Modulating Cyclooxygenase And Lipoxygenase Pathway In Cancer Chemoprevention

Summary

submitted in the fulfillment for the award of the degree of

Doctor of Philosophy

In

Pharmaceutical Sciences

Submitted By

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Enrollment no. 188/11

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(2018)

Summary

Accruing numbers of factors involved in tumorigenesis, mounting evidence indicates that the inflammatory microenvironment accounts for the tumor development. Arachidonic acid (AA) and its metabolites involve the presumed convincing role in cancer biology. Prolonged treatment with the non-steroidal anti-inflammatory drugs (NSAIDs) was well proved evidence to be associated with a lower risk of the several cancers, including mammary gland cancer.

AA is an essential fatty acid that plays a key role in metabolism; cell signaling and inflammation in mammals. The activation of the enzyme phospholipase A2 releases AA from cell membrane, which is further metabolized by the two key enzymes, cyclooxygenase (COX) and lipoxygenase (LOX). The metabolic end products of AA are termed as eicosanoids, which plays an important role in inflammation, development, reproductive process and inflammation-associated diseases including cancer. COX is an enzyme that metabolizes the AA into prostaglandins and is expressed in two isoforms. COX-1 is ubiquitously present in all cells, whereas COX-2 is only expressed in pathological conditions, for instance, in malignant and inflamed cells. COX-2 has been first noted to be up-regulated in colorectal cancer.

The interconnection between inflammation, cancer progression, COX-2 and 5-LOX products has invariably been of interest. Published reports have endorsed the over expression of COX and LOX in several cancers which depicts the link between cancer and inflammation. Emerging evidences proclaimed upsurge production of COX-2 and 5-LOX products in malignant cells. These enzymes interfere with normal physiological function within the cells and take part in the apoptosis, angiogenesis and invasiveness, proliferation and conversion of pro-

carcinogen to carcinogens. While the expression of individual enzyme, the dual inhibition of AA metabolism has also been increasingly appreciated for their importance in cancer progression. Since no experimental data has been exercised in the direction of dual COX and LOX inhibition impeding to cancer, suggesting plethora of biochemical/physiological element needs to be understood and performed. Authors considered it worth elaborating the effect of dual inhibition of AA metabolism on cancer progression. The proposed study presents the insight of differing roles of the enzymes COX-2 and 5-LOX in mammary gland carcinogenesis and emphasizes the potential of DuCLOX inhibition as target chemotherapy for cancer. The monotherapy and combination therapy of zaltoprofen (COX-2 inhibitor) and zileuton (5-LOX inhibitor) were validated for their effect against N-methyl-N-nitrosourea (MNU) induced mammary gland carcinoma in albino wistar rats.

The study is aimed to investigate the possible link between dual inhibition of Arachidonic acid and its correlation with the suppression of tumorigenesis of mammary gland. To elaborate the objectives of the study, we have scrutinized the effect of zaltoprofen and zileuton, and their combination regime on mitochondrial mediated apoptotic pathway, cellular proliferation and angiogenesis. The markers for the above stated parameters were based on biochemical, immunoblotting and qRT-PCR based assay or combination of two. To elucidate further, the study investigated the effect of monotherapy and combination therapy of zaltoprofen and zileuton on cellular metabolic alterations using quantitative NMR.

- Treatment with monotherapy and combination therapy of zaltoprofen and zileuton implicating considerable regulation of autonomic control during

cancer progression. MNU administration was found to be evident for distortion of ECG and HRV profiling reflecting the cardiac risks with the carcinogenic which was restored back to normal after treatment regime.

- The mammary gland tissue was scrutinized for biochemical parameters including thiobarbituric acid reactive substances (TBARs), superoxide dismutase (SOD), catalase, protein carbonyl (PC) and glutathione (GSH). We observed increase in the TBARs (markers for lipid peroxidation) and PC (markers for protein peroxidation) in MNU treated group, which was restored back to normal after the treatments with drugs. MNU administration also downregulated the levels of SOD, catalase, and GSH which were restored back after treatment regime. All in all, DuCLOX-2/5 inhibition favorably regulated oxidative stress markers in MNU induced carcinogenesis.
- The DuCLOX-2/5 inhibition demarcated significant effect upon the cellular proliferation and angiogenesis as evidenced through decreased in alveolar bud count. The mammary gland tissues were found with the increased alveolar bud (AB) count, differentiation (DF) score, and lobules against MNU administration. The increase in AB count and DF score is the reflection of malignant transformation. The treatment regime restored the cellular architecture of the mammary gland tissue.
- Presence of duct, adipocytes, loose connective tissue, dconnective tissue, lymphocytes, cuboidal epithelial cells and myoepithelial cells in control groups. Administration of MNU showed a cellular degeneration and presence of scattered epithelial cells. Restoration of the histopathological architecture was found with the combination therapy when compared to toxic control.

- The immunoblotting and qRT-PCR studies revealed favorable regulation of COX-2 and 5-LOX. As the zaltoprofen and zileuton are already established specific inhibitors of COX-2 and 5-LOX respectively, the immunoblotting and RT-PCR revealed the same.
- The immunoblotting studies revealed the participation of the mitochondrial mediated death apoptosis, i.e, BCL-2, BCL-xl, BAD, BAX, VDAC, Apaf-1, procaspase-9, cytochrome-c. MNU administration was recorded for the upregulated expression of BCL-2, BCL-xl, VDAC, Apaf-1 and procaspase-9, and downregulation of BAX, BAD and cytochrome-c. DuCLOX-2/5 inhibition treatment curtailed down the anti-apoptotic effects of MNU.
- Genomic contributors of the mitochondrial mediated death apoptosis observed through quantitative PCR. The fold change in relative expression of the genes of the cell death pathways, i.e., VDAC, Apaf-1, procaspase-9, cytochrome-c, were found close to normal after administration of treatment regime against MNU administration.
- DuCLOX-2/5 inhibition also upregulated serum levels of caspase 3 and caspase 8, when compared with MNU treatment. In conclusion, DuCLOX-2/5 inhibition treatment curtailed down the proliferative and anti-apoptotic effects of MNU when affirmed through the mitochondrial mediated apoptotic pathway.
- Combination regime restored the metabolic changes to normal when scrutinized through ^{1}H NMR studies. Metabolic changes related to multiple tumor-related metabolic pathways, involving energy metabolism, amino acid metabolism, fatty acid metabolism and choline phospholipid metabolism were found to be distorted on MNU administration. Compared with control group,

MNU treatment had a significant elevation of VLDL/LDL, PUFA, choline, isoleucine, leucine, valine, alanine, proline, tyrosine, phenylalanine, NAG, OAG whereas, they were having decreased levels of glucose, lactate, creatine, TMAO, and formate.

- Zaltoprofen treatment was effective in resetting the elevated serum levels of proline, alanine, valine, lipid metabolites (VLDL/LDL, PUFA), NAG and decreased serum levels of formate, lactate, TMAO, and creatine. Zileuton was well in range to reset the elevated serum levels of isoleucine, leucine, valine, alanine, proline, phenylalanine, lipid metabolites (VLDL/LDL, PUFA), and decreased serum levels of formate, lactate, and creatine. All in all, the combination therapy improved the serum metabolic profiles of branched chain and aromatic amino acids along with choline metabolism.

Henceforth, authors would like to submit that zaltoprofen, zileuton and a combination dose can favorably regulate the metabolic alterations induced by MNU. The study identified DuCLOX-2/5 inhibition as chemopreventive targets of mammary gland cancer. Their specific inhibitors prevented MNU-induced mammary gland carcinogenesis through their inhibitory effects on AA metabolism.