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Research and Development of Cancer Chemopreventive Agents in China

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Abstract Since the late 1970s, a comprehensive search for cancer chemopreventive agents has been established in our Institute. A series of new retinoids have been synthesized and screened on the basis of established methodologies of experimental chemoprevention in vitro as well as in vivo. Pharmacological studies demonstrated that *N*-4-(carboxyphenyl)retinamide (RII) induces cell differentiation of HL-60 cells and inhibits dimethylnitrosamine-induced carcinogenesis of the forestomach in mice, 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced papilloma in mouse skin, and DMBA-induced carcinogenesis of the buccal pouch in Syrian golden hamsters. It significantly promoted lymphoblastic transformation and activated macrophages. In further studies, RII significantly inhibited ornithine decarboxylase activity. After 6 months of chronic toxicological studies in rats and dogs, RII was recommended for clinical trial. Phase II studies found that RII is effective in treating oral and vulvar leukoplakia. It is also effective in treating myelodysplastic syndrome and dysplasia of uterine cervix. The chalcone retinoidal compounds were discovered when the search for new retinoids with less toxicity and higher potency led to third-generation retinoids, which were synthesized and screened. Structure-activity relationship studies found that 3,5-di-*tert*-butyl-4-methoxy-4-carboxyl chalcone (R9158) is the most active inhibitor of a variety of cancer cells. It has no effect on the Colony Forming Unit-Granulocyte/Macrophage (CFU-GM) of bone marrow in mice. In in vivo studies, R9158 showed a remarkable inhibition of chondrosarcoma in rats. It had no cross-resistance to vincristine, but was cross-resistant to all-*trans* retinoic acid. Red ginseng, a processed *Panax* ginseng, is considered a typical tonic in traditional Chinese medicine. Our studies demonstrated that red ginseng extract inhibited DMBA-induced skin papilloma significantly. Experiments showed that glycyrrhetinic acid inhibited croton oil-induced ear edema in mice. It also inhibited epidermal ornithine decarboxylase as well as the rapid DNA damage induced by the carcinogen benzo[*a*]pyrene (B[*a*]P). Our pharmacological studies demonstrated that Chinese gallotannin inhibited the malignant transformation of B[a]P-induced V79 cells in vitro and B[*a*]P-induced pulmonary adenoma in A/J mice in vivo significantly. J. Cell. Biochem. Suppl. 27:7–11

Key words: cancer chemoprevention; *N*-4-(carboxyphenyl)retinamide; chalcone retinoid; red ginseng; glycyrrhetinic acid; Chinese gallotannin

The ultimate purpose of cancer research is to decrease the incidence of cancer and lower the mortality associated with it. In spite of great success in diagnosis and treatment of cancer, total cancer incidence is going up [1]. Reducing cancer incidence is a challenge for the medical community as well as the pharmaceutical community. Cancer chemoprevention uses chemical compounds or natural products to reverse or inhibit malignant transformation of cells and prevent invasion and metastasis. Ideally, cancer chemoprevention would be a less painful, more economical and rational approach to cancer control.

In the late 1970s, the Institute of Materia Medica, Chinese Academy of Medical Sciences, established a comprehensive program to investigate retinoids and plant-originated compounds as cancer chemopreventive agents [2,3]. The search for new retinoids with less toxicity and/or higher potency led to synthesis of a series of retinoids, which were screened on the basis of chemoprevention in vitro as well as in vivo.

N-4-(carboxyphenyl)retinamide (RII) is a derivative of all-*trans*-retinoic acid. Its chemical structure is shown in Figure 1. Pharmacological studies [4–6] demonstrated that RII significantly induces differentiation of HL-60 cells and inhibits dimethylnitrosamine-in-

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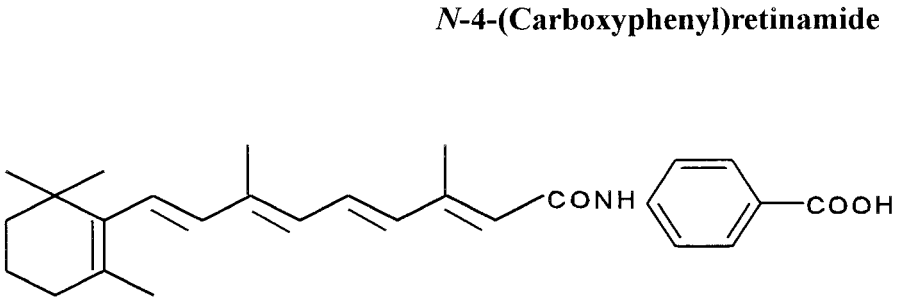

Fig. 1. Chemical structure *N*-4-(carboxyphenyl) retinamide.

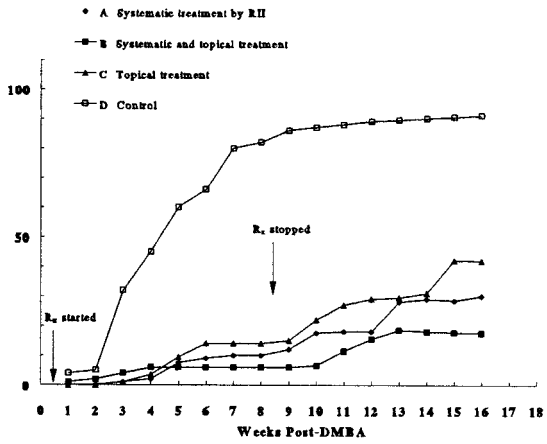

Fig. 2. Inhibition of buccal carcinogenesis caused by DMBA in hamsters.

TABLE I. Effect of RII on Oral Leukoplakia

Group	Cases	CR	PR	NR
Control	30	0	5	25
RII-treated	45	15	23	7
Total	75	15	28	32

TABLE II. Effect of RII on Vulvar Leukoplakia

Hospital	Case	CR	PR	NR
Friendship Hospital, Beijing	40	2	38	0
Hebei Medical College Teaching Hospital	30	6	24	0
Xiang Tan City Hospital	40	6	32	2
Total	110	14	94	2

duced carcinogenesis of the forestomach in mice, suppresses 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced papilloma in mouse skin, and inhibits carcinogenesis in the buccal pouch of Syrian golden hamsters (Fig. 2). It also significantly inhibits the growth of chondrosarcoma in rats. At a concentration of 1 x 10^{-6}M, RII promotes lymphoblastic transformation and activates macrophages, increasing their ability to release hydrogen peroxide (H_2O_2) in the mouse peritoneum. In addition, RII exhibits a significant inhibitory effect on ornithine decarboxylase (ODC) activity induced by croton oil in mouse epidermis. Pharmacokinetic studies on rats detected the parent form of RII in blood 1 h after dosing, reaching a peak at 7 h. The peak concentrations for doses of 100 and 200 mg/kg were 4.79 and 10.21 μg/ml, respectively. The biological half lives ($t_{1/2}$) were 13.0 and 12.5 h, respectively. The areas under the curve (AUCs) were 114.44 and 260.47 mg/L·h, respectively, at the above-mentioned dosage levels.

Seven hours after dosing, tissue distribution studies showed the highest RII concentrations in the intestine and stomach. Blood contained the second highest concentration, followed by liver, ovary, and kidney. The heart, spleen, muscle, testis, and fat tissue contained less RII. The percentage of RII protein binding in serum was 48.1.

After 6 months of chronic toxicological studies in rats and dogs, RII was recommended for clinical trials [7]. Phase I studies indicated that dosages of 60–80 mg/m^2/day were well tolerated by the patients. Phase II studies demonstrated that RII effectively treats oral and vulvar leukoplakia (Tables I, II). RII is also effective in treating myelodysplastic syndrome and dysplasia of the uterine cervix (Tables III, IV). In the case of cervical dysplasia, two courses of treatment were more efficacious than one.

The most common adverse effect of RII in patients was dryness of the mouth and skin. Some patients claimed pruritus and transient night blindness.

TABLE III. Effect of RII on Myelodysplastic Syndrome*

Group	Cases	CR	PR	Improvement	NR
RA	39	3	8	9	19
RAEB	24	1	9	3	11
CMML	4	1	0	2	1
RAEB-T	8	1	1	0	6
Total	75	6	18	14	37

*RA: refractory anemia; RAEB: refractory anemia with excess blasts; RAEB-T: RAEB in transformation; CMML: chronic myelo-monocytic leukemia.

TABLE IV. Effect of RII on Premalignant Lesions of the Uterine Cervix

		Effectiveness			
Treatment course	Number of cases	Cure	Signi-ficant	Effective-ness	No effect
I	27	0	22	2	3
II	27	3	21	2	1

Chalcone Retinoidal Compound

To find new retinoids with less toxicity and higher potency, a series of third-generation retinoids were synthesized and screened [8–12]. Structure-activity relationship studies of these compounds revealed that 3,5-di-*tert*-butyl-4-methoxy-4-carboxyl chalcone (R9158) most actively inhibited growth of a variety of cancer cells [14] (Table V). The EC_{50} for human cancer cell lines (ovarian cancer A_{2780}, hepatoma Bel_{7402}, esophageal cancer CaES17, lung giant cell carcinoma PLA-801C, KB cells, and colon cancer HCT-8) was in the range of 2–3 x 10^{-6} M/L, while some normal cell lines had an EC_{50} in the range of 2–3 x 10^{-5} M/L. R9158 is more potent in tumor growth inhibition than all-*trans* retinoic acid. R9158 did not inhibit the Colony Forming Unit-Granulocyte/Macrophage (CFU-GM) of bone marrow cells in mice. It strongly induced cell differentiation of HL-60 cells and NB_4 cells in vitro. Flow cytometry showed that R9158 exposure arrested cells in the G_1 phase and significantly decreased cell population in the S and G_2/M phases.

In vivo studies demonstrated that R9158 inhibited the growth of rat chondrosarcoma significantly. Dosages of 1.2 and 0.6 mg/kg of R9158 inhibited tumor growth by 99.3 and 95.6%, respectively. It also effectively inhibited Lewis lung carcinoma and melanoma B16 in mice.

TABLE V. Cytotoxic Activity Comparison of R9158 and Retinoic Acid

	EC_{50} (10^{-6} M)		Inhibition ratio
Cell line	R1958	RA	RA/R9158
HCT-8	6.5	125.3	19.4
KB	4.4	60.3	13.7
Lung Giant Cell CA (PLA 801C)	4.3	103	24.2
A_{2780}	5.6	16.2	2.9
Bel_{7402}	5.8	17.7	3.1

It is interesting to note that R9158 has no cross-resistance with vincristine (VCR) in KB/VCR and HCT-8/VCR200 cell lines. Dot blot analysis showed that expression of the multi-drug resistant gene, *mdr*1, decreased when cells were exposed to R9158 at a concentration of 10^{-5} mol/L for 24 h.

Red Ginseng

Traditional Chinese medicine has long depended on nourishing tonics, including red ginseng, a processed *Panax* ginseng . Our studies demonstrated that red ginseng extract inhibited DMBA-induced skin papillomas at dosages of 100, 200, and 400 mg/kg orally (Fig. 3). Red ginseng extract significantly decreased papilloma incidence; the number of papillomas per mouse also decreased dramatically [15,16]. These results coincide with the epidemiological observations of Korean scientists. Ginsenoside Rh_2, one of the characteristic components of red ginseng, exhibited a significant induction of B16 melanoma cells at a concentration of 8 mg/ml.

Curcuma longa L. and Curcumin

Curcuma longa L. is commonly used in traditional Chinese medicine to relieve abdominal pain and as a food additive; it displays antibacterial activity in vitro. The major active principle of this plant is curcumin (Fig. 4). Experimentally, curcumin inhibited *his* revertants induced by methyl methane sulfonate (MMS) in *Salmonella*/microsome plate incorporation assay [16]. It also decreased micronucleus formation induced by endoxan. As an anti-inflammatory and presumed antipromotor of cancer, it decreased ear edema caused by croton oil in mice.

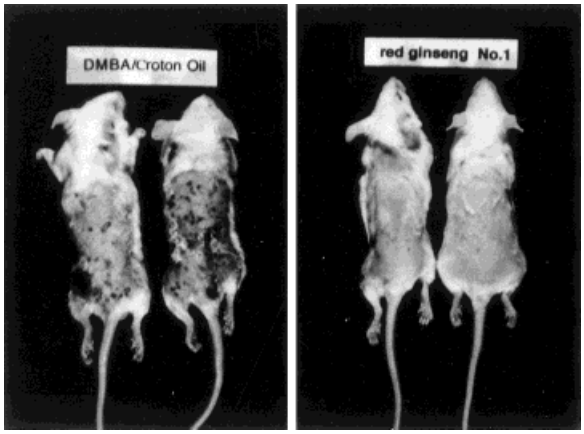

Fig. 3. Chemopreventive effect of red ginseng on skin papillomas induced by croton oil and DMBA.

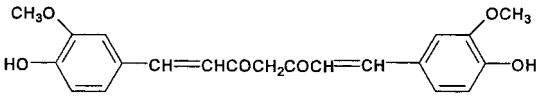

Fig. 4. Chemical structure of curcumin.

Glycyrrhiza uralensis fisch and Glycyrrhetinic Acid

Glycyrrhiza uralensis is widely used as an antidote in traditional Chinese medicine and as a food additive. Our experiments demonstrated that glycyrrhetinic acid inhibited ear edema induced by croton oil in mice. It also inhibited the activity of epidermal ODC at a dosage of 50–200 mg/kg for 3 days [17]. Rapid DNA damage induced by B[*a*]P was significantly controlled by this compound.

Rhus chinensis and Chinese Gallotannin

Rhus chinensis and its major component Chinese gallotannin are widely used as an astringent in traditional Chinese medicine as well as in Western medicine. Recently, Chinese gallotannin was found to have a variety of biological activities, including antitumor, antivirus, and antifungal activities. Pharmacological studies in our laboratory demonstrated that Chinese gallotannin inhibited the malignant transformation of V79 cells induced by benzo[a]pyrene (B[*a*]P) in vitro at concentrations of 5–20 μg/ml. Chinese gallotannin profoundly inhibited B[*a*]P-induced pulmonary adenoma in A/J mice (Table VI). Tumor incidence and number of tumors per mouse in the treated group were significantly lowered (X.G. Chen, unpublished observations). Other experiments indicated that Chinese gallotannin also inhibited DMBA/croton oil-induced skin papillomas in mice. Tumor latency was prolonged and tumor incidence decreased in a dose-dependent manner. In the transplantable tumor system, Chinese gallotannin exhibited significant antitumor activity in sarcoma 180, Lewis lung carcinoma, and colon carci-

TABLE VI. Effect of Chinese Gallotannin on B[a]P-Induced Pulmonary Adenoma in A/J Mice

Group	Dosage (mg/kg)	Pulmonary tumor/mouse
B[a]P	100	12.6 ± 4.48
B[a]P + Gallotannin	100 + 200	2.7 ± 1.58*
B[a]P + Gallotannin	100 + 60	0.9 ± 0.99*

*$P < 0.01$.

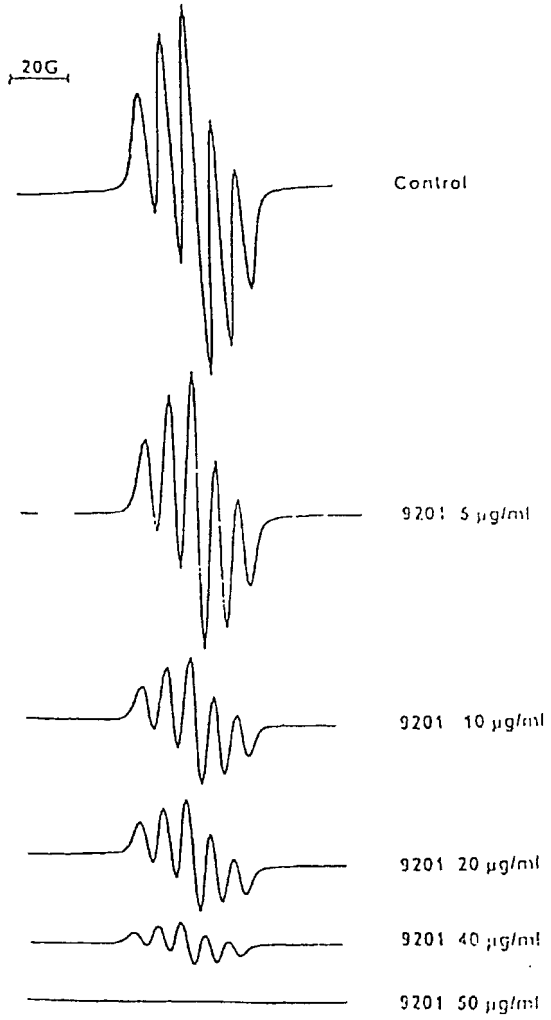

Fig. 5. Effect of Chinese gallotannin on ESR spectra of 1,1-dipheny1–2-picrylhdrazyl (DPPH).

noma 26-bearing mice. Further studies showed that Chinese gallotannin is a strong free radical scavenger in terms of O_2 and 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Fig. 5). Interestingly, it also inhibited cytochrome P-450 A1 and arylhydrocarbon hydroxylase (AHH) activities. Chinese gallotannin showed a strong antimutagenic activity in vitro and decreased chromosome damage induced by cyclophosphamide.

Recipe Antitumor B

Recipe Antitumor B is a complex recipe of traditional Chinese medicine consisting of six medicinal herbs: *Sophora subprostrata Chua et Chen*, *Patrinia villosa* Thumb, *Dictamnus dasycapus, Dyscoria bulbifera L., Prumilla vugalis L*, and *Polygonum historia L*. Lin et al. [18] have reported that Recipe Antitumor B dramatically inhibited esophageal carcinogenesis induced by nitrosamine in rats. Wei and Nishimura [19] and Lin et al. [20] have reported that Recipe Antitumor B has a significant inhibitory effect on DMBA-induced buccal pouch carcinoma in hamsters. On the basis of toxicological studies, this recipe was recommended for clinical trials in Linxian county, Henan Province, China, a high-risk area for esophageal cancer. Long-term (5-year) administration of this recipe to a population at high risk of esophageal cancer significantly reduced cancer incidence. In addition, there were no severe side effects.

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