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ORIGINAL RESEARCH

Anti-human AFP variant monoclonal antibody in radioimmunodetection of primary hepatocellular carcinoma

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Abstract

AIM: To investigate the affinity of AFP-R-LCA monoclonal antibody (AFP-R-LCA McAb) for AFP-positive primary hepatocellular carcinoma (HCC) cells.

METHODS: AFP-R-LCA McAb was labeled by ^{131}I. Eleven cases of HCC with AFP positivity, 6 with AFP negativity, and 4 with hepatitis B-related cirrhosis were investigated by radioimmunodetection.

RESULTS: The ^{131}I-AFP-R-LCA McAb immunoreacted with 9 of the HCC AFP-positive cases (9/11), but with none of the 6 AFP negative HCC cases or of the 4 cirrhosis patients. ^{131}I-AFP-R-LCA McAb at a small dose (7.4×10^7 Bq/300 μg) was associated with no side effects as determined by the liver function test, prothrombin time (Pt) test and thyroid gland function test ($P > 0.05$). Two cases of AFP-positive HCC were not imaged because of large tumor size (diameter > 10 cm) and higher AFP concentration in serum (20000 μg/L).

CONCLUSION: AFP-R-LCA McAb has a strong and special affinity to AFP-positive HCC cells and may be useful as a carrier for radioimmunodetection and radioimmunotherapy.

Key words: Liver neoplasms; AFP; McAb; Radioimmunodetection

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INTRODUCTION

Radioimmunodetection (RAID) and radioimmunotherapy using antibodies against tumor-associated antigens (TAA) are hot topics of tumor targeting research. Since the 1980s, several articles describing such anti-tumor monoclonal antibodies (McAb) have been published. Successful RAID and radioimmunotherapy with the anti-human AFP variant McAb (AFP-R-LCA McAb) against human hepatocellular carcinoma (HCC) were carried out in our laboratory using nude mice xenografts[1]. Based on our findings from those animal experiments, we next used the AFP-R-LCA McAb labeled by ^{131}I to immunodetect 17 human cases of HCC and 4 cases of hepatitis B-related liver cirrhosis.

MATERIALS AND METHODS

Cases and groups

HCC was diagnosed according to clinical findings from CT or MRI, B ultrasonography or AFP concentration in serum, and pathological examinations (after operation). Liver cirrhosis was diagnosed according to clinical findings from B ultrasonography, CT or MRI, liver function test, *etc*. The pre-RAID clinical characteristics are shown in Table 1. All patients included in this study had been admitted to our hospital between March 1, 1995 and September 1, 1996.

Preparation of ^{131}I-AFP-R-LCA McAb

The purification procedure for AFP-R-LCA McAb from ascites fluid was adopted from an earlier study[1]. Briefly, the monoclonal immunoglobulin IgG-containing ascites fluid was precipitated by saturated ammonium sulfate and the purified IgG was separated with a DEAE-Sephacel column. The monoclonal IgG was radioiodinated to a high specific activity with ^{131}I using the chloramine-T method. The product of ^{131}I-AFP-R-LCA McAb was isolated by a Sephadex-G column and then passed through a 0.22 μm filter to remove any bacterial contaminants. After cultivation and Limulus testing to ascertain the absence of bacteria and pyrogens, the product was prepared for clinical use. The labeling rates were 51% to 60%, and the specific activities were 0.11 to 0.33 GBq/mg.

Liver function, thyroid gland function, and prothrombin time (Pt)

Periphery vein blood was collected at 1-3 d before and 2 wk after the RAID.

Methods of RAID

Seven days before and 1 d before the RAID, patients were administered a compound iodine solution to block the thyroid gland. Thirty min before the RAID, patients were injected with 25 mg of phenergan intramuscularly. The radioiodine-labeled monoclonal antibodies, ^{131}I-AFP-R-LCA McAb, were injected intravenously through peripheral veins ($3.7\text{-}7.4 \times 10^7$ Bq/300 μg). The upper abdomen was scanned using emission CT (ECT ILC 3700; Siemens, Germany) and photograms were taken (Omega 500 γ camera) at 24 h, 48 h, 72 h, 120 h and 144 h later.

Table 1 Pre-radioimmunodetection clinical characteristics of hepatocellular carcinoma and control groups

Parameter	HCC group	Control group	
		AFP-negative HCC	Liver cirrhosis
Case	11	6	4
Sex, male/female	11/0	6/0	4/0
Median age in years	52	54	54
Age range in years	35-65	35-65	35-65
Tumor mass of ⩽ 10 cm	9	6	
Tumor mass of > 10 cm	2		
AFP of 2000-10000 μg/L	9		0
AFP of > 10000 μg/L	2		0

Table 2 Differences in liver function and Prothrombin time of patients with hepatocellular carcinoma or liver cirrhosis, before and after intravenous injection of 131I-McAb (x ± s)

Group	*n*	Liver function						Pt, *t*/*s*	
		TB, cB/μmol·L^{-1}		ALT, λB/nmol·S^{-1}·L^{-1}		A/G			
		Before	After	Before	After	Before	After	Before	After
AFP-positive HCC	11	8.1 ± 2.3	8.2 ± 2.1	756 ± 108	748 ± 112	1.4 ± 0.3	1.4 ± 0.2	11.0 ± 2.3	11.0 ± 2.2
AFP-negative HCC	6	6.2 ± 2.0	6.5 ± 2.1	781 ± 116	768 ± 105	1.4 ± 0.2	1.4 ± 0.4	13.0 ± 2.5	13.0 ± 2.6
Liver cirrhosis	4	7.1 ± 2.5	7.5 ± 2.4	914 ± 152	926 ± 146	1.3 ± 0.4	1.3 ± 0.5	12.0 ± 3.1	12.0 ± 3.2

ALT, alanine aminotransferase; HCC, Hepatocellular carcinoma; Pt, Prothrombin time; TB, Total bilirubin.

Statistical analysis

All the clinical parameters are expressed as x ± s, and the Student's *t*-test was used to determine statistical significance of differences.

RESULTS

The ^{131}I-AFP-R-LCA McAb (^{131}I-McAb) was detected in human HCC of 9 of 11 patients with positive AFP status. The imaging of tumors began at 72 h after intravenous infusion of ^{131}I-McAb. After 120 h, the tumor image became clear, but disappearing gradually at 144 h. Besides the detection in the tumor tissues, high concentrations of ^{131}I-McAb were detected in tissues of the heart and spleen. Two patients with AFP-positive HCC were not imaged, as 1 had a large tumor (diameter > 10 cm) and the other had an excessively high concentration of AFP in serum (> 100000 μg/L). There was no positive detection in any of the 6 HCC cases with AFP-negative status or in any of the 4 cases of liver cirrhosis.

Liver function and Pt

There were no obvious differences in liver function or Pt when the levels of before and after intravenous injection of ^{131}I-McAb were compared (Table 2).

Thyroid gland function

There were no obvious differences in thyroid gland function when the levels from before and after intravenous injection of ^{131}I-McAb were compared (Table 3).

The relationship between AFP level and radioimmunoimaging findings of the patients with HCC is presented in Table 4.

DISCUSSION

Radiolabeled antibodies against TAA are gaining acceptance as tools for the detection of neoplasms using the technologies of external scintigraphy and RAID[2,3]. Many reports of RAID for human liver neoplasms, carried out using radioiodine-labeled monoclonal antibodies[4,5], have appeared in the literature. Here, we describe our most recent study that successfully used ^{131}I-AFP-R-LCA McAb for RAID of human liver neoplasms.

We selected AFP-R-LCA McAb labeled by ^{131}I with intravenous delivery. After 72 h, tumor images were obtained, but the findings were more distinct after 120 h. Radioactivity of the tumor tissue and other organs in the area scanned by ECT showed that the tumor was more radioactive than the other tissues (with exception of the heart and spleen). Thus, AFP-R-LCA McAb showed a strong and special affinity for AFP-positive HCC cells. AFP-R-LCA McAb was detected in local area of tumors, with increased intensity over time (within 120 h).

Table 3 Differences in serum FT3 and FT4, before and after intravenous injection of 131I-McAb (x ± s, cB/pmol•L^{-1})

Group	*n*	FT3		FT4	
		Before	After	Before	After
AFP-positive HCC	11	5.2 ± 1.3	5.1 ± 1.2	15.2 ± 3.2	15.1 ± 3.4
AFP-negative HCC	6	5.6 ± 1.5	5.5 ± 1.4	16.2 ± 3.1	16.4 ± 3.0
Liver cirrhosis	4	5.4 ± 1.2	5.3 ± 1.3	15.4 ± 4.5	15.4 ± 4.1

HCC, Hepatocellular carcinoma.

Table 4 Relationship between AFP level and radioimmunoimaging findings of hepatocellular carcinoma patients

AFP, ρB/μg·L^{-1}	*n*	Positive imaging, *n*	Positive percentage, %
2000-10000	6	6	100
10000-100000	3	2	66.7
> 100000	2	1	50

The heart and spleen have a large blood supply, and the blood and spleen belong to the reticuloendothelial system and have a strong non-specific affinity to ^{131}I-AFP-R-LCA McAb. Therefore, the heart and the spleen showed densely under ECT imaging.

Two of the AFP-positive HCC cases in the current study were not imaged because of large tumor size (diameter > 10 cm) with poor blood supply or related necrosis and higher serum AFP concentration (200000 μg/L); these features can thwart ^{131}I-AFP-R-LCA McAb competitively to produce a large amount of immune complexes. The immune complex, itself, can hinder ^{131}I-AFP-R-LCA McAb from getting into the tumor area. Thus, a clear image depends not only on the TAA concentration in serum but also on the tumor's blood supply.

Goldenberg *et al*[6] reported that the serum concentration of AFP may have no influence on RAID, possibly because AFP on the surface of HCC cells is different from AFP circulating in the blood. This conclusion, however, is not consistent with ours.

Successful localization of a radioisotope-labeled monoclonal antibody to a tumor *in vivo* depends not only on the affinity of the antibody for the target cells but also on the speed with which the immunoconjugate passes through the physiological barriers (*i.e.* resistance of the blood vessel wall and phagocytosis by histiocytes) and the speed of its entering into the tumor tissues, as well as the antigen concentration in serum[7,8]. It is a generally accepted practice that tumor images be obtained at 72 h after intravenous infusion of the antibody ligand, but that more distinct images are found within 120 h. Our experimental result agreed with this.

In conclusion, AFP-R-LCA-McAb has a strong and special affinity to AFP-positive HCC cells. The detection of AFP-R-LCA-McAb in tumor tissues of HCC suggests its potential as a carrier for RAID and radioimmunotherapy.

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