AD_____

Award Number: DAMD17-02-1-0579

TITLE: Novel Cox-2 Inhibitor for Breast Cancer Therapy

PRINCIPAL INVESTIGATOR: E. Premkumar Reddy, Ph.D.

CONTRACTING ORGANIZATION: Temple University School of Medicine
Philadelphia, Pennsylvania 19140

REPORT DATE: July 2004

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 2004	Annual (1 Jul 03 - 30 Jun 04)

4. TITLE AND SUBTITLE
Novel Cox-2 Inhibitor for Breast Cancer Therapy

5. FUNDING NUMBERS
DAMD17-02-1-0579

6. AUTHOR(S)
E. Premkumar Reddy, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Temple University School of Medicine
Philadelphia, Pennsylvania 19140

E-Mail: reddy@temple.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

Recent studies have shown that NSAIDS reduce the incidence of human cancers by inhibiting the COX enzymes. Of these, the inducible COX-2 isoform has been shown to be constitutively over-expressed in many tumor types, including those of the breast. The purpose of this study is to develop novel COX-2 inhibitors that can be used in breast cancer therapy. We developed seven classes of novel COX-2 inhibitors that possess tumor growth inhibitory activity. Some of these compounds inhibit the growth of both COX-2 positive and negative tumor cell lines, suggesting that they may target other protein(s) that play an important role in tumor cell proliferation. We have also determined that our most potent COX-2 inhibitor, which is nearly 6-fold more active than Celecoxib, induced irreversible G_1 arrest of tumor cells and ultimately leads to tumor cell apoptosis. These studies suggest that these compounds may have an important role as anti-cancer agents.

14. SUBJECT TERMS
COX-2, Inhibitors, Tumor Growth

15. NUMBER OF PAGES
8

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

Principal Investigator: E. Premkumar Reddy, Ph.D.

Title: Novel COX-2 Inhibitor for Breast Cancer Therapy

INTRODUCTION

Cyclooxygenase-1 and 2 (COX-1 and COX-2) catalyze the formation of prostaglandin H2 by converting arachadonic acid to prostaglandins (PGE) (1). Recent studies have shown that high levels of COX-2 are expressed in a large percentage of tumors, including those of the breast. In particular, constitutive over-expression of COX-2 has been observed in greater than 50% of ductal carcinomas in situ and in several highly metastatic estrogen receptor-negative breast tumor cell lines (2, 3). Furthermore, epidemiological studies have shown that the use of Non-steroidal anti-inflammatories (NSAIDs) can lower the incidence of certain tumor types, including those of the breast and studies in animals have confirmed these findings (4-7, 8-10). Taken together, these studies provide compelling evidence to support the involvement of COX-2 in the development of breast cancer. It is therefore reasonable to conclude that drugs which target COX-2 enzymatic activity can have a profound impact on the treatment of this disease.

Celecoxib, a selective COX-2 inhibitor, has been shown to inhibit 7,12-dimethylbenz (a)anthracene (DMBA)-induced the development of mammary tumors and induced regression in animal model systems (8,9). Because these studies demonstrate that COX-2 specific NSAIDs can act as both anti-carcinogenic and anti-neoplastic agents with respect to breast cancer, and because these types of NSAIDs are devoid of side effects, there is a need to develop new and improved agents to treat this disease.

BODY

Synthesis of novel COX-2 NSAIDs: To achieve the first aim of the proposal, we have synthesized additional series of compounds aimed at identifying the most potent COX-2 inhibitor to be used in breast cancer therapy. These compounds belong to three classes: (i) the 18000 series compounds are additional derivatives of SKU-46 and include 8 compounds (18200, 18210, 18220, 18230, 18160, 18170, 18180 and 18190); (ii) the 53000 series of compounds are derivatives with a hydrazone backbone and include 20

compounds (53010, 53020, 53030, 53040, 53050, 53060, 53070, 53090, 53100, 53110, 53120, 53130, 53140, 53150, 53160,53210, 53220, 53230 and 53240).

Effect of the 18000 and 53000 series compounds against COX-2 enzymatic activity: The inhibitory effect (reported as the IC_{50} value) of these drugs against the COX-2 (ovine) enzyme was analyzed using a COX inhibitory screening assay kit as described by the manufacturer (Cayman Chemicals, MI) (12). This assay directly measures the production of PGE_{2a}, which is produced by stannous chloride reduction of COX-derived $PGEH_2$ via an enzyme immunoassay. This type of assay has been demonstrated to be more reliable than peroxide inhibition-based assay systems (12). Celecoxib, which has an IC_{50} of 1.71μM, and our most potent inhibitors 9250A and 9310A, which have IC_{50} values of 0.85μM and 0.29μM, respectively, were used as controls in all assays. The results of these studies show that while the 18000 series of compounds were unable to inhibit COX-2 activity, three compounds belonging to the 53000 series (53010, 53050 and 53060) were able to inhibit the enzyme at concentrations of 6μM, 13μM and 13μM, respectively. While none of these compounds can inhibit COX-2 as well as 9310A or 9250A which are our most potent inhibitors, we are currently synthesizing additional analogs of these three derivatives with the goal of further improving their COX-2 inhibitory activity.

Effect of the 18000 and 53000 series compounds on breast tumor cell viability: To test the anti-tumor effects of these compounds, we next grew COX-2 positive and negative cell lines in the presence of the 18000 and 53000 series compounds. The results of these studies showed two compounds, 18040 and 18050 had GI_{50} values of 10.3μM and 8.5μM, respectively in COX-2 negative cell lines and 7μM and 23.4μM, respectively, in COX-2 positive cells. Celecoxib, in these assays had GI_{50} values of 13.1μM and 15.9μM in COX-2 negative and positive tumor cell lines, respectively. We are currently determining the GI_{50} values for the 53000 series of compounds. These results suggest that compound 18040 is more efficient than Celecoxib in inducing death of tumor cells. Because 18040 induces cell death in both COX-2 positive and negative

tumors, we are currently investigating the mechanisms by which this compound induces tumor cell death.

Effects of compounds 9250A and 9310A on cell cycle progression: As stated above, two compounds (9250A and 9310A) are extremely potent COX-2 inhibitors. Their IC_{50} values of 0.85μM and 0.29μM, respectively, are 2-5.9-fold more potent than Celecoxib, which has an IC_{50} value of 1.71μM. To determine the effects of these compounds on cell cycle progression, COX-2 positive and negative tumor cell lines were synchronized at late G_1/S phase with aphidicolin (1μg/ml). After 24 hours, the media was replaced with fresh medium containing DMSO (control) or 9250A or 9310A. Cells were collected at 0, 24, 48 and 72 hour time points and the cell cycle distribution determined by flow cytometric analysis. As expected, the control-treated cells re-entered the cell cycle after the removal of aphidicolin. Similar results were obtained in cells that were treated with 9250A, although a greater percentage of cells remained in G_1 throughout the experiment. However, at the 24hour time point, cells that were treated with 9310A remained arrested in the G1 phase. In addition, a noticeable percentage of the cells were beginning to enter the apoptotic pathway. At the 48-hour time point, the number of apoptotic cells had doubled, and by 72 hours, nearly all of the cells were apoptotic. These studies show that 9310A induces an irreversible G_1 arrest that ultimately leads to apoptosis.

KEY RESEARCH ACCOMPLISHMENTS

We have synthesized additional classes of COX-2 inhibitors that possess tumor growth inhibitory activity. As with our other series of compounds, some of these new compounds inhibit the growth of both COX-2 positive and negative cells, suggesting that they may target other protein(s) that play an important role in tumor cell proliferation.

We have also determined that our most potent COX-2 inhibitor, which is almost 6-fold more active than Celecoxib, induced G_1 arrest of tumor cells and ultimately activates their apoptotic pathway.

REPORTABLE OUTCOMES

Boominathan R., Reddy M.V.R., Cosenza, S.C., Sheikh, M.S. and Reddy, E. P. 2004. Novel COX-2 Inhibitors with Enhanced Anti-tumor Activity. 20th Annual Meeting on Oncogenes, Frederick MD, June 2004,

Pallela, V.R., Boominathan, R., Venkatapuram, P, Reddy, E. P. and Reddy, M.V.R. 2004. Synthesis of Styryl Acetophenylsulfides: Novel Cyclooxygenase Inhibitors. 227th ACS National Meeting.

CONCLUSIONS

The involvement of COX-2 in breast tumor growth has necessitated the development of specific COX-2 NSAIDs. In terms of breast cancer therapy, it is necessary to develop new therapeutic agents that possess both growth inhibitory and pro-apoptotic properties that are more efficacious than the present group of drugs (which were originally developed to treat inflammation). Our results to-date show that we have developed novel agents that inhibit COX-2 and have growth inhibitory and pro-apoptotic activities against breast tumor cells. These studies suggest that these compounds may play an important role as anti-cancer and chemopreventive agents.

REFERENCES

1. Vane, J.R. and Botting, R.M. (1998). Overview: The Mechanism of Action of Anti-inflammatory Drugs. In "Clinical Significance and Potential of Selective COX-2 Inhibitors." Vane, J. and Botting, R., eds. William Harvey Press, UK, 1-17.

2. Soslow, R.A., Dannenberg, A.J., Rush, D., Woerner, B.M., Khan, K.N., Masferrer, J. and Koki, A.T. (2000). COX-2 is Expressed in Human Pulmonary, Colonic and Mammary Tumors. Cancer 89: 2637-2645.

3. Liu, X.H. and Rose, D.P. (1996). Differential Expression and Regulation of Cyclooxygenase-1 and 2 in Two Human Breast Cancer Cell Lines. Cancer Res. 56: 5125-5127.

4. Prescott, S.M. and Fitzpatrick, F.A. (2000). Cyclooxygenase-2 and Carcinogenesis. Biochim. Biophys. Acta 1470: M69-78.

5. Taketo, M.M. (1998). Cyclooxygenase Inhibitors in Tumorigenesis. Part I. J. Natl. Cancer Inst. 90: 1529-1536.

6. Taketo, M.M. (1998). Cyclooxygenase Inhibitors in Tumorigenesis. Part II. J. Natl. Cancer Inst. 90: 1609-1620.

7. Williams, C.S. Mann, M. and DuBois, R.N. (1999). The Role of Cyclooxygenases in Inflammation, Cancer and Development. Oncogene 18: 7908-16.

8. Badawi, A.F., El-Sohemy, A., Stephen, L.L., Ghoshal, A.K. and Archer, M.C. (1998). The effect of Dietary n-3 and n-6 Polyunsaturated Fatty Acids on the Expression of Cyclooxygenase 1 and 2 and Levels of p21ras in Rat Mammary Glands. Carcinogenesis 19: 905-910.

9. Alshafie, G.A., Abou-Issa, H.M., Siebert, K. and Harris, R.E. (2000). Chemotherapeutic Evaluation of Celecoxib, a Cyclooxygenase-2 Inhibitor, in a Rat Mammary Tumor Model. Oncol. Rep. 7: 1377-1381.

10. Nakatsugi, S., Ohta, T., Kawamori, T., Mutoh, M., Tanigawa, T., Watanabe, K., Sugie, S., Sugimura, T. and Wakabayashi, K. (2000). Chemoprevention by Nimesulide, a Selective Cyclooxygenase-2 Inhibitor, of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)-Induced Mammary Gland Carcinogenesis in Rats. Jpn. J. Cancer Res. 91: 886-892.

11. Liu, C.H., Chang, S., Narko, K., Trifan, O.C., Wu, M., Smith, E., Haudenschild, C., Lane, T.F. and Hla, T. (2001). Overexpression of Cyclooxygenase-2 is Sufficient to Induce Tumorigenesis in Transgenic Mice. J. Biol. Chem. 267: 18563-18569.

12. Gierse, J.K., Koboldt, C.M., Walker, M.C., Seibert, K. and P.C. Isakson (1999) Kinetic basis for selective inhibition of cyclooxygenases. The Biochemical Journal, 339, 607-614

13. Huang Y, He Q, Hillman MJ, Rong R, Sheikh MS. (2111) Sulindac sulfide-induced apoptosis involves death receptor 5 and the caspase 8-dependent pathway in human colon and prostate cancer cells. Cancer Res. 2001, 61:6918-24.