Hepatocellular Carcinoma

Prevention of Second Primary Tumors by an Acyclic Retinoid, Polyprenoic Acid, in Patients with Hepatocellular Carcinoma

Key Words
Hepatocellular carcinoma · Cancer chemoprevention · Retinoid · Differentiation induction · Apoptosis · Clonal deletion · α-Fetoprotein

Introduction

Retinoid is a collective term which indicates vitamin A (retinol) and its derivatives. Retinoid has a variety of functions such as growth promotion, maintenance of reproduction and dark adaptation. Retinoid also regulates cell differentiation and tissue morphogenesis. Since abnormality in the differentiation induces cellular atypia and that of the morphogenesis induces structural atypia, retinoid has important effects which inhibit carcinogenesis.

It is well known that hepatoma arises from chronic liver diseases associated with viral infection or alcohol abuse. Abnormal metabolism of retinoids was revealed in hepatocarcinogenesis. Furthermore, not only experimental but also clinical studies suggest that repairs of these abnormalities may suppress hepatocarcinogenesis.

Altered Retinoid Metabolism in Hepatocarcinogenesis

In the analysis of retinoid concentration in the tissue of human and experimental hepatomas, it was shown that the concentration is reduced in premalignant and malignant lesions [1, 2]. It is suggested that premalignant and malignant tissues lose or catabolize retinoids which suppress hepatocarcinogenesis.

Basic Mechanisms of Suppression of Hepatocarcinogenesis by Acyclic Retinoid

Hepatoma arises from chronic viral or alcoholic liver diseases. The liver with such chronic liver disease likely has multiple and independent clones of premalignant cells. These clones develop multicentric carcinogenesis. Such a characteristic pattern of carcinogenesis is ex-
Table 1. Prevention of second primary tumors (SPT) with acyclic retinoid (AR) in patients with hepatoma – time course of changes in serum AR, AFP-L3 and liver SOL.

|                    | Entry placebo | Entry active | 12 months placebo | 12 months active | Follow-up (median 38 months) |
|--------------------|---------------|--------------|-------------------|-----------------|-----------------------------|
|                    |               |              |                   |                 | placebo | active | placebo | active |
| Serum AR, ng/ml    | 0             | 0            | 0                 | 45              | –       |       |
|                    |               |              |                   | (n = 39)        |         |        |
| Serum AFP (n=21)   |               |              |                   |                 |         |        |
| Total, ng/ml       | 29            | 23           | 42                | 25              | –       | –       |
| AFP-L3, ng/ml      | 0             | 0.74         | 0                 | 0               | –       | –       |
| AFP-L3-positive cases, % | 4 (19%) | 5 (24%) | 12 (57%) | 1 (5%) | – | – |

Liver SOL

|                  |                   |                   |                   |          |          |          |          |
|------------------|-------------------|-------------------|-------------------|----------|----------|----------|----------|
| Positive cases, %| 0 (n=45)          | 0 (n=44)          | 9 (20%)           | 4 (9%)   | 20 (44%) | 7 (16%)  |          |
| SPT              |                   |                   |                   |          |          |          |          |

Data were derived from references 8 and 9, respectively.

1 Polyprenoic acid (3,7,11,15-tetramethyl-2,4,6,10,14-hexadecapentaenoic acid).
2 Lectin-reactive AFP measured by lectin-affinity electrophoresis and antibody affinity blotting.
3 Not done.

*p < 0.05, **p < 0.01.

explained by a concept of field canerization which postulates that multiple cancers arise from a field that is exposed to a continuous carcinogenic insult. In order to prevent hepatocarcinogenesis in liver cirrhosis or occurrence of second primary hepatomas after curative treatments, deletion and/or inhibition of such clones is essential. Clonal deletion is realized by eradication of premalignant cells in the promotion, conversion or progression step and clonal inhibition is realized by inhibition of development of transformed cells to the next step.

In attempts to develop novel synthetic compounds for cancer chemoprevention, we found a 20-carbon polyprenoic acid (3,7,11,15-tetramethyl-2,4,6,10,14-hexadecapentaenoic acid) [4] that binds to the cellular retinoic acid-binding protein and has relatively low toxicity. This agent suppresses cell growth and the production of α-fetoprotein (AFP), and induces cell differentiation [5] and apoptosis [6,7] in human hepatoma-derived cell lines. Furthermore, it inhibits chemically induced hepatocarcinogenesis in rats and spontaneous hepatoma in mice [2]. It action is mediated in part through RAR and RXR [5]. This compound can be called an open-chain or acyclic retinoid.

The acyclic retinoid deletes premalignant or malignant clones by two possible mechanisms. One mechanisms is that the cascade of transcription factors induce the differentiation of premalignant or malignant clones, through a signal that begins with the binding of the retinoid to RXR [5]. It is followed by the recovery of programmed cell death, deletion and/or inhibition of such clones is essential. Clonal deletion is realized by eradication of premalignant cells and premalignant clones must be finally deleted. The other is that, in a short period, the retinoid causes apoptosis in the promotion, conversion or progression step and clonal inhibition is realized by inhibition of development of transformed cells to the next step.

Clinical Experiences of Acyclic Retinoid on Hepatocarcinogenesis

All cirrhotic patients have a high risk since the annual incidence of primary hepatomas in these patients is 7 or 8%. Moreover, the patients who received the curative
treatment of the initial hepatoma have a higher incidence of second primary hepatomas at 20–25% a year.

We conducted a randomized controlled study to test whether polypropenoic acid prevents second primary hepatomas, which associate with multicentric carcinogenesis, after curative surgical resection or the percutaneous injection of ethanol into the initial hepatoma. Acyclic retinoid (600 mg/day) and placebo were orally given for 12 months in 44 and 45 patients, respectively. Since toxic symptoms were noticed only one in active and two in placebo groups, respectively, acyclic retinoid could be used in safety without side effects. Mean serum acyclic retinoid was found to be 45 ng/ml at 1 year (table 1).

After a median follow-up of 38 months, 12 patients (27%) in the active group developed a new HCC, whereas 22 (49%) did in the placebo group (p = 0.04). The acyclic retinoid particularly prevented second primary HCC (7 cases, 16%) as compared with placebo (20 cases, 44%) (p = 0.004). Analysis with Cox proportional-hazards model demonstrated the agent as a single independent factor to reduce the second primary occurrence (adjusted relative risk for the agent being 0.31; 95% confidence interval, 0.12–0.78) [8].

In this clinical study (table 1), we also demonstrated that abnormal clone was suppressed and/or eradicated by the retinoid by measuring an isoform of serum AFP as a possible marker of the clone [9]. AFP has a microheterogeneity due to structural variations in its sugar chain [10]. AFP-L3, an isoform of AFP, is reactive with lens culinaris agglutinin and is known to suggest the presence of latent hepatoma cells in the cirrhotic liver [11] and in the remnant liver after the curative removal of preceding hepatoma [12].

Acyclic retinoid not only deleted AFP-L3 from patients who had been positive for AFP-L3 at entry but also prevented the appearance of AFP-L3 in patients who had been negative at entry (p < 0.01). In contrast, placebo significantly raised the incidence of AFP-L3-positive patients after a 12-month administration from that at entry (p < 0.05) [9]. This result shows that the abnormal clone which produces AFP-L3 received clonal suppression as well as clonal deletion [9], suggesting that basic mechanism of suppression of hepatocarcinogenesis by acyclic retinoid as described earlier is realized in clinical cases. We expect that the theoretical strategy to prevent hepatocarcinogenesis by the retinoid is applicable for other cancers since the retinoid induces apoptosis to several kinds of cancer cell lines [unpubl. data].

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