THE EFFECTS OF INDOMETHACIN ON HEPATOMA (HEP G2) CELL LINE

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ABSTRACT

Objective: *Antiproliferative effectiveness of nonsteroidal anti-inflammatory drugs (NSAID) has been shown on colon, esophagus, stomach carcinoma, and CML cell lines in several experimental studies. In this study, effects of 100 and 200 µM/L indomethacin doses, an indol-derived non-selective NSAID, on hepatocellular cancer cell line (Hep G2) were investigated.*

Material and Method: *This study was done by addition of indomethacin in different concentrations (100 mM/L and 200 mM/L) into Hep G2 in vitro. After 96 hours, study and control groups were evaluated for cell counts, proliferative index (PI) and apoptosis rates.*

Results: *At the end of the study, cell counts were found to be 775 ± 837/µL in the control group, 437 ± 354/µL in indomethacin 100 µM/L group and 187 ± 356/µL in indomethacin 200 µM/L group. In flow cytometric evaluation, cell PI were determined as 47.56 ± 9.46% at the dose of 100 µM/L indomethacin , and 48.86 ± 16.47% at the dose of 200 µM/L indomethacin, while the cell PI in the control group was found to be 49.61 ± 14.88%. Apoptosis rates detected with flow cytometric methods were 0.75 ± 0.88% in the control group, 0.44 ± 0.78% in indomethacin 100 µM/L group and 1.0 ± 2.0% in indomethacin 200 µM/L group.*

Conclusion: *No statistically significant differences were detected between the study groups and the control group for cell counts, proliferation and apoptosis rates. These results show that indomethacin at the doses used in this study, has no effect on apoptosis, proliferation and cell counts of Hep G2 cells.*

Key Words: *Indomethacin, hepatoma, cell line.* ***Nobel Med 2011; 7(1): 84-87***

HEPATOMA (HEP G2) HÜCRE SERİSİ ÜZERİNE İNDOMETAZİN'İN ETKİLERİ

ÖZET

Amaç: Deneysel çalışmalarda, nonsteroidal antiinflamatuvar ilaçların (NSAİİ) antiproliferatif etkinliği kolon, özefagus, mide kanseri ve KML hücre dizilerinde gösterilmiştir. Bu çalışmada indol türevi non selektif NSAİİ olan indometazinin 100 ve 200 .ıM/L dozlarında hepatosellüler kanser hücre serisi (Hep G2) üzerine etkilerini araştırdık.

Materyal ve Metod: Bu çalışmada in vitro Hep G2 üzerine indometazinin farklı dozlarını (100mM/L and 200mM/L) çalıştık. Doksan altı saatlik inkübasyon süresi sonunda çalışma ve kontrol gruplarının hücre sayısı, proliferatif indeks (Pİ) ve apopitozis oranları değerlendirildi.

Bulgular: Çalışmanın sonucunda hücre sayısı, kontrol grubunda 775 ± 837/µL, indometazin 100µM/L dozunda 437 ± 354/µL ve 200 µM/L dozunda 187 ± 356/µL tespit edildi. Flow sitometrik analizde Pİ kontrol grubunda %49,61 ± 14,88'iken, 100µM/L indometazin grubunda %47,56 ± 9,46 ve 200µM/L indometazin grubunda da %48,86 ± 16,47 tespit edildi. Apopitozis oranları flow sitometri ile çalışıldı.

Kontrol grubunda %0,75 ± 0,88, indometazin 100µM/L grubunda %0,44 ± 0,78 ve indometazin 200µM/L grubunda %1,0 ± 2,0 bulundu.

Sonuç: Çalışma grupları ve kontrol grubu arasında hücre sayısı, proliferasyon ve apopitoz oranları değerlendirildiğinde istatistiksel anlamlı bir fark tespit edilmedi. Sonuç olarak indometazin Hep G2 hücrelerinde çalışmamızda kullandığımız dozlarda hücre sayısı, proliferasyon ve apopitoz üzerine etkili değildir.

Anahtar Kelimeler: İndometazin, hepatoma, hücre serisi. **Nobel Med 2011; 7(1): 84-87**

INTRODUCTION

It has been reported that NSAID may have antiproliferative and apoptotic effects on cancer cells and may lead to tumoral regression.[1,2] Although these effects of NSAIDs were reported in tumoral cell cultures from colorectal origin in the beginning, similar results were also reported for breast, prostate and pancreas cancer cell lines.[2-4] Perhaps as a clinical reflection of these data, determining low incidence, and mortality for colorectal carcinoma in individuals who take NSAID regularly is noticeable in epidemiological studies.[5] The objective of this study was to investigate the possible apoptotic and antiproliferative effectiveness of indomethacin on Hep G2.

MATERIAL and METHOD

Karadeniz Technical University Medical Faculty, Internal Medicine Department, Hematology-Oncology Laboratory conducted this study. The study was approved by the institutional ethics committee. Tests in this study were performed three times.

Cells and Culture: Human hepatoma cell line Hep G2 cells (ATCC) were cultured in RPMI-1640 medium (Sigma, R 6504) containing 10% fetal bovine serum (FBS) (Biochrom Cat. No:S 0113), supplemented with penicillin (100u/ml) and streptomycin (100µg/ml) at 37°C in a humidified 5% CO_2 atmosphere, and 10^5 cells/mL were incubated with different concentrations of the drug (control, indomethacin 100µM/L, indomethacin 200µM/L) for 96 hours. At the end of 96 hours, trypsination procedure was performed with 0.25% tripsine, and obtained cells were evaluated.

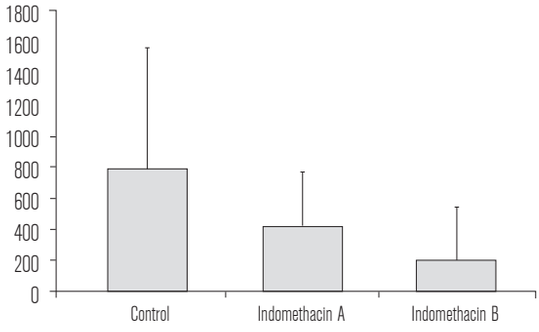

Figure 1. Comparison of cell counts between study groups and control

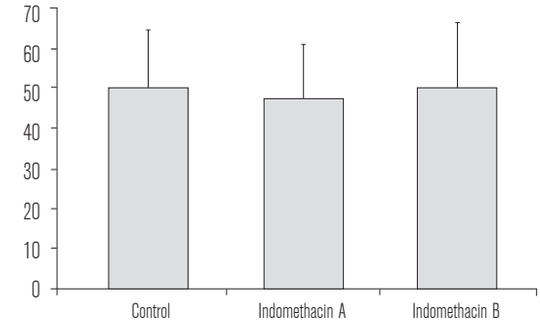

Figure 2. Comparison of proliferation between study groups and control

Cell count: After 96 hours of incubation, cultured mononuclear cells were counted by an autoanalyzer (System 9000 Hematology analyzer, Spain). →

Table: Comparison of results in Indomethacin groups with control.

	Cell number (/mL)	Proliferative index (%)	Apoptosis (%)
Control	775 ± 837	49.61 ± 14.88*	0.750 ± 0.88*
Indomethacin A	437 ± 354	47.56 ± 9.46*	0.443 ± 0.78*
Indomethacin B	187 ± 356	48.86 ± 16.47*	1.05 ± 2.0*

*: non-significant

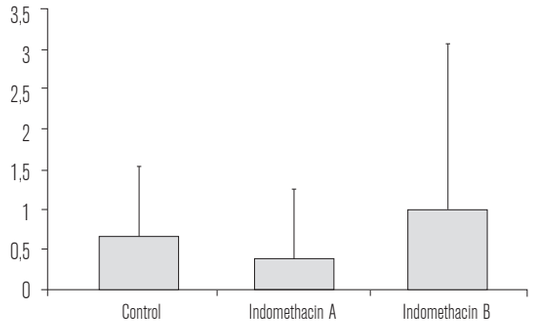

Figure 3. Comparison of apoptosis between study groups and control

Flow Cytometric Analysis of Apoptosis and Proliferation: The Coulter DNA-prep reagent system (Miami FL, USA) was used to stain the DNA of the cultured cells with propidium iodide (PI) for the quantitative measurement of cellular deoxyribonucleic acid (DNA) content by flow cytometry. The reagents were used in conjunction with the Coulter DNA Prep workstation (Florida USA). Flow cytometry was performed on Coulter Epics Elite Flow cytometry (Florida USA). Data were analyzed for apoptosis and cell cycle, using the Multicycle Software (Phoenix Flow Systems, San Diego, CA). Apoptosis ratio of cultured mononuclear cells was measured as the percentage of hypodiploidic peak. The proliferation ratio of cultured cells was assessed using the formula below:[6]

$$\text{Proliferative index (\%)} = 100 \times \frac{\text{Cell number in mitosis} + \text{Cell number in S-phase}}{\text{Total cell number}}$$

Statistical analysis

The analysis of variance (ANOVA) was used as a statistical method. Tukey HSD was used for post HOC. Data were presented as mean ± standard error of mean. $P<0.05$ was accepted as the level of statistical significance.

RESULTS

In this study, cell counts, PI and apoptosis rates were determined in control and study groups (Table). Cell counts were found to be 775 ± 837/µL in the control group, 437 ± 354/µL in indomethacin 100µM/L group and 187 ± 356/µL in indomethacin 200 µM/L group. No statistically significant differences were observed. While apoptosis percentage was 0.75 ±0.88 in control group, it was found to be 0.443 ± 0.78 in indomethacin 100µM/L group, and 1.05 ± 2.0 in indomethacin 200µM/L group. These results have not created a statistically significant difference. While proliferative indexes were calculated as 47.56 ± 9.46% at the indomethacin dose of 100µM/L and 48.86 ± 16.47% at the indomethacin dose of 200µM/L, proliferative index of the cells observed in the control group was found as 49.61 ± 14.88%. No significant differences were was found between three groups.

DISCUSSION

In our study, it has been observed that indomethacin has no significant effects on proliferation, apoptosis and number of cells. In a study performed on K562 cell lines, while the apoptotic cell percentage was 1.92% at the end of 72 hours in the control group, apoptotic cell counts were found to be 3.08% at the dose of 100µM/L indomethacin, 12.67% at the dose of 200µM/L and 49.83% at the dose of 400µM/L.[7] In this study, a statistically significant increase in apoptosis has been detected after the dose of 200µM/L indomethacin. The biological effects of indomethacin on the proliferation and apoptosis of liver cancer cell line has been reported before.[8] In another study, which investigated the efficiency of indomethacin (100µM/L) on colon carcinoma cells that express COX or not, no significant effects on apoptosis of COX-2 expressing and non-COX-2 expressing cells were observed, while at 400 and 600µM/L doses significant increases in apoptosis rates were found.[9] It is of interest that the fact that a significant difference in apoptosis could not have been shown with low doses in both studies is very similar to our findings.

In the studies performed to demonstrate the apoptotic effectiveness of indomethacin and to explain its mechanisms of actions various findings were obtained with various cell groups. In a study performed by Zhou et al.[10] with 400µM/L of indomethacin on AGS and MKN-28 cells, which are gastric cancer cell lines, antiproliferative effectiveness was observed along with an increase in apoptosis; and this effect has been associated to an increase in bax and bak levels displaying some apoptotic effectiveness. On the other hand, Hong et al.[11] studied gene expressions related with apoptosis in colon cancer cell lines (HT-29) after 4 hours exposure to indomethacin, and reported that there was no alteration in expressions of Fas, bcl-2,→

bax and c-myc. Yamamato et al.[12] investigated the apoptotic effectiveness of sulindac, indomethacin, ibuprofen and acetylsalicylic acid (ASA), and reported that only sulindac and ASA had induced apoptosis via NFκB inhibition on HTC 15 and HT 29 cell lines. However, at low doses of indomethacin (25µMol/L) as used in our study, they have reported that NFκB inhibition and induction of apoptosis were not observed.

In our study, we observed that indomethacin has no significant effect on proliferation of HepG2 cells. However, its effects on cell cycle were demonstrated in various different studies. In a study, performed by Smith et al,[9] indomethacin were used at the doses of 400, 600µmol/L on colon cancer cell lines expressing COX-2 such as HT-29 FU, HCA-7 and on non-COX-2 expressing lines such as SW480, HTC 116; and it has been reported that it caused an arrest in phase G1. In another study, head-neck cancer cell lines were used, and indomethacin given at the doses of 100 and 200µmol/L had not showed any significant effect on proliferative index when compared to control group. One of the explanations for the fact that no effects were observed on the proliferation in our study might be the relatively low dose of indomethacin used in the study.

Studies conducted on different cell lines with various doses have not elucidated the antiproliferative and apoptotic effects of NSAIDs so far. In our study, it was shown that the administration of indomethacin at the doses of 100 and 200µM/L had no effects on the inhibition of proliferation and induction of apoptosis in HepG2 cell lines. Though the decrease observed in cell counts and in apoptosis rates were not found to be statistically significant. Another reason of negative results might have been too high standard deviations of the groups. that the study was restricted only on the level of cell culture, and no any molecular results were found. More comprehensive studies are needed.

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