Pure & Appl. Chem., Vol. 63, No. 1, pp. 157–160, 1991.
Printed in Great Britain.

Acyclic retinoids and cancer chemoprevention

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Abstract Human trials of cancer chemoprevention employing antitumor promoter retinoids are currently progressing worldwide and are targeting mainly skin, lung, uterine cervix or breast cancers. Although the inhibitory effects of various synthetic retinoids on experimental cancers and tumor-derived cell lines are promising, several problems with adverse effects such as mucocutaneous disorders, teratogenicity or hyperlipidemia still remain to be solved before clinical application. In an attempt to develop new analogs that are much safer in clinical use, we have synthesized acyclic retinoids which bind to cellular retinoic acid-binding protein (CRABP) with affinities as high as that of all-trans retinoic acid. Among these acyclic retinoids, 3,7,11,15-tetramethyl-2,4,6,10,14-hexadecapentaenoic acid or E-5166 showed a better therapeutic index than the trimethyl methoxyphenyl analog of retinoic acid (TMMP or Etretinate) on mouse skin papilloma and, in particular, on experimental liver tumors induced by 3'-methyl-4-dimethylaminoazobenzene in rats as well as in spontaneous hepatoma-bearing mice (C3H/HeNCrj). E-5166 also regulated a human hepatoma-derived cell line: PLC/PRF/5 (Alexander cell) in terms of both cell growth and alpha-fetoprotein production. Following these observations, application of E-5166 has cleared the Phase I trial in cirrhotic patients who are regarded as a high-risk group for hepatoma, that is now the third leading cause of male cancer death in Japan.

INTRODUCTION

It is well established that vitamin A or retinol is a nutrient essential for the support of growth and life of higher animals, in particular, for vision and reproduction. On the other hand, the integrity of normal cell growth and differentiation in epithelial tissues can also be achieved by retinoic acid, which is irreversibly formed from retinol.

One of the current topics concerning vitamin A and its derivatives, collectively called retinoids, has focused on their action as anti-tumor promoters, and practical applications of retinoids are on trial for the purpose of cancer chemoprevention in man (ref.1). In the present paper, basic research and clinical application have shown an acyclic retinoid, originally found in our laboratory, to be one of the most effective and safe retinoids yet available.

GENERAL METABOLISM OF RETINOIDS IN RELATION TO THEIR SPECIFIC BINDING PROTEINS

Vitamin A is generally obtained from food either direct or as provitamin A (mainly beta-carotene). Retinyl esters, which are absorbed by intestinal epithelia, are converted into retinol, re-esterified, and released with chylomicrons into the thoracic lymphatics. Following uptake by hepatic parenchymal cells, retinyl esters are hydrolyzed and transferred for storage to fat-storing cells or stellate cells in the liver. Depending on vitamin A requirements, retinol is secreted from the liver in association with its specific carrier protein, retinol-binding protein (RBP), and delivered to target tissues. Plasma RBP level is strictly regulated by two mechanisms, (1) synthesis and secretion by the liver, and (2) degradation in the kidney.

At the target cells, retinol is taken up through a cell surface receptor for RBP and generally converted into active metabolites, one of which is retinoic acid. For the translocation of retinol and retinoic acid inside the cells, intracellular binding proteins, with their strict ligand specificities, have been identified as listed in Table 1.

Table 1. Vitamin A and its specific binding proteins

	Plasma	Cytosol
Retinol	RBP	CRBP, CRBP(F)
Retinoic acid & its analogs	Albumin	CRABP, CRBP(F)
Retinyl esters	Lipoprotein	?

Among these proteins, cellular retinol-binding protein (CRBP) and cellular retinoic acid-binding protein (CRABP) are generally distributed in various tissues which need retinoids to maintain their functions, while the F-type cellular retinol-binding protein, (CRBP(F)) appears exclusively in a limited number of organs including fetal liver (ref.2), regenerating liver (ref.3), and hepatoma (ref.4). Tissue levels of CRBP, CRABP and CRBP(F) in experimental hepatocarcinogenesis are given in Table 2. In addition, cellular retinol-binding protein, type III :CRBP(III) has recently been purified and partially characterized from bigeyes of tuna fish, and its endogenous ligand was identified as retinol (ref.5).

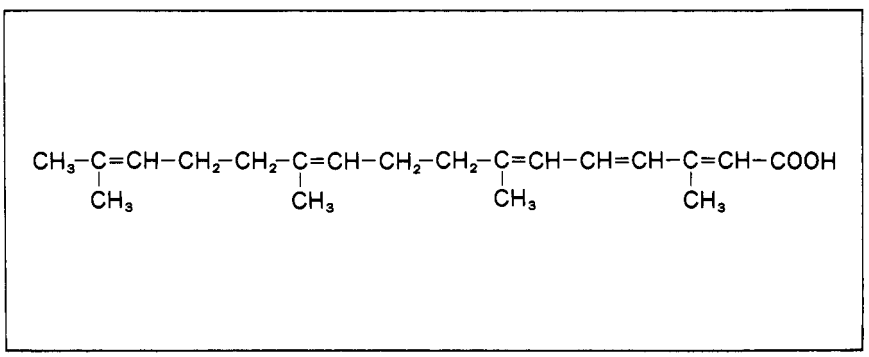
Table 2. Concentrations of cellular retinoid-binding proteins CRBP, CRABP, and CRBP(F) during hepatocarcinogenesis

Group	CRBP	CRABP	CRBP(F)
		pmol/g, mean	
Control (0-time)	121,8	ND	ND
Hyperplastic nodule (10 weeks)	102.1	2.1	35.6
Hepatoma (20 weeks)	25.7	0.2	15.4
Surrounding tissue (20 weeks)	43.1	ND	ND

ND; not detectable. Each value represents a mean of 5 rats treated with 3'-methyl-4-dimethylaminoazobenzene.

A NOVEL ACYCLIC RETINOID

In general, binding with these carrier proteins as mentioned above seems to be essential for retinoids to exert their functions. In the process of developing novel acyclic retinoids, we employed the binding affinity of the compounds to CRABP as a screening criterion. Among those surveyed, 3,7,11,15-tetramethyl-2,4,6,10,14-hexadecapentaenoic acid or E-5166 showed the highest affinity for CRABP (ref.6). Hence, E-5166 was selected thereafter as a promising candidate for basic research on cancer chemoprevention.



Chemical fomula of E5166

INHIBITORY EFFECTS OF THE ACYCLIC RETINOID ON SKIN PAPILLOMA AND HEPATIC TUMORS

SKIN PAPILLOMA: Skin papilloma was induced by topical applications of dimethylbenzanthracene followed by croton oil. When the number of papillomas reached 6-20, E-5166 or beta-carotene were given by a stomach tube 5 days per week for 7 to 28 days. Administrations of both E-5166 and beta-carotene resulted in significant regressions in skin papillomas (Table 3)(ref.7). The 50% effective dose (ED_{50}) of E-5166 was found to be 104mg/kg. The minimal toxic dose was 320 mg/kg, so the therapeutic index of E-5166 was calculated to be 3.1, which was better than 0.2 for retinoic acid and 2.0 for the TMMP analog. Therefore, E-5166 is clearly favorable as a cancer chemopreventive agent, on the basis of its wide safety margin.

Table 3. Antitumor effects of E-5166 and beta-carotene on skin papillomas in mice

Treatment	Percent cumulative diameter of papilloma		
	0	7	14 days
Control	100	105	112
E-5166	100	82[a]	69[a]
Beta-carotene	100	97[a]	96[a]

Each value represents mean of 6 mice.
a; $P<0.05$ as compared with control group.

HEPATOMA: Inhibitory effects of E-5166 were examined in two distinct models of hepatocarcinogenesis: i.e., chemically induced tumors by 3'-methyl-4-dimethylaminoazobenzene in rats (ref.8) and spontaneous or genetically determined hepatoma in C3H/HeNCrj mice (ref.9). As shown in Table 4, E-5166 as well as TMMP significantly inhibited the incidence of hepatoma in both models. Of importance is the finding that TMMP induced significantly growth retardation and marked hypertriglyceridemia, while no adverse effect was noted in animals treated with E-5166 (Table 4). Hence, it is also established that E-5166 has a better therapeutic index particularly for a long-term cancer chemoprevention.

Table 4. In vivo effects of TMMP and E-5166 on rat hepatocarcinogenesis induced by 3'-MeDAB and on blood biochemical data for the hepatoma-bearing rats

Treatment	Gamma-GTP positive tumor		Blood biochemical data			
	Number/cm^2	Area(%)	gamma-GTP (U/l)	ALT (U/l)	Albumin (g/dl)	Triglyceride (mg/dl)
Control	0.85	9.6	33.6	92.3	3.98	357.9
TMMP	0.50[a]	5.6[a]	13.8[a]	70.7[a]	4.16[a]	512.2[a]
E-5166	0.53[a]	6.1[a]	25.6[a]	71.5[a]	4.16[a]	354.4

Each number represents a mean of 40 rats.
a; $P<0.05$ as compared with control.

PHARMACOKINETICS OF THE ACYCLIC RETINOID

When [7-^{14}C]-E-5166 was administered orally to mice bearing papillomas, the labeled compound was rapidly absorbed within 10 hr; 50% of the absorption was accounted for by the portal vein and 30% by the lymphatics. [^{14}C]-E-5166 tended to be accumulated gradually in the papilloma between 5 and 24 hr after administration, the effect being higher there than in every other organ except the liver. Autoradiographic examination 8 hr after the administration also revealed a large accumulation of the radioactivity in papilloma tissues, in addition to the gastrointestinal tract, liver, brown adipose tissue and submaxillar gland (data not shown). Moreover, radiochromatographic analysis revealed that E-5166 in the papilloma was in the "free" form, whilst the compound in normal epidermis was in the triglyceride form (data not shown). Hence, it is strongly suggested that E-5166 is markedly converted into the "free" or active form in the papilloma, presumably as a result of enhanced hydrolysis.

In C3H/HeNCrj mice bearing spontaneous hepatomas, the ratio of the radioactivity of [^{14}C]-E-5166 accumulated in tumor cells over that in non-cancerous, surrounding parenchymal cells was higher than 1.0, whereas the ratio was below 0.5 in the case of retinoic acid (data not shown). This result strongly suggests a selective incorporation of E-5166 into tumor cells, supporting the efficacy of the compound for inhibition of hepatocarcinogenesis.

CLINICAL APPROACH TO CHEMOPREVENTION OF HEPATOMA WITH THE ACYCLIC RETINOID

Preliminary to applying E-5166 to man, the effects of the compound on the human hepatoma-derived PLC/PRF/5 cell line was investigated. E-5166 significantly reduced the cell growth, and further inhibited the secretion of alpha-fetoprotein (AFP) at much lower concentrations (1uM) than those required for growth inhibition (10uM). Furthermore, a gene transcriptional switch from AFP to albumin by addition of E-5166 was also suggested (Y.Fukutomi, et al. manuscript submitted).

More important is the recent unpublished observation by Omori et al. in our group that only a single dosing of E-5166 at the proper time significantly reduced the incidence of spontaneous hepatoma in C3H/HeNCrj mice. The previous report that marked and long-lasting alterations in mRNA pattern were induced in testicular cells by a single injection of retinoids (ref.10), also supports the contention that "once a life (or more practically, only a limited period)", rather than "life-long", administration of the retinoid may well be effective for the cancer chemoprevention.

BIOCHEMICAL CHARACTERISTICS OF HEPATOMA IN RELATION TO VITAMIN A METABOLISM

A reduction in the retinoid content in hepatoma was initially described in humans (ref.4), and confirmed in experimental liver cancers (ref.7). Mechanisms of retinoid depletion in hepatoma are proposed as follows: (1) reduced uptake of retinoids by the cells due to alterations of membrane receptors for chylomicron remnants, (2) enhanced degradation of retinoids, for example, the conversion of retinol into inactive anhydroretinol (ref.7). Moreover, retinoid depletion was found to develop during the precancerous stage of chemically induced hepatocarcinogenesis with a concomitant appearance of CRABP and/or CRBP(F), which were thought to act as receptors for synthetic retinoids such as E-5166. In addition, tissue levels of CRABP and CRBP(F) were found to be significantly higher in the precancerous stage than in established hepatoma (Table 2), suggesting that the administration of E-5166 should be targeted to this stage, which corresponds to liver cirrhosis, in human hepatocarcinogenesis.

CONCLUSION

A novel synthetic acyclic retinoid, E-5166, is a compound identified through the screening of binding affinities to CRABP and/or CRBP(F). Basic experiments concerning the inhibitory effects and pharmacokinetics of E-5166 in hepatocarcinogenesis are almost finished and then reveal the excellent efficacy and safety of the compound. We have cleared the Phase I clinical study of E-5166 and are preparing an early Phase II trial in cirrhotic patients, who are regarded as a group with high risk of hepatoma.

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