Inhibition of Breast Cancer Bone Metastasis and Pancreatic and Colon Cancer by Synthetic Curcumin Analogs

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Curcumin (diferuloylmethane) is a polyphenol derived from the plant *Curcuma longa*, commonly called turmeric (Figure 1). Curcumin is the principle component of all curry powders & pastes, added to nearly every meat & vegetable dish in India, and 460,000 tons produced in 1994-95.

The compound has been reported to have anti-oxidative, anti-tumor promoting, anti-thrombotic and anti-inflammatory properties. The pleiotropic effects of curcumin are attributed at least partly to inhibition of the transcription factors, nuclear factor-κB (NF-κB), AP-1 and Egr-1 and their downstream signals [1,2].

Tissue factor (TF), a transmembrane receptor for coagulation factor VII/VIIa, is aberrantly expressed in human cancers [3,4]. It has been reported that the dietary pigment curcumin reduces endothelial tissue factor gene expression by inhibiting binding of AP-1 to the DNA and activation of NF-kappa B. [5,6].

We developed a method of the targeted delivery of paclitaxel to TF by conjugating the drug with coagulation factor VIIa inhibitor for preventing clotting. The factor VIIa inhibitor was provided by Drs. Lars C. Petersen, Ulla Hedner and Mirella Ezban, Novo Nordisk, Denmark. [7]. During this process, we decided to simplify the structure of curcumin and tried to find the active portion of the structure (Figure 2).

Based on the active moiety, we prepared over 100 monocarbonyl analogs of curcumin (MACs) to preserve the remarkable diversity of curcumin’s biological actions and improve its weak potency, low solubility and poor bioavailability, and screened for anticancer and anti-inflammatory activities. (Figure 2)

We screened all analogs against a breast cancer and a prostate cancer cell lines. The National Cancer Institute (NCI) kindly screened 13 MACs against the NCI-60 cancer cell line panel [8,9]. We came up with the same analog EF24 that was most active. The first active analog was EF24 which was synthesized by Eva M. Ferstl, Ph.D. of the Professor Dennis C. Liotta’s laboratory in the Department of Chemistry, Emory University. [8]. Since then, we have developed much more active analogs; EF31 and UBS109 [10]. (Figure 3).
The National Cancer Institute (NCI) screened 13 MACs against the NCI-60 cancer cell line panel during the early phase of synthesis. (Figure 4).

One of the first MACs, EF24, exhibited much stronger anticancer activity than cisplatin with less toxicity. The mean growth inhibitory concentration GI50 of EF24, curcumin and cisplatin were measured to be 0.7 µM, 7.3 µM, and 9.5 µM, respectively. EF24 showed much stronger anti-cancer activity than cisplatin. (Figure 5) [8].

The NCI subcontracted the Mayo Clinic Department of Medicine to perform a pharmacokinetic study of EF24 [11].

EF24 10 mg/kg administered to male mice iv, ip & orally.

EF24 absorption was rapid. Plasma concentrations of nearly 1000 nM detected 3 min after ip & oral dosing. Plasma half-life values of 74, 177 & 219 min were estimated for iv, oral and ip admin, respectively EF24 bioavailability 60% and 35% in mice after oral and ip administration, respectively.

Curcumin (MW. 368 g/mol) 100 mg/kg ip to mice; 2.3 µg/ml plasma in 15 min.

EF24 Toxicology – Mayo Clinic/NCI

MTD (NCI): IV 200 mg/kg;
IP 400 mg/kg;
oral >160 mg/kg vs IP 10 mg/kg for cisplatin (20–40x!).

All nude xenograft mice gained weight during drug treatment No organ damage (liver, kidney, spleen) upon sacrifice and pathology exam

Effect of EF-24 on gene expression suggested by DNA microarray [12] revealed two-pronged function: anti-cancer & anti-angiogenesis activities and may be cytoprotective & anti-carcinogenic activities (Table 1).

Since then, we have developed much more active analogs. UBS109 (Figure 6) was synthesized by Ustun B. Sunay, Ph.D. of the Professor Dennis C. Liotta’s laboratory in the Department of Chemistry, Emory University [10]. UBS109 is the most water soluble among the MACs. The water solubility seems to correlate with anti-cancer activity of the analogs. UBS109 shows the excellent activity against xenografts of head and neck squamous cell carcinoma, pancreatic cancer, colon cancer, and breast cancer [10,13-17]. (Table 2).

EF24, EF31 and UBS109 do not kill normal breast cell MCF-10A at concentrations range from 0 to 20 µM, whereas these analogs killed 100% of MDA-MB-231 breast cancer cells and Mia-Paca pancreatic cancer cells at 0.3-5 µM (Figure 7) [10].

Therapy of breast cancer metastasis

The bones, lungs, liver, and brain are frequent sites of breast cancer metastasis [18]. Breast cancer bone metastasis occurs in 65-70 % of patients with advanced breast cancer [19], leading to severe pathological bone fractures, pain, hypercalcemia, and spinal cord and nerve-compression syndromes [20,21], which are a common problem.
cause of morbidity and mortality. Tumor invasion into bone tissues is associated with osteoclast and osteoblast recruitment, resulting in the liberation of growth factors from the bone matrix, which can feed back to enhance tumor growth resulting in the vicious cycle of bone metastasis [18-23].

- Bone is one of the most common sites (65-70%) of metastasis. Hence, the prevention of metastasis is the key to save patients.
- An estimated 90% of deaths due to breast cancer are a consequence of metastatic disease.
- An estimated 20% to 30% of women diagnosed with invasive breast cancer will have a recurrence and may eventually die of their disease.
- Furthermore, MACs including EF24, EF31 and UBS109 inhibit NF-κB by suppressing IκB kinase-α and -β (IKK-α and -β) [10,13].

Recently, we demonstrated that the UBS109 has preventive effects on bone loss induced by breast cancer cell lines. This bone loss was prevented by p.o. and i.p. administration of UBS109 in vivo. UBS109 has suppressive effects on osteoclastogenesis by antagonizing RANKL-induced NF-κB activation and potent stimulatory effects on osteoblastogenesis and mineralization through activation of Smad signaling [15-17].

**UBS109 Inhibition of breast cancer bone metastases.**

We demonstrated that UBS109 suppressed the differentiation of RAW264.7 osteoclast precursors into mature osteoclasts (Figure 8) [15] and inhibited bone destruction and osteolysis by breast cancer MDA-MB-231 cells (Figure 9,10) and [16]. Furthermore, UBS109 directly stimulates osteoblastogenesis and mineralization in bone marrow cells from normal nude mice in vitro as demonstrated by Alizarin red stain (Figure 11). UBS109 concentration 1.25 µM killed breast cancer cells MDA-MB-231 at 100% in vitro. (Figure 12).

**UBS109 Inhibition of breast cancer lung metastases.**

Since breast cancer lung metastasis is a major problem in addition to bone metastasis, we studied the efficacy of UBS109 on lung metastasis of breast cancer MDA-MB-231 cells. We injected breast cancer cells into the tail vein of athymic nude mice to generate a lung metastasis (colonization) model for the study of UBS109 activity. UBS109 at 15 mg/kg i.p. administration inhibited lung metastases.

Lung metastases of breast cancer was produced by injecting luciferase gene containing MDA-MB-231 breast cancer cells 0.6 x 10^6 cells/0.1 ml, i.v./6 week-old female nude mouse, nu/nu. One week later, animals were treated with UBS109 5 or 15 mg/kg body weight, or vehicle (0.5% carboxymethyl cellulose sodium [CMC]) with 10% DMSO in sterile water) intra-peritoneal injection once a day for 5 days a week for 6 weeks (n = 6). All mice gained weight in the same degree and fashion (Figure 13).

Figure 8: UBS109 suppresses the differentiation of RAW264.7 osteoclast precursors into mature osteoclasts. Receptor activator of NF-κB (RANK) and its ligand (RANKL) [15].

**Figure 9:** Inhibition by UBS109 of bone destruction induced by breast cancer MDA-MB-231 bone metastatic cells. [16], a, b, control; c, UBS109: 50 mg/kg, po, d, 150 mg/kg, po, e, 10 mg/kg, ip, f, 20 mg/kg, ip.

**Figure 10:** UBS109 inhibition of breast cancer cell-induced bone destruction. [16]. Osteolyses in the bone cortex from the vehicle-treated mice (Fig. 10a and 10b), but no osteolysis in the bones from UBS109 treated mice. a, b, control, c. UBS109: 50 mg/kg, po, d. 150 mg/kg, po, e, 10 mg/kg, ip, f. 20 mg/kg, ip.

DMSO in sterile water) intra-peritoneal injection once a day for 5 days a week for 6 weeks (n = 6). All mice gained weight in the same degree and fashion (Figure 13)

UBS109 at 15 mg/kg inhibited breast cancer colonization in the lungs (Figure 14). Lung weight from mice that contain breast cancer treated with UBS109 at 15 mg/kg was significantly less than the 5 mg/kg and vehicle treated mice (Figure 15). Hematoxylin-eosin stain of breast tumors (MDA-MB-231) in the lungs of mice shows smaller tumor nodules at UBS109 15 mg/kg treated mice. (Figure 16). Mean concentrations of UBS109 in the plasma following intraperitoneal dosing of 15 mg/kg showed 1-40 ng/ml (Figure 17).

**Lung Metastasis of Breast Cancer MDA-MB-231-containing**
Luciferase gene. [Unpublished data, Shoji et al 2018]

Experiment
Day 1: 0.6 x 10^6 cells/0.1 ml, iv/mouse x 35 mice,
Day 4: Treatment started: UBS109, 5 or 15 mg/kg, ip, once daily for 5 days/week for 6 weeks.
Day 33: luciferase activity imaged.
Day 40: sacrificed and lung weight determined.

Novel therapeutic intervention for Pancreatic cancer using synthetic curcumin analog UBS109

Pancreatic ductal adenocarcinoma (PDA) is the fourth most
common cause of cancer death, the overall 5-year survival for PDA is less than 5%, and a median survival is for 4–6 months. Current chemotherapeutic drugs include gemcitabine (Gemzar) fluourouracil (5FU) albumin-bound paclitaxel (ABRAXANE)

We tested the cytotoxic activity of UBS109, EF24, HSP90 inhibitor (STA9090), gemcitabine, Akt inhibitor (MK2206) and p38 MAPK inhibitor (SB203580) against four human pancreatic cancer cell lines in vitro. (Figure 18) [Adams KB, 2015]. The cytotoxic activity ranking of various inhibitors against four human pancreatic cancer cells in vitro as demonstrated in Figure 18. [12]. The cytotoxic activity ranking of various inhibitors against four human pancreatic cancer cells in vitro as demonstrated in Figure 18. [12]

1. UBS109
2. EF31
3. EF24
4. HSP90 inhibitor (STA9090)
5. gemcitabine
6. Akt inhibitor (MK2206)
7. p38 MAPK inhibitor (SB203580).

We also tested the efficacy of UBS109 (25 mg/kg, i.v.) and EF31 (25 mg/kg, i.v.) once per a week for 3 weeks against xenografts of MiaPaCa-2 human pancreatic cancer (n = 6) (Figure 19) [13,14]. UBS109 and EF31 significantly inhibited the xenografts and UBS109 is better than EF31.

We then compared UBS109 (25 mg/kg, i.v.) with oxaliplatin (5 mg/kg) plus 5FU (30 mg/kg) (n = 6/group) against xenografts of human colon cancer cell lines HT-29 and HCT-116. UBS109 is better than one of the current regimens oxaliplatin plus 5FU. However, a combination of UBS109, and oxaliplatin plus 5FU inhibited better than UBS109 or oxaliplatin plus 5FU alone the xenografts of HT-29 and HCT-116 (Figure 20) [24-26].

Mechanisms of Action

During the earlier phase of the investigation, we demonstrated that EF24 bound with GSH and thioredoxin less than 100 seconds and induced oxidative stress (Mechanism 1) and depolarization of the mitochondrial membrane, which is the early irreversible step of apoptosis (Mechanism 2). Subsequently, we demonstrated that UBS109 inhibited NF-κB and its nuclear translocation by suppressing IKK-α and IKK-β (Mechanism 3 and 4) and also significantly inhibited angiogenesis (Mechanism 5,6). Our synthetic curcumin analogs inhibit multiple targets just like curcumin, which is more advantageous than a single target inhibition.

Conclusion and Future Prospective

It is of interest how the course of our research has taken turns. We initially wanted to make a targeted drug delivery using curcumin as a drug carrier. Instead, we have ended up on developing synthetic curcumin analogs for anticancer and anti-inflammatory agents. UBS109 demonstrated a strong cytotoxic activity but less toxicity as compared with other chemotherapeutic agents. We have to do a pre-IND (independent new drug) toxicology work on UBS109 by the

Mechanism 3

Mechanism 5. UB5109 inhibits angiogenesis in pancreatic cancers. 

References


Mechanism 4.

Mechanism 4. UB5109, EF31 and curcumin inhibit activation of NF-κB 

Mechanism 6.

Mechanisms of Action of Monocarbonyl Analogs of Curcumin (MACs):
UBS109, EF24, and EF31

1) Bind GSH and thiolate, generating ROS, inducing oxidative stress, mitochondrial membrane depolarization, activating caspase 3, phosphorylating receptor and apoptosis.

2) Inhibit inflammatory reactions and the DNA binding activity of NF-κB by suppressing IκKα and IκKβ, thereby inhibiting the downstream effectors such as COX-2, inflammatory cytokines, VEGF, and full length TNF and alternatively up-regulating TNF and preventing downstream.

3) Inhibit osteoclastogenesis and osteolytic bone destruction. 

4) Induce a G2/M arrest after 48 h of treatment.

5) Inhibit angiogenesis by downregulating HIF-1α protein levels & transcriptional activity and protein levels of VEGFA, FGF-2, Ang-1 and Ang-2.

6) Inhibit DNA methyltransferase (DNMT-1), HSP90 and enhance SPARC, P16 and E-cadherin expression and induce hypomethylating DNA.

7) Induce the DNA binding activity of p53 and p14.

Mechanism 5

Mechanism 5

FDA-approved company to apply for the IND of UBS109 to the FDA and go on for clinical trials for treatment of metastatic breast cancer, pancreatic cancer as well as colorectal cancer.

References


