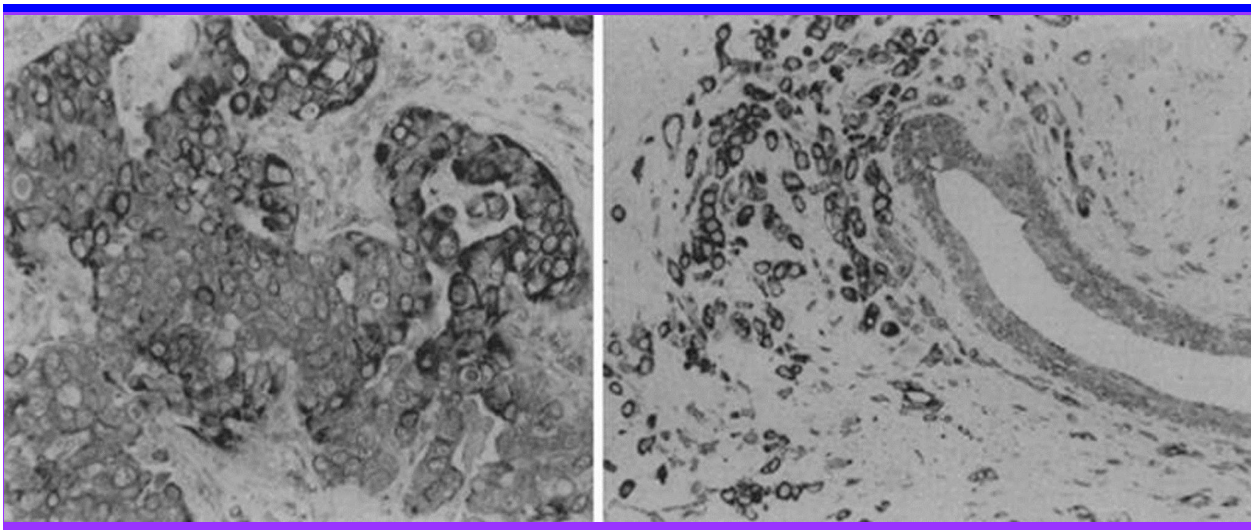

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Targeting breast cancer using retinoic acid triflоromethyl chalcone: A promising therapeutic strategy in the treatment of breast cancer

Hao Ding[1], Ben-Zhong Wang[2], Hua-Qing Zhu[3], Liu-Yi Dong[4], Yu-Fang Gu[1] and Yu Zhao[1]

Department of [1]Plastic Surgery and [2]Breast Surgery, The First Affiliated Hospital of Anhui Medical University, Hefei 230 022, China; Department of [3]Biochemistry and [4]Pharmacology, Anhui Medical University, Hefei 230 022, China.

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Abstract

The study was devised to investigate the effect of retinoic acid triflоromethyl chalcone (RAFC) on mammary carcinogenesis in female rats. The data revealed a significant decrease in number of rats with mammary tumor, number of tumors per rat and tumor volume by 54, 72 and 75% respectively in RAFC group compared to control group. The ibuprofen treated rats also showed a significant decrease in number of rats with tumor, number of tumors per rat and tumor volumes by 43, 55, and 59%, respectively. Treatment of rats with RAFC also increased the latency period of tumor induction significantly. Median detection period (50% of tumors) was 92, 83 and 56 days respectively in the rats from RAFC, ibuprofen and control groups respectively after DMBA induction. These results demonstrate that RAFC possesses strong chemopreventive activity against mammary carcinogenesis.

Introduction

In Western countries breast cancer is a most frequent malignant neoplasm among women. There were 234,580 new breast cancer cases detected and 40,030 estimated deaths in the United States alone in 2013 (Siegel et al., 2013). Despite intensive cancer control efforts, it remains the second leading cause of cancer deaths among American women (Singletary et al., 1998). The main cause of death in these patients was its metastases at distant sites. Owing to difficulty in predicting metathesis development many women are over-treated and suffer toxic side effects of chemotherapy (Weigelt et al., 2005). Recent epidemiological studies suggested the presence of an inverse association between regular intake of NSAIDs and the relative risk of breast cancer (Harris et al., 2005; Harris et al., 1995; Harris et al., 1996). Animal studies have also demonstrated the effects of NSAIDs against mammary carcinogenesis (Lee et al., 1992; McCormick et al., 1995), and in our laboratories, the common over-the-counter compound ibuprofen produced highly significant reductions in tumor size and tumor burden associated with inhibition of the genetic expression of COX isoforms (Joarder et al., 1997; Robertson et al., 1998; Alshafie et al., 1999).

Regulation of cell cycle behaviour is well known by retinoids and related compounds like all-trans retinoic acid (Krupitza et al., 1995; Defer et al., 1997). Despite of the biological promise of RA to treat breast cancer (Huang et al., 1988), head and neck cancer (Lehman et al., 1988), ovarian adenocarcinoma (Szuts et al., 1991), human malignant gliomas (Jeong et al., 2006), and acute promyelocytic leukemia (Chung et al., 2012), its poor aqueous solubility (0.1 µM at pH 7.3) (Crocetti et al., 2012; Tanaka et al., 2013) under *in vivo* restricts its clinical applications. One of the techniques to overcome this drawback is the development of polymeric micelles (Stummeret al., 2009), like glycol chitosan micelle. RA-incorporated GC nanoparticles inhibit the proliferation of HuCC-T1 cholangiocarcinoma cells at higher than 20 µg/mL of RA concentration (Norden et al., 2006). In the present study more active anlogues of retinoic acid,

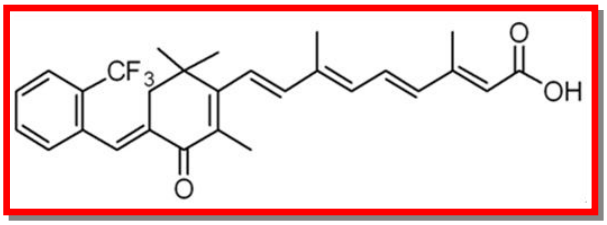

Figure 1: Structure of retinoic acid trifloromethylchalcone

retinoic acid trifloromethyl chalcone (Figure 1) was used to investigate its effect on breast cancer.

Materials and Methods

Reagents and chemicals

Retinoic acid, 7,12-dimethylbenz(a)anthracene (DMBA) and all other reagents with the highest purity were purchased from Sigma Chemical Co. (St. Louis, MO). The parent drug (retinoic acid) was then made one step modification to get its floro-chalcone. Ibuprofen oral suspension was purchased as Motrin (100 mg/5 mL; McNeil) oral suspension.

Dietary and tumor induction protocols

Female Sprague Dawley rats 150 in number were supplied by Laboratory Animal Services Centre at the Chinese University of Hong Kong. The rats were randomly assigned into three different groups of 50 each. The control group of rats were fed standard diet alone, those of ibuprofen group were fed standard diet containing 1,200 mg/kg body weight ibuprofen and those in RAFC group were fed standard diet containing 1,200 mg/kg body weight RAFC for 110 days. All the animals received 20 mg of DMBA intragastrically by gavage. The experiment was terminated after 15 weeks and during this period general health of rats, weight gain and food consumption were monitored. The animals were palpated twice a week for detection of mammary tumors after 28 days of DMBA treatment. The clinical parameters like number of rats with tumor, time of first tumor appearance, number of tumors per rat, relative tumor size, and location of every tumor were recorded weekly. After end of experiment, a micrometer caliper was used to measure diameter of all the tumors. From the diameter tumor volume was calculated using the formula $V = 4/3\ \pi r^3$. The animals were sacrificed and gross examination of stomach, kidneys, and liver was performed. All tumors, stomach and both kidneys of each rat were rejected and fixed in 10% buffered formalin. Prior to histological evaluation samples were embedded in paraffin blocks. The histological evaluation was performed using H & E staining. HPLC was used to determine the level of RAFC and ibuprofen in the serum of animals after the completion of experiment.

Statistical analysis

Descriptive statistics on body weights, tumor latency, number of rats with tumor, number of tumors per rat, and tumor volumes were examined and compared among the control, ibuprofen and RAFC groups. The statistical significance of comparisons between the three treatment groups were obtained using x2 tests, Fisher's exact test, ANOVA, and multiple mean comparison procedures (Elston et al., 1994).

Results

There was no significant difference in the initial and final average body weight of animals of control, ibuprofen and RAFC groups (Table I).

The histopathological evaluation of the tumors excised from the rats of control, ibuprofen and RAFC group showed that all the 150 tumors from control and 72 tumors from ibuprofen groups were adenocarcinomas. Among 22 tumors from RAFC group, 17 were adeno-carcinomas, and 3 were non-malignant fibroadenomas (Figure 2).

The examination of rats revealed a significant decrease in number of rats with mammary tumor (Figure 3), number of tumor/rat (Figure 4) and tumor volume by 54, 72 and 75% respectively in RAFC group compared to control group (Table II). Out of 50 rats in RAFC group, 17 had malignant tumors and 4 rats suffered from fibroadenomas. The tumor volume in these rats was very small. On the other hand, in control group all the 50 rats suffered from malignant tumors. Most of the rats had multiple tumors and the tumor volume was larger. The ibuprofen treated rats also showed significant decrease in number of rats with tumour, number of tumors per rat and smaller tumor volumes by 43, 55 and 59% respectively. Treatment of rats with RAFC also increased the latency period of tumor induction significantly. Median detection period (50% of tumors) was 92, 83 and 56 days respectively in the rats of RAFC, ibuprofen and control group respectively after DMBA induction (Table II).

The mean RAFC level in serum was 4.3 mg/mL. However, the level of RAFC in rats without tumor was

Table I

Initial and final body weight of HPDH and ibuprofen treated rats

Group	Initial weight (g)[a,b]	Final weight (g)[b,c]
Control	166.3 ± 1.7	281.1 ± 2.4
RAFC	169.8 ± 1.5	283.6 ± 2.6
Ibuprofen	167.5 ± 2.9	281.7 ± 1.3

[a]Mean weight ± SE; [b]No significant statistical variation was observed in the means of treatment; (initial weights; p=0.17; final weights, p=0.32)

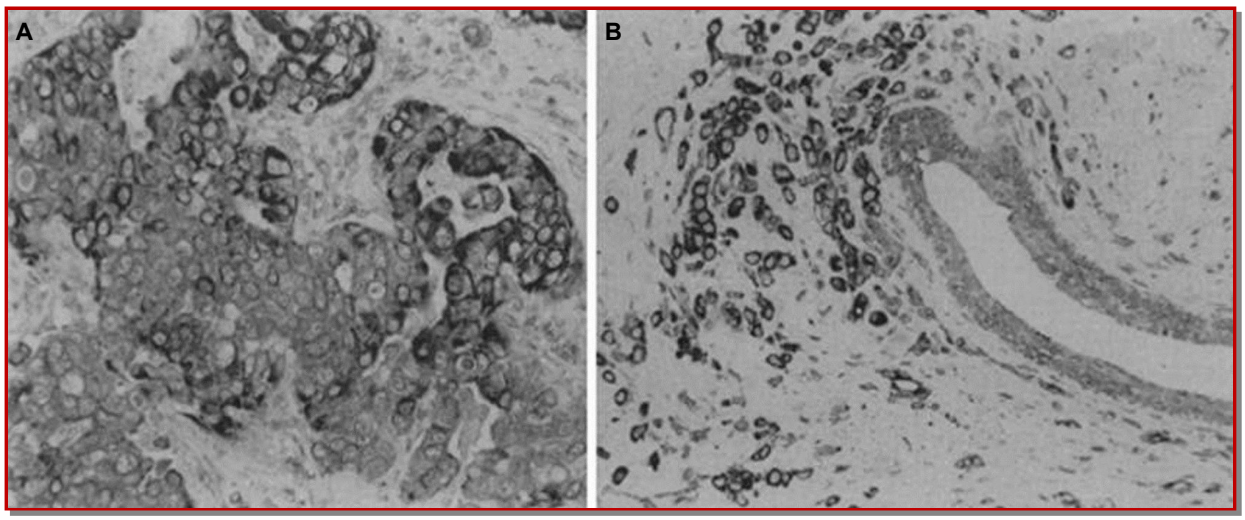

Figure 2: Adenocarcinoma from (A) control group of rat and (B) RAFC group of rat

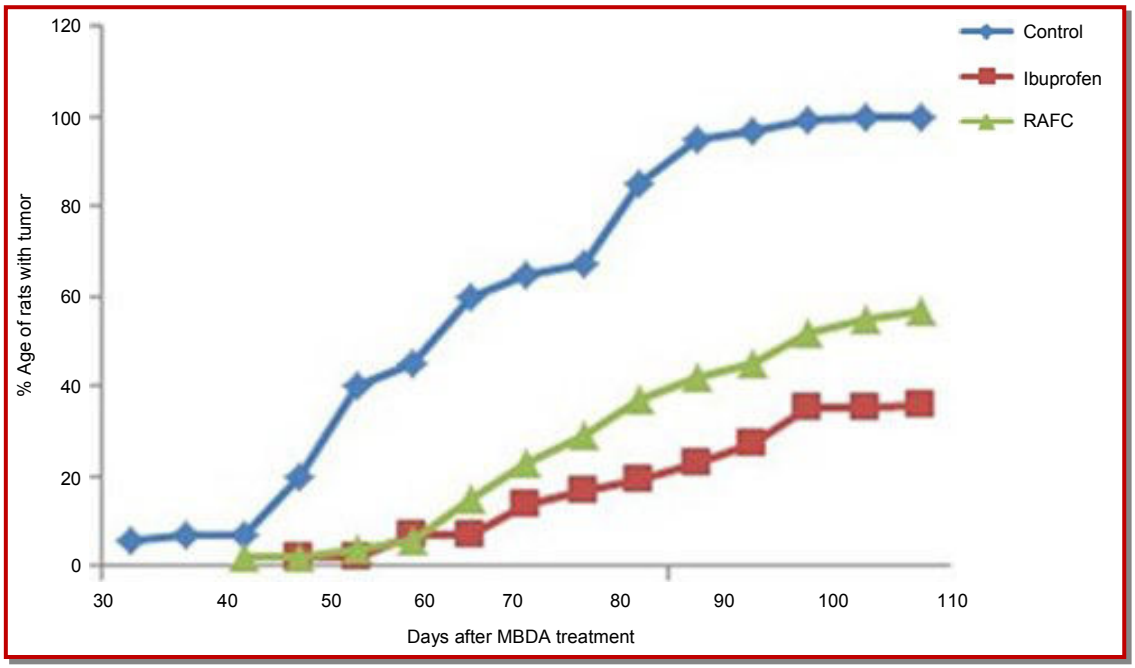

Figure 3: Effects of RAFC and ibuprofen on breast cancer incidence. The control group received Teklad powdered chow diet, the RAFC group received chow plus 1,200 mg/kg RAFC, and the ibuprofen group received chow plus 1,200 mg/kg ibuprofen

slightly more (4.6 mg/mL) than those with tumor (4.1 mg/mL). The ibuprofen levels in serum ranged from 5–11 mg/mL.

Discussion

RA is used to treat breast cancer (Huang et al., 1988), head and neck cancer (Lehman et al., 1988), ovarian adenocarcinoma (Szuts et al., 1991), human malignant gliomas (Jeong et al., 2006), and acute promyelocytic leukemia (Chung et al., 2012). But its poor aqueous solubility (Crocetti et al., 2012; Tanaka et al., 2013) under *in vivo* limits its clinical applications. Since RAFC possesses more bioavailabity we devised an experiment to investigate the effect of RAFC in the treatment of mammary carcinogenesis. Our results demonstrate that RAFC is a potential chemopreventive agent against the development of chemically-induced breast cancer. The observed chemopreventive effects of the RAFC exceeded those of the ibuprofen. The exact mechanism by which RAFC suppresses mammary tumor is not clearly known but it works similar to that of ibuprofen. Treatment of animals with RAFC did not lead to any toxic effect.

The animals were palpated twice a week for detection of mammary tumors after 28 days of DMBA treatment.

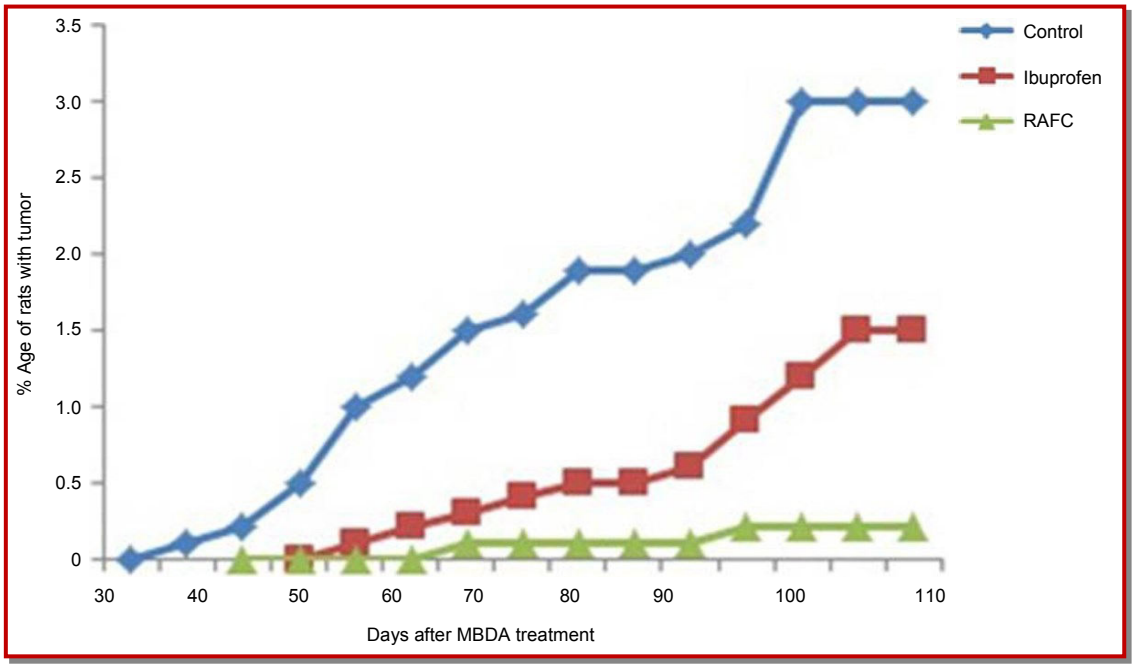

Figure 4: Effects of RAFC and ibuprofen on tumor burden. The control group received Teklad powdered chow diet, the RAFC group received chow plus 1,200 mg/kg RAFC, and the ibuprofen group received chow plus 1,200 mg/kg ibuprofen

Table II

Effects of RAFC and ibuprofen on the number of rats with tumor, number of tumors per rat, and tumor volume after DMBA-induced rat mammary tumors

Group	Latency period (days)	Number of rats with tumor (%)		Number of tumors per rat[c]	Tumor volume (cc)[d]
		Cancer[a]	All tumors (cc)[b]		
Control	58	100	100	3.2 ± 60.2	1.6 ± 0.4
RAFC	95[f]	32 (68)[e]	60 (40)[e]	1.6 ± 0.3 (52)[e]	0.7 ± 0.2 (57)[e]
Ibuprofen	86[f]	60 (40)[e]	60 (40)[e]	1.6 ± 0.3 (52)[e]	0.7 ± 0.2 (57)[e]

[a]The frequency of animals that developed breast cancer; [b]All tumors includes animals in the RAFC treatment group that developed fibroadenomas; [c]Mean number of tumors/animal 6 ± SE; [d]Mean tumor volume 6 ± SE; [e]Statistical significance relative to the control group at p=0.001; Reductions in the incidence rates, tumor burden, and tumor volume for the experimental diets relative to the control diet are given in parentheses

The clinical parameters like number of rats with tumor, time of first tumor appearance, number of tumors per rat, relative tumor size, and location of every tumor were recorded weekly. A micrometer caliper was used to measure tumor diameters from which tumor volumes were calculated using the formula $V = 4/3\pi r^3$. H & E staining was used for histological evaluation of the tumors and HPLC was used to determine the level of RAFC and ibuprofen in the serum of animals at completion of the experiment. All these results indicated that RAFC can act as effective therapeutic agent in the treatment of mammary carcinogenesis.

In conclusion, administration of RAFC, suppressed the number of rats with tumor, number of tumors per rat, and volume of malignant breast tumors induced by DMBA in female rats. These results suggest that celecoxib may be an effective chemoprevention agent against human breast cancer.

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Author Info
Ben-Zhong Wang (Principal contact)
e-mail: benzhong125@gmail.com

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