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Hepatic Extraction of Adriamycin in Patients with Hepatocellular Carcinoma*

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Abstract—*Adriamycin (ADR) is used in the treatment of hepatocellular carcinoma (HCC). HCC frequently occurs in association with cirrhosis of the liver. ADR undergoes an extensive hepatic metabolism and biliary secretion. Therefore ADR elimination could be impaired in patients with HCC. In this study we measured the hepatic extraction of ADR by catheterization of the hepatic veins in five patients with HCC associated with cirrhosis or chronic hepatitis. In all patients the hepatic extraction ratio of ADR was very low (<0.10) and in four patients it was 10% or less of that of indocyanin green. This suggests that an impairment of ADR hepatic transport exists in these patients.*

INTRODUCTION

ADRIAMYCIN (ADR) is a glycosidic antibiotic of the anthracycline group with a broad spectrum of antitumoral activity [1]. The main toxic effect of ADR is the development of chronic cardiomyopathy which limits the total amount of drug administered to the patient [1]. ADR undergoes an extensive hepatic metabolism and biliary secretion [1]. It has been shown in patients with hepatic metastasis and elevated bilirubin that the plasma disposition of ADR was reduced and the toxicity was increased [2].

ADR is used in the treatment of hepatocellular carcinoma (HCC) with inconsistent efficiency [3]. In Western countries HCC frequently occurs in association with liver cirrhosis. The pharmacokinetics of many drugs metabolized by the liver are impaired in cirrhotics. However, a recent study has shown that ADR pharmacokinetics were not significantly modified in HCC patients [4]; however, the hepatic extraction ratio and the hepatic clearance of ADR were not measured. The aim of this study was therefore to measure the hepatic extraction of ADR in 5 patients with HCC.

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MATERIALS AND METHODS

Patients

Five male patients were studied (age range 45–65). All were French natives. The diagnosis of HCC and cirrhosis (patients 2–5) or chronic hepatitis (patient 1) was biopsy-proven.

Surgical non-resectability was evident. In two patients (patients 1 and 4) the tumour involved the two lobes of the liver and in three patients the tumour was limited to the right lobe. The biological characteristics of the patients are indicated in Table 1. No patients had received prior chemotherapy. ECOG performance scale was less than 2. No patient had underlying heart disease. All patients were given only one ADR dose for the study except patient 1 who was treated subsequently with this drug.

Drugs

ADR (doxorubicin chlorhydrate) was obtained from Laboratoire Roger Bellon, Neuilly, France. Indocyanin Green (ICG) was obtained from Hynson, Westcott and Dunning Inc., Baltimore, MD, U.S.A.

Protocol

ADR was injected i.v. in 5 min. ADR doses ranged between 35 and 60 mg/m^2. Other drugs were discontinued at least 48 hr before the investigation. After the i.v. administration of ADR, the right hepatic vein was catheterized under fluoroscopic control as described pre-

Table 1. Biological characteristics of HCC patients

Patient	Bilirubin (μM)	Alcaline phosphatases (UI)	AST* (UI)	ALT† (UI)	Albumin (g/l)	PT‡ (%)	Creatinine (μM)
1	10	360	14	56	25	84	69
2	14	290	79	88	36	62	74
3	15	238	22	24	32	95	109
4	24	217	31	17	36	100	79
5	25	315	43	84	37	40	86

*AST: serum aspartate amino-transferase.
†ALT: serum alanine amino-transferase.
‡PT: prothrombin time % of control.

viously [5]. ICG 0.5 mg/kg (0.2 ml/kg) was bolus injected in an antecubital vein.

Blood samples were drawn simultaneously from the hepatic vein and from the femoral artery, 4, 8, 12 and 16 min after ICG injection. Samples were centrifuged, the plasma was collected and separated into two fractions; one sample was used for the determination of ADR concentration and the other for the determination of ICG concentration. The circulating blood volume was measured the same day with a dilution isotopic technique using ^{51}Cr-labelled red blood cells.

Drug analysis

ICG concentration in the plasma was determined according to the method of Nielsen [6]. The addition of ADR to blank plasma did not interfere with the measurement of ICG. ADR concentration in the plasma was determined by an HPLC assay. In brief, plasma samples were kept frozen at -20°C until analysis. Daunorubicin was used as an internal standard (1 μg/ml). Plasma was brought to pH 3.5 with HCl 1 N.

Extraction was performed on 1 ml of plasma with 10 ml of chloroform (Merck), propanol 2 (Carlo-Erba) (1:3, v/v). Contact time was 10 min with agitation. The organic layer was evaporated at 50°C under nitrogen flow. Chromatographic separation was performed using a micro-Bondapack C column (Waters).

The elution solvent was a mixture of acetonitrile (Carlo-Erba) distillated water (1:3, v/v) containing 6.6% orthophosphoric acid (Merck). Fluorescence was detected using a Perkin-Elmer 204 A fluorescence detector.

Detection was performed at excitation and emission wavelengths of 470 and 580 nm respectively. The limit of sensitivity was 5 ng/ml. The coefficient of variation in this assay was less than 2%. ADR recovery was 75%. The addition of ICG to blank plasma did not interfere with the measurement of ADR.

Calculations

The hepatic extraction ratio of ICG (E_{ICG}) was calculated as:

$$E_{ICG} = \frac{C_{PICG} - C_{HICG}}{C_{PICG}},$$

where C_{PICG} and C_{HICG} represent the plasma concentration of ICG in the femoral artery and the right hepatic vein respectively. The hepatic clearance of ICG (HCl_{ICG}) was calculated from the rate constant (k) of the plasma ICG concentration–time curve and from the plasma volume (PV) as [7]:

$$HCl_{ICG} = k \times PV.$$

The hepatic plasma flow (Q_P) was then calculated as [7]:

$$Q_P = \frac{HCl_{ICG}}{E_{ICG}}.$$

The hepatic blood flow (Q_B) was calculated as:

$$Q_B = \frac{Q_P}{1 - Ht}.$$

The hepatic extraction ratio of ADR (E_{ADR}) was calculated as:

$$E_{ADR} = \frac{C_{PADR} - C_{HADR}}{C_{PADR}},$$

where C_{PADR} and C_{HADR} are the plasma concentration of ADR in the femoral artery and the right hepatic vein respectively. Plasma concentrations of ADR were mean values calculated from 1–4 plasma samples drawn at 4-min intervals.

RESULTS

The hepatic extraction ratio, hepatic plasma clearance of ICG and hepatic blood flow are shown in Table 2. The hepatic extraction ratio of ICG ranged from 0.109 to 0.419. Plasma concentrations and the hepatic extraction ratio of ADR are shown in Table 3. The hepatic extraction ratio of ADR ranged from 0.03 to 0.105. In all the patients except patient 1, the hepatic extraction ratio of ADR was 10% or less of the hepatic extraction of ICG. The hepatic clearance of ADR ($Q \times E_{ADR}$) was reduced in the same proportion.

Except in patient 1, no tumour response was recorded after the administration of ADR.

DISCUSSION

The main finding of this study was that the hepatic extraction ratio of ADR was very low and was considerably less than the hepatic extraction ratio of ICG.

Table 2. Hepatic extraction ratio, hepatic plasma clearance of ICG and hepatic blood flow in HCC patients

Patients	E_{ICG}*	Q_B† (ml/min)	HCl_{ICG}‡ (ml/min)
1	0.109	4610	327
2	0.356	1027	230
3	0.419	1379	376
4	0.253	2734	467
5	0.223	1547	138
normal values§	0.620	1200 ± 100	496

*E_{ICG} = hepatic extraction ratio of ICG.
†Q_B = hepatic blood flow.
‡HCl_{ICG} = hepatic plasma clearance of ICG.
§Data from literature.

In the rabbit the hepatic extraction ratio of ADR has been estimated to be 0.6 [8]. In man the hepatic extraction ratio of ADR has been determined in five patients with breast cancer and hepatic metastasis and two patients with adenocarcinoma of the bile ducts involving the liver [9], and has been estimated to range from 0.45 to 0.50. ADR pharmacokinetics have been studied in HCC patients, but clearance of ADR was not measured. Hepatic extraction of a drug depends of the following factors: (a) the hepatic blood flow; (b) the arrangement of intrahepatic microcirculation; and (c) the intrinsic removal capacity of all cells exposed to the drug. Hepatic extraction is inversely related to hepatic blood flow [10]. Hepatic blood flow was increased in only two patients (1 and 4) and therefore cannot be considered as the main factor responsible for the marked decrease in ADR extraction. Since the measured extraction ratio of ADR was only 10% or less than that of ICG, it is unlikely that disturbances in intrahepatic microcirculation only were responsible for these findings. Therefore we suggest that an impairment of the cellular transport of ADR exists in these patients. The hepatic vein from which blood was drawn drained both the tumour and non-tumoral regions. Therefore it is impossible to assess the relative contribution of the tumour and the associated liver disease to the marked reduction of ADR extraction. Whatever the exact mechanism, the reduction of the hepatic clearance of ADR should be taken into consideration when interpreting ADR pharmacokinetics in patients with HCC.

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Table 3. Hepatic extraction ratio of ADR in HCC patients

Patients	ADR dosage (mg/m²)	Time of sampling	C_{PADR}* (ng/ml)	C_{SADR}† (ng/ml)	E_{ADR}‡
1	40	6.0 hr	19 ± 1	17 ± 1	0.105
2	35	2.5 hr	28 ± 1	27 ± 2	0.035
3	60	3.5 hr	35 ± 4	33 ± 1	0.057
4	60	6.0 hr	88 ± 1	83 ± 2	0.060
5	50	20 min	340	350	-0.030

*C_{PADR}: Mean ± S.D. plasma concentration of ADR in the femoral artery.
†C_{SADR}: Mean ± S.D. plasma concentration of ADR in the right hepatic vein.
‡E_{ADR}: Mean hepatic extraction ratio of ADR.

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