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Possible Antiproliferative Effect of Tolfenamic Acid on Human LNCaP Prostate Cancer Cell Lines

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ABSTRACT

Prostate cancer is a common type of cancer. Contemporary treatment modalities require that new, effective methods or chemicals should be developed. Tolfenamic acid (TA) is a nonsteroidal anti-inflammatory medicine stimulating apoptosis in cancer cells like pancreas, oesophagus and lungs. This study aims to determine the role of TA in cell viability and apoptosis in glioma cells (T-98G) and androgen-sensitive human prostate cancer cell line (LNCaP). In our study, 1, 5, 10, 25, 50, and 100 µM doses of TA were applied to T-98G and LNCaP cell lines for periods of 24 and 48 h. Subsequent cell vitality was tested with MTT Atest. The effective doses were analysed by flow cytometry and real-time PCR techniques. MTT spectrophotometric tests showed no significant effects on the survival rate of T-98G cells in 24 and 48 h. Therefore, T-98G cells were not analysed by flow cytometry and RT-PCR. When LNCaP cells were exposed to 1, 5, 10, 25, 50 and 100 µM doses of TA for 24 h, the rates of living cells were determined as 91%, 83%, 82%, 76%, 61% and 49%, respectively. When the same doses were applied for 48 h, the living cell rates were 90%, 83%, 82%, 78%, 52%, and 47%, respectively. Following applications of 25, 50 and 100 µM TA to LNCaP cells for 24 h, the apoptotic values (0.60% for the control group) were found 2%, 4%, and 17%, whereas the values for 48 h were 10%, 10% and 64% (0.48% for the control group), respectively. TA induced apoptosis of LNCaP cells depending on the dose and duration of exposure. According to PCR results, caspase-9 activity increased incrementally in groups given 50 µM of TA. The increase in the apoptosis of LNCaP cells may be due to the rising amount of TA concentration. In our study, in 25, 50, and 100 µM TA groups, cyclooxygenase-2 (COX-2) decreased incrementally compared to the control group. Considering overstimulation of COX-2 paths in emergence of cancer or in metastasis, this study shows TA has an antiproliferative and stimulating effects on apoptosis of LNCaP cells based on the dose and duration of exposure.

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Authors' Contribution
RK, SK and GK conceived and designed the study. All authors were involved in experimental studies. RK and SK analyzed the data. RK and SK wrote the article.

Key words
Tolfenamic acid, Prostate cancer, LNCaP, Apoptosis, MTT.

INTRODUCTION

Prostate cancer can be defined as the malign growth of the organ volume as a result of the imbalance between cell proliferation and cell death in the prostate gland. While prostate cancer is usually restricted to the elderly, it may also be reported in those below 40, though rarely. Moreover, it accounts for 32% of all cancers. As for its incidence, the risk increases in line with age, particularly after 40 (Tanagho and Mcanich, 2004). This disease is also the cancer type with the highest prevalence for males across the USA. While it is the 7th most common cause of death in Japanese males, it constitutes 9% of all male deaths in Europe (Kaygusuz *et al*., 2007). Studies report that prostate cancer is triggered by testosterone, suprarenal gland derived or exogenous androgens (Vis and Schroder, 2009). Androgen-dependent cancers tend to diminish in the absence of androgens while androgen-independent tumours continue to grow despite surgical operations and medical castration (Oh, 2000). Therefore, initial medical intervention involves androgen-inhibiting cures, but these suppressants are withheld in progressive prostate cancer. Instead, secondary hormonal therapies and cytotoxic chemotherapies are commenced (Calabro and Sternberg, 2007). However, stimulation of apoptotic pathways in prostate cancer is recommended as a curative method for advanced cases (Tang and Porter, 1997; Cho *et al*., 2004). Recent studies have suggested that TA, a member of small non-steriodal antinflammatory drugs (NSAIDs), may exhibit anti-cancer activities in some cancer models by suppressing metastasis and inhibiting cell growth (Abdelrahim *et al*., 2006a, 2007; Lee *et al*., 2008; Choi

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et al., 2011). TA has been reported to stimulate apoptosis in pancreatic cancer models and growth of tumour just as it does in colorectal cancer cells, an indicative of its anti-cancer properties (Abdelrahim *et al*., 2006b, 2007; Lee *et al*., 2008). Furthermore, TA has been reported to inhibit cell growth in lung, breast and ovarian cancers (Basha *et al*., 2011a, b; Papineni *et al*., 2009).

The purpose of this study is to determine the effects of TA upon cell viability and apoptosis on androgen-sensitive human prostate cancer cell line (LNCaP), as well as shedding light upon the processes regarding the anti-cancer activities of TA and its relevant mechanisms. In our study, we used human glioma (T98G) results by making a comparison with those of other cancer studies in order to determine efficacy of TA.

MATERIALS AND METHODS

In this study, doses of 1, 5, 10, 25, 50 and 100 µM were assessed at the 24th and 48th h using MTT and then effective concentrations were subjected to the flow cytometry assay and qRT-PCR techniques in order to evaluate the effects of TA upon T-98G and LNCaP cells. The experiment was divided into two different groups: 24th h and 48th h. These also include control group, DMSO group (complete medium with a final concentration of 0,1 % DMSO, solvent) and 1, 5, 10, 25, 50 and 100 µM doses of TA. The experiments were repeated three times for each group and consisted of 24 samples. The cells were placed in an incubator with 100% of humidity and 5% of CO_2 of 5% at 37°C after they had been inoculated in the flasks with a medium. Proliferation of cells was observed through a reverse light microscope (Olympus).

Determination of cell vitality through the MTT method

The cells were harvested from the bottom of the flasks through a 0.25% of trypsin-EDTA after they had been stained with trypan blue. The number of living cells occurring in 1mL was determined with a cell counter. Afterwards, an inoculation process was achieved in such a way that each of the 96 wells would include 1x10^4 cells. T98G and LNCaP cells were added at 24th and 48th h separately in doses of 1, 5, 10, 25, 50 and 100 µM and then the toxicity upon the cells was determined with the MTT (Sigma) spectrophotometric method defined by Mossmann (1983).

Measurement of apoptosis with the flow cytometric assay

Once the cell counting had been completed, the effects of TA on the apoptosis of LNCaP cells were investigated by inoculating 1x10^6 LNCaP in each of the flasks of 25 cm^2 at 24th and 48th h. At the end of the experiment, the cells harvested with 0,25% of trypsin were placed in tubes and then rotated for 5 min at 1200 RPM at 4°C. Following this process, the fluid part on the top was thrown into the tubes in a way that each tube would include 2 mL. Once the number of cells had been determined following the coating with trypan blue, the necessary processes were performed in line with the protocol of Annexin V Apoptosis Assay (Invitrogen). The results were evaluated using a FACSCalibur flow cytometer in the haematology laboratory in Faculty of Medicine at Eskişehir Osmangazi University. The results demonstrated that neither PI nor FITC coating was observed in living cells [FITC(-)/ PI(-)]. While [(FITC(+)/ PI (-)] occurs in early apoptotic cells, it disappears in late apoptotic cells [FITC(+)/ PI (+)].

Real Time-PCR (RT-PCR) technique

In order to determine the effects of TA upon the caspase-9 and COX-2 gene expressions of LNCaP cells, inoculation was achieved in the flasks of 25 cm^2 in such a way that each would include 1x10^6 cells for 24 h. As a result, RNA was isolated from the LNCaP cells harvested from the flasks. These RNA concentrations were measured using NanoDrop 1000 (Thermo Scientific, Waltham, MA, ABD). RNA was diluted down to 1000 µg/mL. cDNA was synthesized in each of the RNA samples using a thermal cycle machine and a Reverse Transcriptase cDNA Synthesis Kit (Roche Nano Lightcycler; Roche Diagnostics) according to the instructions issued by its producer. Amplification was determined using Dual Label TaqMan probes. Caspase 9, COX-2 (target genes) and β-actin (reference gene) mRNA levels were measured using Nano Lightcycler (Roche Germany) with the help of Dual Label (TaqMan probe).

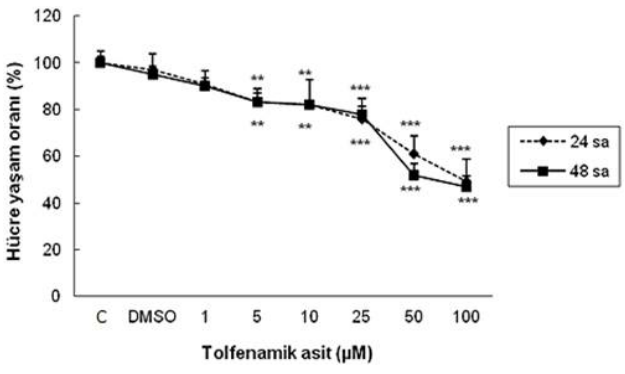

Fig. 1. The effects of TA application in doses of 1, 5, 10, 25, 50 and 100 µM at 24th and 48th h upon LNCaP cell survival. Compared to control group, the results are: **, $p < 0.01$; ***, $p < 0.001$.

Statistical analysis

IBM SPSS 21 was used for statistical evaluation. The groups were first analysed based on whether they showed a normal distribution using the Shapiro-Wilk Normality Test. Those exhibiting normal distribution were then subjected to the one-way ANOVA test before the differences observed between the groups could be evaluated via the Tukey test. As for the data showing abnormal distribution, they were evaluated via the Kruskal-Wallis test and these evaluations were subjected to the Dunn test. Those with the P-value smaller than 0.05 were considered to be of statistical significance. The results were expressed in x ± SE terms.

RESULTS

According to the data obtained by the MTT method, the amount of DMSO used as dissolvent caused no changes in the cell survival rate. Also, no effects of TA in doses of 1, 5, 10, 25, 50 and 100 µM could be determined upon T-98G cells. For this reason, there was no need for analysing T-98G cells with either the flow cytometer assay or RT-PCR. The cell survival rates were calculated as 91%, 83%, 76%, 61% and 49%, respectively following the application of TA to LNCaP cells in abovementioned doses for 24 h. At 48th h, the cell survival rates observed for TA doses were 90%, 83%, 82%, 78% and 52%, respectively (Table I). Our flow cytometer data revealed that the highest early apoptosis rate was observed in 100 µM of TA at 24th h (17.36%) in comparison with that of the control group (Table I). This rate increased up to 10.18% for 25 µM dose of TA and to 64.38% for 100 µM at 48th h for in comparison with that of the control group (0.48%) (Table II). Early apoptosis levels observed in cancerous LNCaP cells at 48th h were higher than those observed at 24th h (Figs. 2, 3). The conclusion was that TA stimulates apoptosis in LNCaP cells depending on doses and application time of TA. According to PCR results, no statistically significant difference could be found between the study groups as far as the caspase-9 level is concerned. On the other hand, caspase-9 activities in the group of 50 µM of TA were understood to have increased multi-fold while those in the group of 100 µM of TA decreased multi-fold (Table III). No statistical significance was determined between COX-2 level groups; even so, in the groups given 50 and 100 µM of TA, there was multi-fold increase compared COX-2 with control group (Table III).

Table I.- Comparison of apoptosis results obtained with the flow cytometer in LNCaP cells in doses of 25, 50 and 100 µM of TA for 24 h. The experiments have been repeated three times.

LNCaP cell (%)	Control	25 µM TA	50 µM TA	100 µM TA	p
Live	93.44±2.91	68.77±15.09	65.89±18.55	54.92±19.13	0.076
Early apoptosis	0.60±0.17	1.79±0.27	3.59±2.06	17.36±8.03	0.004
Late apopotosis or necrosis	5.41±2.47	21.67±40.94	30.15±14.55	26.39±14.46	0.102

Table II.- Comparison of apoptosis results of LNCaP cells in doses of 25, 50 and 100 µM of TA for 48 h. The experiments have been repeated three times.

LNCaP cell (%)	Control	25 µM TA	50 µM TA	100 µM TA	p
Live	90.2±1.62	58.27±10.61	59.76±12.7	24.09±31.35	0.013
Early apoptosis	0.48±0.36	10.56±2.19	10.18±0.2	64.38±38.73	0.014
Late apoptosis or necrosis	7.98±1.24	14.02±1.74	13.28±1.16	10.29±6.57	0.207

Table III.- Comparison of caspase-9 and COX-2 gene expression results with those of Control Group in LNCaP cells subjected to TA application.

Mean relative fold increase	Control	25 µM TA	50 µM TA	100 µM TA	p
Caspase-9	1.3±1.1	1.5±1.4	2.8±1.3	0.4±0.5	0.16
COX-2	1.2±1.2	1.0±1.0	0.35±0.14	0.10±0.08	0.33

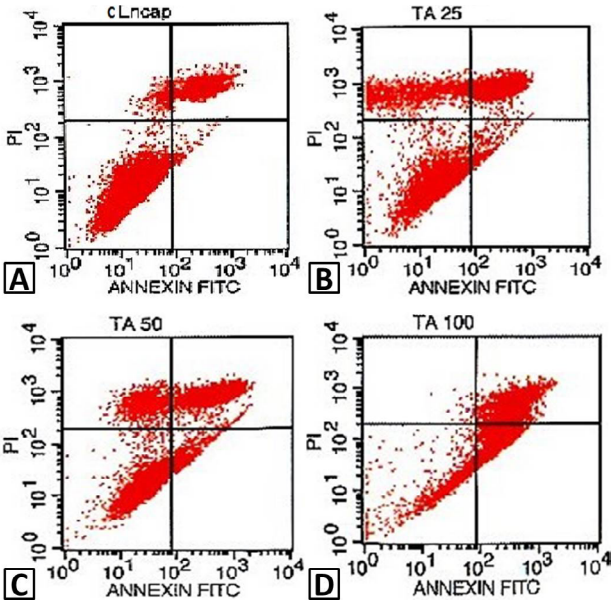

Fig. 2. Determination of cell percentage of early apoptosis in cancerous LNCaP cells following TA application (A, Control; B, 25 µM; C, 50 µM; D, 100 µM) for 24 h with LNCaP with the flow cytometric method. Live cells FITC (-) / PI (-), early apoptotic cells (FITC (+) / PI (-), necrotic or late apoptotic cells FITC (+) / PI (+).

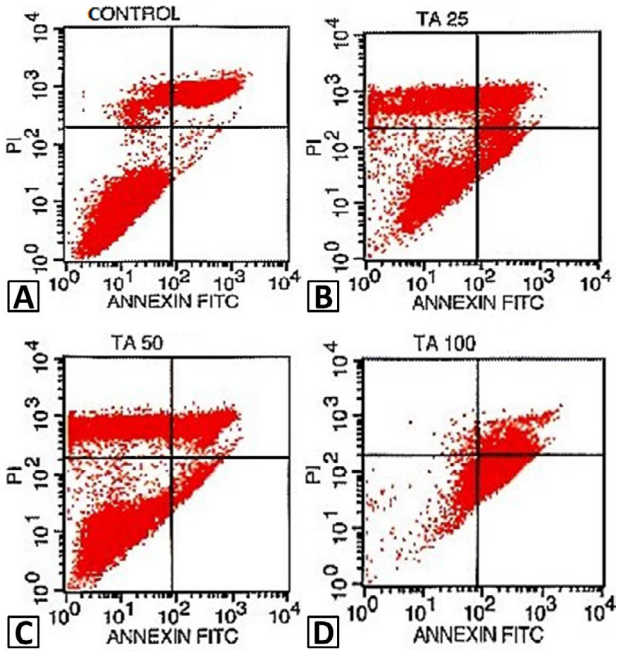

Fig. 3. Determination of cell percentage of early apoptosis in cancerous LNCaP cells following TA application (A, Control; B, 25 µM; C, 50 µM; D, 100 µM) for 48 h with LNCaP. Live cells FITC (-) / PI(-), early apoptotic cells (FITC (+) / PI (-), necrotic or late apoptotic cells FITC (+) / PI (+).

DISCUSSION

There is an increasing number of *in vivo* and *in vitro* scientific studies on the relationship between cancer and TA. In the present study, we used two human cancer cell lines (LnCaP and T98G) to determine the cytotoxic and apoptotic effects of TA. TA showed no significant inhibitory effects on cell survival of T98G cells, whereas our results showed a remarkable decrease in cell survival rates in LnCap cells. Various studies have shown that TA supresses cell proliferation, tumour growth and metastasis in pancreas cancer (Abdelrahim *et al*., 2006a, b, 2007), oesophagus cancer (Papineni *et al*., 2009), lung cancer (Colon *et al*., 2011) and ovarian cancer (Basha *et al*., 2011b).

In a study by Sung *et al*. (2012) which used squamous cancer cell lines occurring in the head and neck areas, the cytotoxic effects of TA were bigger compared to other NSAIDs and TA was a more powerful stimulant for apoptosis. It is interesting to note that while TA showed low toxicity in normal keratinocyte cells as much as 100 µM, it only increased mortality rate when applied in high doses. Colon *et al*. (2011) showed in one of their studies on lung cancer in *in-vivo* rat modals that TA in doses of 25 and 50 mg/kg inhibited cell proliferation and cell survival in A549 and CRL5803 cancerous cells. When compared with other NSAIDs, TA causes a low, well-tolerated gastric effect and a high therapeutic index. Also, its antipyretic and analgesic effects have been shown not only in several animal experimental models but also in long-term treatments of migraine, rheumatoid arthritis, and osteoarthritis.

Early apoptosis measurements done in investigating the cell-death mechanism of TA in LNCaP cells have revealed that apoptosis shows variation based on the dose and duration of TA application. Early apoptosis measurements were the highest in the group of 100 µM TA concentration at 24th h following TA application (17.36%) and at 48th h (64.38%) when compared to those of control group (Tables I, II). Studies have suggested that TA stimulates apoptosis in colorectal cancer cells in addition to the models investigating metastasis and tumour growth in pancreatic cancer (Abdelrahim *et al*., 2006a, b; Lee *et al*., 2008). However, there is lack of sufficient knowledge in the literature about what effects TA actually has, including its molecular effects, the apoptosis of prostate cancer cells (Choi *et al*., 2011). In a study by Sung *et al*. (2012) TA significantly stimulated the loss of mitochondrial membrane potential, thus interfering with mitochondrial membrane damage in TA-related apoptosis.

While the exact mechanism of apoptosis still remains unclear, the most important phenomenon as regards apoptosis is considered to be activation of caspases

(Choen, 1997; Lee *et al*., 2000). In several cases, caspase activation has been considered inescapable for cell apoptosis (Salvesen, 2002). As many as 400 caspase substrates have been identified for DNA metabolism and repair work, cell proliferation, and regulation of the cell life cycle (Fischer *et al*., 2003), as well as the proteins involved in apoptosis, and this number increases as new tumour-inhibiting proteins are added to the list (Lin *et al*., 2010; Shen *et al*., 2010). Caspase 2, 8, 9, and 10 trigger apoptosis (Ozawa *et al*., 2002; Wang, 2000). Our findings indicate that caspase-9 activity increased multi-fold in the group where 50 µM of TA concentration was applied, but this increase was of no statistical significance (Table III). On the other hand, our findings also showed that, though statistically insignificant, COX-2 decreased multi-fold in 50 and 100 µM TA groups when compared to control group (Table III). COX-2, a highly crucial medium for inflammation, is suspected to play a role in the development of prostate cancer (Bakhle, 2001). Clinical results in the literature have led us to speculate that upregulation of COX-2 must be one of the basic steps to the development of cancer (Prescott and Fitzpatrick, 2000). COX paths are key regulators of cell growth and angiogenesis, and studies are still being carried out due to the likelihood of COX-inhibiting drugs being used as potential anti-cancer drugs (Hyde and Missailidis, 2009; Ölgen *et al*., 2002).

NSAIDs also decrease the risk for some cancers to develop as well as for metastasis (Coogan *et al*., 2000; Leitzmann *et al*., 2002). In addition to anti-proliferative properties, NSAIDs also have COX-inhibiting properties and increases the number of apoptotic paths and antiangiogenic activities (Tarnawski and Jones, 2003; Lincova *et al*., 2009). Nelson and Harris (2000) showed that a regular daily intake of ibuprofen or aspirin decreases the risk for prostate cancer by 66%, which could be attributed to COX-2 restriction, an isomorph of COX enzyme that is suspected to contribute to the development of prostate cancer because it converts arachidonic acid into pro-inflammatory prostaglandins (Gupta *et al*., 2000; Nelson and Harris, 2000). The number of the investigations into whether inhibition of COX decreases the risk for cancer development and prevents growth of cancerous cells has greatly increased in recent years (Ding *et al*., 2003).

CONCLUSION AND SUGGESTIONS

Our study is the first of its kind in that TA has been shown to inhibit cancerous LNCaP cells considering the fact that COX-2 paths are excessively stimulated in development or spreading of cancer. It also shows that TA stimulates early apoptosis depending on the dose and time of application via the MTT spectrophotometric method. We, therefore, suggest that TA and relevant compounds should be investigated as potential drugs for prostate and some other cancers for clinical use.

Statement of conflict of interest

The authors declare no conflict of interest.

REFERENCES

Abdelrahim, M., Baker, C.H., Abbruzzese, J.L. and Safe, S., 2006a. Tolfenamic acid and pancreatic cancer growth, angiogenesis, and Sp protein degradation. *J. Natl. Cancer Inst*., **98**: 855-868. https://doi.org/10.1093/jnci/djj232

Abdelrahim, M., Safe, S., Baker, C. and Abudayyeh, A., 2006b. RNAi and cancer: Implications and applications. *J. RNAi Gene Silenc*., **2**: 136-145.

Abdelrahim, M., Baker, C.H., Abbruzzese, J.L., Sheikh-Hamad, D., Liu, S., Cho, S.D., Yoon, K. and Safe, S., 2007. Regulation of vascular endothelial growth factor receptor-1 expression by specificity proteins 1, 3, and 4 in pancreatic cancer cells. *Cancer Res*., **67**: 3286-3294. https://doi.org/10.1158/0008-5472.CAN-06-3831

Bakhle, Y.S., 2001. COX-2 and cancer: A new approach to an old problem. *Br. J. Pharmacol*., **134**: 1137-1150. https://doi.org/10.1038/sj.bjp.0704365

Basha, R., Baker, C.H., Sankpal, U.T., Ahmad, S., Safe, S., Abbruzzese, J.L. and Abdelrahim, M., 2011a. Therapeutic applications of NSAIDS in cancer: Special emphasis on tolfenamic acid. *Front. Biosci. (Schol. Ed.),* **3**: 797-805.

Basha, R., Ingersoll, S.B., Sankpal, U.T., Ahmad, S., Baker, C.H., Edwards, J.R., Holloway, R.W., Kaja, S. and Abdelrahim M., 2011b. Tolfenamic acid inhibits ovarian cancer cell growth and decreases the expression of c-Met and survivin through suppressing specificity protein transcription factors. *Gynecol. Oncol*., **122**: 163-170. https://doi.org/10.1016/j.ygyno.2011.03.014

Calabro, F. and Sternberg, C.N., 2007. Current ındications for chemotherapy in prostate cancer patients. *Eur. Urol.,* **51**: 17-26. https://doi.org/10.1016/j.eururo.2006.08.013

Cho, S.D., Jiang, C., Malewicz, B., Dong, Y., Young, Cc.y., Kang, K.S., Lee, Y.S., Ip, C. and Lü, J., 2004. Methyl selenium metabolites decrease prostate-specific antigen expression by inducing protein degradation and suppressing androgen-stimulated transcription. *Mol. Cancer Ther*., **3**: 605-611.

Choen, G.M., 1997. Caspases, the executioners of apoptozis. *Biochem. J*., **326**: 1-16. https://doi.

org/10.1042/bj3260001

Choi, E.S., Shim, J.H., Jung, J.Y., Kim, H.J., Choi, K.H., Shin, J.N., Ae, J.S., Cho, N.P. and Cho, S.D., 2011. Apoptotic effect of tolfenamic acid in androgen receptor-independent prostate cancer cell and xenograft tumor through specificity protein 1. *Cancer Sci*., **102**: 742-748. https://doi.org/10.1111/j.1349-7006.2011.01871.x

Colon, J., Basha, M.R., Madero-Visba, R., Konduri, S., Baker, C.H., Herrera, L.J., Safe, S., Sheikh-Hamad, D., Abudayyeh, A., Alvarado, B. and Abdelrahim, M., 2011. Tolfenamic acid decreases c-Met expression through Sp proteins degradation and inhibits lung cancer cells growth and tumor formation in orthotopic mice. *Invest. New Drugs*, **29**: 41-51. https://doi.org/10.1007/s10637-009-9331-8

Coogan, P.F., Rosenberg, L., Palmer, J.R., Strom, B.L., Zauber, A.G., Stolley, P.D. and Shapiro, S., 2000. Nonsteroidal anti-inflammatory drugs and risk of digestive cancers at sites other than the large bowel. *Cancer Epidemiol. Biomark. Prev*., **9**: 119-123.

Ding, X.Z., Hennig, R. and Adrian, T.E., 2003. Lipoxgenase and cyclooxgenase metabolism: New insights in treatment and chemoprevention of pancreatic cancer. *Mol. Cancer*, **2**: 1-12. https://doi.org/10.1186/1476-4598-2-1

Fischer, U., Janicke, R.U. and Schulze-Osthoff, K., 2003. Many cuts to ruin: A comprehensive update of caspase substrates. *Cell Death Differ*., **10**: 76-100. https://doi.org/10.1038/sj.cdd.4401160

Gupta, S., Srivastava, M., Ahmad, N., Bostwick, D.G. adn Mukhtar, H., 2000. Over-expression of cyclooxygenase-2 in human prostate adenocarcinoma. *The Prostate*, **42**: 73-78. https://doi.org/10.1002/(SICI)1097-0045(20000101)42:1<73::AID-PROS9>3.0.CO;2-G

Hyde, C.A.C., and Missailidis, S., 2009. Inhibition of arachidonic acid metabolism and its implication on cell proliferation and tumour-angiogenesis. *Int. Immunopharmacol*., **9**: 701-715. https://doi.org/10.1016/j.intimp.2009.02.003

Kaygusuz, G., Tulunay, O., Baltaci, S. and Gogus, O., 2007. Microvessel density and regulators of angiogenesis in malignant and nonmalignant prostate tissue. *Int. Urol. Nephrol*., **39**: 841-850. https://doi.org/10.1007/s11255-006-9144-z

Lee, D., Scott, A.L. and Jerry, A.L., 2000. Potent and selective nonpeptide inhibitors of caspases 3 and 7 inhibit apoptozis and maintain cell functionally. *J. biol. Chem*., **275**: 16007-16014. https://doi.org/10.1074/jbc.275.21.16007

Lee, S.H., Bahn, J.H., Choi, C.K., Whitlock, N.C. and Baek, S.H., 2008. ESE-1 / EGR-1 pathway plays a role in tolfenamic acid-induced apoptosis in colorectal cancer cells. *Mol. Cancer Ther*., **7**: 3739-3750. https://doi.org/10.1158/1535-7163.MCT-08-0548

Leitzmann, M.F., Stampfer, M.J., Ma, J., Chan, J.M., Colditz, G.A., Willett, W.C. and Giovannucci, E., 2002. Aspirin use in relation to risk of prostate cancer. *Cancer Epidemiol. Biomark. Prev*., **11**: 1108-1111.

Lin, S.Y., Hsueh, C.M., Yu, S.L., Su, C.C., Shum, W.Y., Yeh, K.C., Chang, G.C. and Chen, J.J., 2010. HLJ1 is a novel caspase-3 substrate and its expression enhances UV-induced apoptosis in non-small cell lung carcinoma. *Nucl. Acids Res*., **38**: 6148-6158. https://doi.org/10.1093/nar/gkq412

Lincova, E., Hampl, A., Pernicova, Z., Starsichova, A., Krcmar, P., Machala, M., Kozubik, A. and Soucek, K., 2009. Multiple defects in negative regulation of the PKB/Akt pathway sensitise human cancer cells to the antiproliferative effect of non-steroidal anti-inflammatory drugs. *Biochem. Pharmacol*., **78**: 561-572. https://doi.org/10.1016/j.bcp.2009.05.001

Mossmann, T., 1983. Rapid colorimetric assay of cellular growth and survival: Application to proliferation and cytotoxicity assay. *J. Immun. Method*, **65**: 55-63. https://doi.org/10.1016/0022-1759(83)90303-4

Nelson, J.E. and Harris, R.E., 2000. Inverse association of prostate cancer and non-steroidal antiinflammatory drugs (NSAIDs): Results of a case-control study. *Oncol. Rep*., **7**: 169-170.

Oh, W.K., 2000. Chemotherapy for patients with advanced prostate carcinoma: A new option for therapy. *Cancer*, **88**(12 Suppl): 3015-3021. https://doi.org/10.1002/1097-0142(20000615)88:12+<3015::AID-CNCR18>3.0.CO;2-M

Ozawa, H., Keone, R.W. and Marcilla, A.E., 2002. Thetapeutic stragies targetting caspase inhibition following spinal cord injury in rats. *Exp. Neurol*., **177**: 306-313. https://doi.org/10.1006/exnr.2002.7998

Ölgen, S., Bıçak, I. and Nebioğlu, D., 2002. Angiogenesis and new aspects of cancer chemotheraphy. *Fac. Pharm. J.,* **31**: 193-214.

Papineni, S., Chintharlapalli, S., Abdelrahim, M., Lee, S.O., Burghardt, R., Abudayyeh, A., Baker, C., Herrera, L. and Safe, S., 2009. Tolfenamic acid inhibits esophageal cancer through repression of specificity proteins and c-Met. *Carcinogenesis*, **30**:

1193-1201. https://doi.org/10.1093/carcin/bgp092

Prescott, S.M. and Fitzpatrick, F.A., 2000. Cyclooxygenase-2 and carcinogenesis. *Biochim. Biophys. Acta*, **1470**: M69-78.

Salvesen, G.S., 2002. Caspases and apoptosis. *Essays Biochem*., **38**: 9-19. https://doi.org/10.1042/bse0380009

Shen, S.M., Yu, Y.,Ying, L.W., Cheng, J.K., Wang, L.S. and Chen, G.Q., 2010. Downregulation of ANP32B, a novel substrate of caspase-3, enhances caspase-3 activation and apoptosis induction in myeloid leukemic cells. *Carcinogenesis*, **31**: 419-426. https://doi.org/10.1093/carcin/bgp320

Sung, U.K., Yoo S.S., Hye, S.H., Seung, J.B., Seong-Ho, L. and Chul-Ho, K., 2012. Tolfenamic acid ınduces apoptosis and growth ınhibition in head and neck cancer: Involvement of NAG-1 expression. *PLoS One*, **7**: 0034988. https://doi.org/10.1371/journal.pone.0034988

Tanagho, E.A. and Mcanich, J.W., 2004. *Smith's general urology, lange* (trans. ed. G. Kazancı). 16 Baskı, Nobel Tıp Kitabevleri, İstanbul, pp. 410-418.

Tang, D.G. and Porter, A.T., 1997. Target to apoptosis: A hopeful weapon for prostate cancer. *The Prostate*, **32**: 284-293. https://doi.org/10.1002/(SICI)1097-0045(19970901)32:4<284::AID-PROS9>3.0.CO;2-J

Tarnawski, A.S. and Jones, M.K., 2003. Inhibition of angiogenesis by NSAIDs: Molecular mechanisms and clinical implications. J. mol. Med., **81**: 627-636. https://doi.org/10.1007/s00109-003-0479-y

Vis, A.N. and Schroder, F.H., 2009. Key targets of hormonal treatment of prostate cancer. Part 1: the androgen receptor and steroidogenic pathways. *BJU Int*., **104**: 438-448. https://doi.org/10.1111/j.1464-410X.2009.08695.x

Wang, K.K., 2000. Calpain and caspase can you tell the differance? *Trends Neurosci*., **23**: 20-26. https://doi.org/10.1016/S0166-2236(99)01479-4