifically in adenocarcinomas (3, 4).

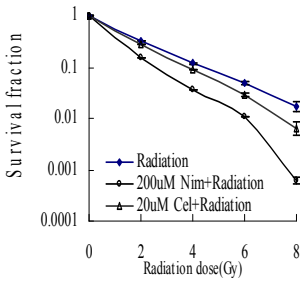
Radiotherapy has been the important treatment for NSCLC. In recent studies, newer molecules that target specific pathophysiology or molecular pathways have been tested for the radiation sensitizers (5). COX-2 inhibitors are shown to enhanced radioresponse of cultured human cancer cell lines and immunodeficient mice (6, 7). However, little is known about the molecular and biochemical mechanisms how NSAIDs enhance radioresponse of tumor cells.

Nimesulide (methanesulfonamide, N-(4-nitro-2-phenoxyphenyl)), selective COX-2 inhibitors, is a drug with anti-inflammatory, anti-pyretic and analgesic properties. Nimesulide has the specific affinity to inhibit the inducible form of cyclooxygenase (COX-2) rather than the constitutive form (COX-1) (8), and is well tolerated by adult, elderly and pediatric patients (9). Nimesulide was found also to have a chemopreventive activity against colon, urinary bladder, breast, tongue, and liver carcinogenesis (10). In this study, we examined whether nimesulide can increase radiation-induced cell death and its mechanism in NSCLC cells A549.

2. Methods and Results

1. The COX-2 selective inhibitor, nimesulide enhances radiation-induced apoptosis in lung cancer cells

A.



B.

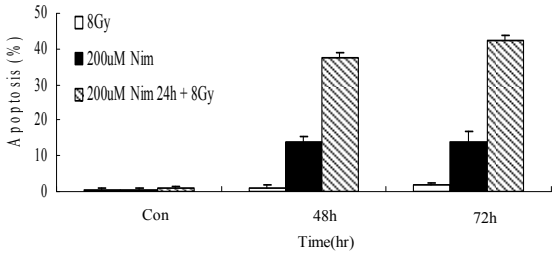
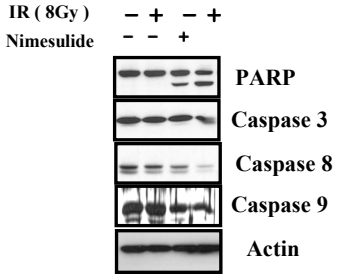


Figure 1 Nimesulide enhanced the intrinsic radiosensitivity of A549 cells. (A) Clonogenic survival curves of irradiated A549 cells treated with nimesulide (200 μM) or celecoxib (20 μM). Cells were treated with nimesulide or celecoxib for 24hr before irradiation (B) Apoptosis by nimesulide (200 μM) in combination with radiation (8Gy). Cells were stained with propidium iodide and analyzed by flow cytometry. Cells were treated with nimesulide (200 μM for 24h) before radiation (8 Gy), then cultured for additional 24h and 48 h (48 h and 72h after nimesulide treatment, respectively). Error bars represent the standard error of the mean ($\pm$SD) of three independent experiments

2. Apoptosis by combined treatment of nimesulide and radiation is caspase –dependent manner.

A.



B.

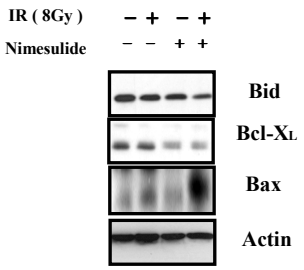


Figure 2. Western blot analysis of pro-caspases and PARP (A), and Bid, Bcl-xL, Bax (B). Cells were treated with nimesulide (200 μM for 24h) before radiation (8 Gy), then cultured for additional 24h (48 h after nimesulide treatment). Results are representatives of three or four experiments

3. Nimesulide in combination with radiation delayed tumor growth in xenograft model

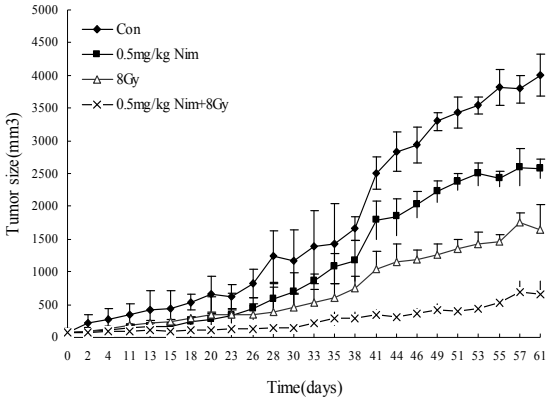


Figure 3. Combined effect of nimesulide and radiotherapy against the tumor growth of A549 human tumor xenografts in nude mice. Tumors were treated with vehicle (DMSO) or 0.5 mg/kg nimesulide on day O. After 24 h, radiation (8 Gy) was delivered locally using ^{60}Co irradiator on mice xenografts. Data are shown as the means ± SD of three independent experiments (n=5 mice/group in each experiment). Statistically significant differences on day 39: control vs 0.5 mg/kg, $P < 0.01$; others, $P < 0.001$.

3. Conclusion

Many studies showed that non-steroidal anti-inflammatory drugs (NSAIDs) have radiosensitizing effect on cancer cells *in vitro* and *in vivo*. However, little is known about the biological mechanism of NSAIDs-induced radiosensitizing effect. In this study, we showed the combined treatment of nimesulide and radiation significantly increased the intrinsic radiosensitivity of human non-small cell lung cancer cell line, A549. Combination of nimesulide and radiation increased apoptosis of A549 cells in the caspase-dependent manner. The protein level of pro-apoptotic protein, Bax was remarkably increased and anti-apoptotic Bcl-x_L expression was decreased by the combined treatment of nimesulide and radiation. Furthermore, caspase-8 inhibition protected the activation of caspase-3, PARP cleavage and apoptosis, indicating that nimesulide induced increase of radiosensitivity is initiated by caspase-8. This antitumor effect was confirmed when animals were treated with a combination of nimesulide (0.5mg/kg) and radiation. Combined therapy showed tumor regression in xenografts, Therefore, our results indicated that nimesulide has a role of radiosensitizer *in vitro* and *in vivo.*

REFERENCES

1. Pairet M, and Engelhardt G. Distinct isoform (COX-1 and COX-2) of cyclooxygenase : possible physiological and therapeutic implications. Fundam. Clin. Pharmacol. 10:1-17, 1996
2. Soslow RA, Dannenberg AJ, Rush D, Woerner BM, Khan KN, Masferrer J, and Koki AT. COX-2 is expressed in human pulmonary, colonic, and mammary tumors. Cancer 89: 2637-45, 2000.
3. Liao Z, Milas L, Komaki R, Stevens C, Cox JD. Combination of a COX-2 inhibitor with radiotherapy or radiochemotherapy in the treatment of thoracic cancer. Am J Clin Oncol. 26(4):S85-91, 2003.
4. Vartanian RK, Weidner N. Correlation of intratumoral endothelial cell proliferation in breast carcinoma. Am J Pathol. 144:1188-94, 1994.
5. Kvols LK. Radiation sensitizers: a selective review of molecules targeting DNA and non-DNA targets. J Nucl Med 46:187S-90S, 2005.
6. Furuta Y, Hunter N, Barkley T Jr, Hall E, Milas L. Increase in radioresponse of murine tumors by treatment with indomethacin. Cancer Res. 48:3008-13, 1988.
7. Maltese A, Maugeri F, Bucolo C. Rapid determination of nimesulide in rabbit aqueous humor by liquid chromatography. J Chromatogr B Analyt Technol Biomed Life Sci. 804(2):441-3, 2004
8. Davis R, and Brogden RN. Nimesulide. An update of its pharmcodynamic and phamacokinetic properties, and therapeutic efficacy. Drugs, 48:431-54, 1994
9. Shaik MS, Chatterjee A, Singh M. Effect of a selective cyclooxygenase-2 inhibitor, nimesulide, in the growth of lung tumors and their expression of cyclooxygenae-2 and peroxisome proliferator activated receptor-gamma. Clin Cancer Res. 10(4):1521-29, 2004