Transcatheter Arterial Chemoembolization Therapy for Hepatocellular Carcinoma Using Polyactic Acid Microspheres Containing Aclarubicin Hydrochloride

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ABSTRACT

Transcatheter arterial chemoembolization therapy using polyactic acid microspheres containing aclarubicin hydrochloride (ACR) was performed in 62 patients with primary hepatocellular carcinoma. These microspheres were about 200 μm in diameter and contained 10% (w/w) aclarubicin. A single dose of polyactic acid microspheres containing ACR (50–100 mg of ACR) was administered 1 to 8 times with a mean of 2.2 doses (a total of 160 treatments) in 62 patients. Antitumor effects were observed from the decrease in serum α-fetoprotein levels (82.1% of the patients) and in two dimensional size of tumor on computed tomography (93.6%). The cumulative survival rate was 54.3% at 1 year, 24.6% at 2 years, and 19.2% at 3 years, respectively, among 59 patients with unresectable tumors. In 3 resected liver specimens, there was a significant accumulation of ACR in the tumor, and severe necroses of the tumors were observed histologically. Systemic toxicity was mild and all patients tolerated this treatment. These results suggest that transcatheter arterial chemoembolization with the use of polyactic acid microspheres containing ACR is a useful tumor-targeting chemotherapy and is effective in the treatment of hepatocellular carcinoma.

INTRODUCTION

Surgical therapy is the only radical treatment for HCC, and the survival rate among patients in whom tumors were detected and resected early has been high. However, this cancer is often associated with liver cirrhosis in Japan, and therefore the rate of resection has not increased significantly (1, 2). Moreover, despite the recent progress in diagnostic imaging, approximately 70% of the detected cancers are unresectable (3–5). These inoperable HCCs used to be treated by ligation of the hepatic artery or with anticancer drugs given by continuous intraarterial infusion, by one shot intraarterial injection, or by systemic i.v. administration. However, none of these methods produced satisfactory results. In 1968, Dopman et al. (6) first used transcatheter arterial embolization with gelatin sponge fragments for arteriovenous malformation of the spinal cord. In 1976, Goldstein et al. (7) applied this procedure to the treatment of abdominal tumors including HCC. This mode of therapy has subsequently been shown to have epoch-making antitumoral effects. In 1979, Yamada et al. (8) treated 19 patients with unresectable HCC by TACE with gelatin sponge fragments containing anticancer drugs and obtained a good survival rate in patients with unresectable HCC in Japan. These achievements have established TACE as a procedure not only for inoperable patients but also for a preoperative use. We developed biodegradable polyactic acid microspheres (9–13) containing aclarubicin-HCl (14–16), an anthracycline anticancer antibiotic for TACE, after preliminary studies in animals (17).

MATERIALS AND METHODS

Preparation of PLA-ACRs

PLA-ACRs were prepared in the pharmacy of the Kumamoto University Hospital. Briefly, aclarubicin hydrochloride and isopropyl myristate, a medium-chain fatty acid ester, were dissolved in 7.5% polyactic acid-methylene chloride. The resultant solution was dispersed in 1% gelatin solution and sterilized in an autoclave for 20 min at 120°C; this mixture was then stirred with a magnetic stirrer at 500 rpm for 1 h. The resultant microspheres were collected by filtration through a membrane filter (3 μm pore diameter), rinsed 2 to 3 times (in 1 liter of distilled water), and dried under reduced pressure for 5 days. These steps were carried out aseptically in a clean bench. Polyactic acid, the base for microspheres, is biodegradable, can be molded into a solid form, and is metabolized into H₂O and CO₂ in vivo. ACR is a new derivative of doxorubicin, developed in 1975. Unlike doxorubicin, the cytotoxic action of ACR is time dependent in low concentrations, with inhibition of RNA synthesis (18–20). ACR was provided by Sanraku Ocean Co. (Tokyo, Japan), and PLA was supplied by Mitui Toatsu Co. (Ohmuta, Japan). PLA was an L form with a molecular weight of about 35,000. The PLA-ACRs were about 200 μm in mean diameter and contained ACR at about 10% and isopropyl myristate at about 25% concentration.

In Vivo Studies on Release of ACR from PLA-ACRs

The amount of ACR released into circulating venous blood following injection of PLA-ACRs into the hepatic artery was determined in comparison with an aqueous solution of ACR. Determination of ACR in blood was carried out by high performance liquid chromatography (21) at the Central Laboratories of Sanraku Ocean Co.

Determination of Plasma ACR Levels in Dogs. The abdomens of adult mongrel dogs weighing 5–9 kg were opened under anesthesia with pentobarbital, and a 5 French vascular catheter was introduced through the gastroduodenal artery, with the tip placed into the proper hepatic artery. PLA-ACRs or the aqueous solution of ACR were administered in doses of 1.5 mg/kg of ACR through this catheter. Subsequently, 3-ml blood samples were serially collected from the carotid vein through the catheter retained in the inferior vena cava. These specimens were placed in heparinized test tubes and immediately centrifuged for 10 min at 3000 rpm. Plasma was separated and frozen at −20°C until determination.

Determination of Plasma ACR Levels in Humans. Blood samples were serially collected from the antecubital vein in patients who received PLA-ACRs or the aqueous solution of ACR (1 mg/kg of ACR) through the catheter introduced into the hepatic artery via the femoral artery by the technique of Seldinger (22). The specimens were similarly treated and frozen at −20°C until determination.

Clinical Study

Sixty-two patients (TACE-micropheres group) with advanced HCC were treated with PLA-ACRs at our department and affiliated hospitals between April 1984 and March 1988. All patients gave consent to the treatment protocol. Diagnoses were made primarily by imaging (angiography, ultrasonography, computed tomography), serum examinations including AFP, and histological examination in some of the patients.

The age of the patients ranged from 43 to 76, with a mean of 60.9 years, and the male to female ratio was 6:1. Twenty-five (40.3%) of 62 patients were in stage I, 18 (29.0%) in stage II, and 19 (30.7%) in stage III, respectively, according to Okuda’s stage classification (3), and were judged to be unresectable on admission (Table 1). A total of 160

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treatment courses were given to the 62 patients. A single dose of PLA-ACRMs (50–100 mg of ACR) was administered 1 to 8 times, with a mean of 2.2 doses. These 62 patients were treated with PLA-ACRMs alone (38 cases) and additional anticancer drugs (24 cases). In the latter group, Adriamycin was given in combination to 2 patients, cis-platinum to 6, mitomycin C to 1, and Adriamycin plus cis-platinum to 10 (Table 2). Three patients who underwent resection following the TACE therapy were not included in the calculation of survival rate. PLA-ACRMs were administered as follows. A 6.5 French vascular catheter was introduced through the femoral artery under local anesthesia according to Seldinger’s technique. First, portography was carried out through the superior mesenteric artery with the aid of 20 µg of prostaglandin E1 as a vasodilator to determine the presence or absence of occlusion of the portal trunk. Then, a catheter was selectively introduced through the celiac artery into the hepatic artery, and PLA-ACRMs (50–100 mg of ACR) suspended in a mixture of about 10 ml of contrast medium and physiological saline were gently infused while monitoring by fluoroscopy to avoid reflux. The serial treatment consisted of 2–3 doses of PLA-ACRMs per course given at intervals of 2–4 weeks. Patients under long-term follow-up received the treatment at intervals of 6–12 months. Fig. 1 shows angiograms taken before and after treatment with PLA-ACRMs in one patient. Embolization occurred at the level of the 3rd to 4th order intrahepatic arterial branches. A posttreatment angiogram shows disappearance of the tumor stain without occlusion of the main hepatic arterial branches.

Evaluation of Anticancer Effects

Efficacy was assessed by (a) the rate of decrease in serum AFP levels, (b) the rate of tumor regression on CT, and (c) prognosis in term of cumulative survival rate. Tumors resected following treatment with PLA-ACRMs were submitted to histopathological examination.

RESULTS

Behavior of Drugs in Circulating Blood

Determination of Plasma ACR Levels in Dogs. Fig. 2 shows the plasma concentrations of ACR in blood following injection of PLA-ACRMs or the aqueous solution of ACR into the hepatic artery of dogs. The drug given in the aqueous solution resulted in a biphasic disappearance curve; the plasma concentration decreased rapidly during the first hour after administration, followed by a slow decrease. In contrast, the plasma levels of ACR administered in the form of microspheres were practically constant after 1 h up to 24 h. This suggests that the microspheres released the drug continuously at a relatively constant rate. By comparison, the area under the plasma concentration curve during the first 12 h for the group given PLA-ACRMs was approximately 36.0% of that of the group receiving the aqueous solution.

Determination of Plasma Levels in Humans. As illustrated in Fig. 3, the patients given the aqueous solution showed a biphasic

Table 2  Tissue concentration of aclarubicin and necrotic ratio in main tumor in resected liver specimen following preoperative administration of PLA-ACRMs in 3 patients with hepatocellular carcinoma

<table>
<thead>
<tr>
<th>Case</th>
<th>Total dose of ACR (mg)</th>
<th>Artery used</th>
<th>Period from last administration to hepatectomy (days)</th>
<th>Extent of necrosis in main tumor (%)</th>
<th>Tissue concentration of ACR (µg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main tumor</td>
<td>Nontumorous lesion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------</td>
<td>------------------------</td>
<td>-------------</td>
<td>------------------------------------------------------</td>
<td>------------------------------------</td>
<td>----------------------------------------</td>
</tr>
<tr>
<td>67 yr, M</td>
<td>50</td>
<td>CHA*</td>
<td>43</td>
<td>50</td>
<td>281.0</td>
</tr>
<tr>
<td>62 yr, M</td>
<td>100</td>
<td>RHA</td>
<td>17</td>
<td>75</td>
<td>258.95</td>
</tr>
<tr>
<td>58 yr, F</td>
<td>50</td>
<td>PHA</td>
<td>23</td>
<td>95</td>
<td>226.82</td>
</tr>
</tbody>
</table>

* CHA, common hepatic artery; RHA, right hepatic artery; PHA, proper hepatic artery.

Fig. 1. (A) Pretreatment hepatic arteriogram showing a hypervascular nodule of hepatocellular carcinoma in the right lobe of liver. (B) Angiogram after treatment with PLA-ACRMs demonstrating marked diminution in vascularity peripheral to the 3rd to 4th intrahepatic arterial branches.
INTRAARTERIAL INFUSION OF PLA-ACR MICROSPHERE

![Graph showing plasma levels of aclacinomycin following administration of aclacinomycin hydrochloride (1 mg/kg) in saline solution or microspheres into the hepatic artery.]

Fig. 3. Plasma levels of aclacinomycin following administration of aclacinomycin hydrochloride (1 mg/kg) in saline solution or microspheres into the hepatic artery.

Table 3 Side effects associated with hepatic arterial administration of PLA-ACRms in 62 patients

<table>
<thead>
<tr>
<th>Side effects</th>
<th>Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nausea and vomiting</td>
<td>44 (65.7)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>27 (40.3)</td>
</tr>
<tr>
<td>Fever (&gt;38°C)</td>
<td>25 (38.8)</td>
</tr>
<tr>
<td>Hyperamylasemia</td>
<td>22 (34.3)</td>
</tr>
<tr>
<td>Jaundice (persistent)</td>
<td>6 (8.9)</td>
</tr>
<tr>
<td>Gastrointestinal bleeding</td>
<td>2 (3.0)</td>
</tr>
<tr>
<td>Severe liver dysfunction</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>Myelosuppression</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Table 3: Side effects associated with hepatic arterial administration of PLA-ACRms in 62 patients.

PLA-ACRms group was approximately 27.0% of that in the aqueous solution group.

Clinical Study

Determination of Tissue Concentrations of ACR

In the 3 resected livers, the tissue concentrations of ACR in the cancerous region were 281.0, 258.95, and 226.82 µg/g (tissue weight), respectively, and the corresponding figures in the noncancerous region were 39.6, 2.88, and 2.56 µg/g. Thus, the ACR in the cancerous region was approximately 21 times higher on the average. In the liver resected after the longest period (43 days) following the final administration, the tissue concentration was approximately 9 times as high (Table 3).

Efficacy Evaluation

Rate of Decrease in Serum AFP Levels. The serum AFP levels were higher than normal (<20 ng/ml) in 49 (73.1%) of the 62 cases given PLA-ACRms. In 39 (79.6%) of the 49 cases, the levels were higher than 200 ng/ml. Thirty-two (82.1%) of the...
39 patients showed decreases after treatment. The decreases in 28 (71.8%) of the 39 patients were marked, more than 50% of the pretreatment level of AFP (Fig. 4).

Rates of Tumor Regression on CT. In 47 cases tumor size could be measured and the rate of tumor regression was determined in two dimensional size on CT. Regression occurred in 44 (93.6%) of the 47 cases, to a tumor size less than 50% in 20 (42.6%) (Fig. 5). Fig. 6 illustrates one patient in whom dramatic regression of the tumor occurred following administration of PLA-ACRms.

Prognosis and Cumulative Survival Rate (Fig. 7). By the method of Kaplan and Meier, the survival rate was at 54.3% for 1 year, 24.6% at 2 years, and 19.2% at 3 years. According to the clinical stage, the 1-year survival rate was 62.3% and the 2-year survival rate was 38.2% in stage I; 45.9 and 17.2%, respectively, in stage II; and 37.0 and 0%, respectively, in stage III. Thus, the PLA-ACRms group showed relatively good prognoses.

Histopathological Examination. The histological changes in the tumor were examined in 3 resected patients after PLA-ACRms treatment. The rate of necrosis in the main tumor assessed in mass volume was between 50 and 95% (Table 3). Fig. 8 shows a case of 55-year-old female with HCC resected following administration of PLA-ACRms. Most of the main tumor showed coagulation necrosis and fibrosis. In the surrounding connective tissue, tumor cells were sporadically seen in which the nuclei were pyknotic with vacuoles in the cytoplasm, suggesting decreased viability of tumor cells. The nontumorous area of the liver showed cirrhosis (Fig. 8).

Side Effects

Early common postoperative complications were nausea and vomiting (65.7%), epigastric pain requiring analgesics (40.3%), fever higher than 38°C (38.8%), and hyperamylasemia (34.3%). However, all these symptoms were transient and subsided after conservative treatment. Hyperamylasemia occurred only among those in whom the drug was infused into the common hepatic artery. With regard to the liver function test, aspartate aminotransferase, alanine aminotransferase, and total bilirubin returned to the preoperative levels nearly simultaneously with the improvement of clinical symptoms (Fig. 9) and changes in plasma indocyanine green clearance were not so remarkable.
DISCUSSION

The specific hemodynamics of HCC in the liver are a major factor for the efficacy of the present therapy, because the HCC is nearly 100% dependent on the hepatic artery whereas normal liver has dual blood supply, both portal and arterial (23, 24). For this reason, embolization of the hepatic artery, the feeding vessel, results in selective avascular necrosis of the cancerous region. However, most patients with HCC have associated liver cirrhosis in Japan and they show a marked decrease in the hepatic reserve following embolization. TACE with gelatin sponge fragments and/or metal coil embolizing the relatively large arteries such as the 1st to the 2nd order intrahepatic arterial branches induces serious complications. To facilitate synergistic effects of embolization and chemotherapy with minimal complications, we developed a new embolizing agent, PLA-ACRms, which stays in blood vessels for a long period producing embolization at the level of peripheral arteries such as the 3rd to 4th branches, where extraanatomical collaterals would not form as a defense response. These conditions permitted more frequent repeated treatments with PLA-ACRms, resulting in greater effects and better survival rates.

The venous concentrations of this drug in dogs and humans indicated that the drug is released into circulating blood slowly and in small quantities, so that undesirable systemic effects of this drug are expected to be much reduced. In confirmation the resected livers showed concentrations of the drug in the cancerous area about 21 times higher than that in the intact area. Thus, our procedure may be regarded as a tumor-targeting chemotherapy. We evaluated the effect of treatment in terms of the rate of decrease in serum AFP levels and regression of tumor on CT; both parameters demonstrated significant antitumor effects of PLA-ACRms. However, in some cases, AFP levels rose again, and tumor diameter increased, after a certain period. In such cases, the treatment was repeated. The cumulative survival rates in the patients receiving TACE of PLA-ACRms were significantly better than those treated with systemic chemotherapy in the same period in our department (n = 43; cumulative survival rate, 6-month rate of 11.6% and 1-year rate of 0%). Yamada et al. (25) in 1987 reported the following results of TACE using gelatin sponge fragments: a 1-year cumulative survival rate of 51%; a 2-year rate of 24%; a 3-year rate of 12%; a 4-year rate of 8%; and a 5-year rate of 6%. Other newly developed embolizing agents including mitomycin C microcapsules (26), Lipiodol-styrene maleic acid neo-carzinostatin (27, 28), and albumin microspheres (29) showed similar effects. The results of our therapy are similar to, or better than, those obtained with these new drugs. However, the stages of disease are not the same and comparison is difficult. Finally, the use of PLA-ACRms in TACE in the treatment of HCC reduced side effects with no serious complications such as bone marrow suppression, myocardial disturbance, fatal hepatic insufficiency, and necrosis of the gallbladder and showed marked antitumor effects, and our experiences suggest the TACE using PLA-ACRms to be a useful preoperative and palliative treatment for advanced HCC.

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