
The Effect of Non-Steroidal Anti-Inflammatory Drugs on Osteosarcoma Cells

Lee M. Zuckerman¹, William L. Frames¹, Joseph G. Elsissy¹, Troy G. Shields¹, Ikenna Nwachuku¹, Rosaliade Necochea-Campion², Hamid R. Mirshahidi², Nadine L. Williams¹, Saied Mirshahidi²

¹ Orthopaedic Surgery, Loma Linda University Medical Center, Loma Linda, CA, United States

² Biospecimen Laboratory, Loma Linda University Medical Center, Loma Linda, CA, United States

INTRODUCTION AND OBJECTIVES:

Osteosarcoma (OS) is an aggressive malignancy that is the most common primary bone tumor in children. Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used in orthopaedic surgery to reduce pain and inflammation. NSAIDs have been shown to be toxic to certain malignancies such as colorectal, breast, and pancreatic cancers, as well as acute myeloid leukemia, but are not well-studied in OS. The purpose of this study is to assess whether ketorolac and indomethacin induce apoptosis in OS cells, compare this to a control and explore the underlying mechanism.

METHODS: A rat OS cell line (UMR-108) was exposed to various concentrations of both ketorolac and indomethacin. Both ketorolac and indomethacin were compared to a pH buffered saline control. The background death rate of the control was subtracted from the test samples. Cell viability, cytotoxicity, apoptosis induction, DNA fragmentation and the expression of apoptosis-related markers were examined by MTT assay, colony formation assay, flow cytometry, agarose gel electrophoresis, and western blot respectively.

RESULTS: The results demonstrate that ketorolac and indomethacin induce apoptosis of rat OS cells in a dose-and time-dependent manner. Apoptosis was confirmed by cell morphology and annexin positivity. Both NSAIDs induced morphological changes in OS cells and decreased their capacity to form colonies. The molecular data showed that the NSAIDs affected expression of Bcl-2, survivin, and PARP. Caspase-3 was also decreased as shown in Figure 1.

CONCLUSIONS: This study showed that both ketorolac and indomethacin caused a decrease in OS cell viability and an increase in apoptosis in a time-and dose-dependent manner. NSAIDs are well-studied, inexpensive and readily available with well known side effect profiles. Due to these characteristics, additional in vitro and in vivo research studies are warranted to identify the use of these medications in the treatment of OS in the clinical setting.

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Send correspondence to: jelsissy@llu.edu

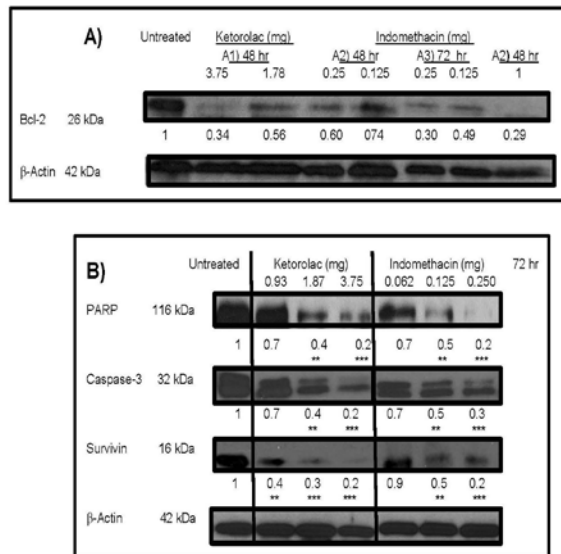


Figure 1. Expression of apoptotic proteins in OS tumor cells after being exposed to NSAIDs. UMR-108 cell were exposed to A1) 1.78 and 3.75 mg of ketorolac for 48 hr, A2) 0.125, 0.250 and 1 mg of indomethacin for 48 hr and A3) 0.125, 0.250 for 72 hr. B) Cell were exposed to 0.93, 1.87 and 3.75 mg of ketorolac and 0.062, 0.125 and 0.250 mg of indomethacin for 72 hr. Then total protein was isolated. Equal amounts of protein from each sample were loaded and separated through 12% SDS-PAGE gels and then transferred to PVDF membranes. The following antibodies were used; PARP, caspase-3, Bcl-2, survivin, and beta-actin. The bands were visualized by enhanced chemiluminescence kit instructions. Data were normalized to corresponding values of Beta-actin densitometry. (***) $p < 0.001$, ** $p < 0.01$).

REFERENCES:

1. Picci, P. Osteosarcoma (osteogenic sarcoma). Orphanet J Rare Dis, 2007. 2: p. 6.
2. Wang W, Li X, Meng FB, Wang ZX, Zhao RT, Yang CY. Effects of the Long Non-Coding RNA HOST2 On the Proliferation, Migration, Invasion and Apoptosis of Human Osteosarcoma

Cells. Cell Physiol Biochem, 2017. 43(1): p. 320-330.

3. Arndt CA, Rose PS, Folpe AL, Laack NN. Common musculoskeletal tumors of childhood and adolescence. Mayo Clin Proc, 2012. 87(5): p. 475-87.
4. Maki RG. Ifosfamide in the neoadjuvant treatment of osteogenic sarcoma. J Clin Oncol, 2012. 30(17): p. 2033-5.
5. Ferrari, S., et al., Neoadjuvant chemotherapy with high-dose Ifosfamide, high-dose methotrexate, cisplatin, and doxorubicin for patients with localized osteosarcoma of the extremity: a joint study by the Italian and Scandinavian Sarcoma Groups. J Clin Oncol, 2005. 23(34): p. 8845-52.
6. Dahlin, D.C. and M.B. Coventry, Osteogenic sarcoma. A study of six hundred cases. J Bone Joint Surg Am, 1967. 49(1): p. 101-10.
7. Bacci, G., et al., Local and systemic control for osteosarcoma of the extremity treated with neoadjuvant chemotherapy and limb salvage surgery: the Rizzoli experience. Oncol Rep, 2000. 7(5): p. 1129-33.
8. Jaffe, N. and E. Frei, Osteogenic sarcoma: advances in treatment. CA Cancer J Clin, 1976. 26(6): p. 351-9.
9. Mirabello, L., R.J. Troisi, and S.A. Savage, Osteosarcoma incidence and survival rates from 1973 to 2004: data from the Surveillance, Epidemiology, and End Results Program. Cancer, 2009. 115(7): p. 1531-43.
10. Diaz-Rodriguez, L., et al., Effects of indomethacin, nimesulide, and diclofenac on human MG-63 osteosarcoma cell line. Biol Res Nurs, 2012. 14(1): p. 98-107.