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Phase I and Pharmacological Study of Intra-arterial Hepatic Administration of Pirarubicin in Patients with Advanced Hepatic Metastases

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Intra-arterial hepatic (i.a.h.) administration of the doxorubicin analogue pirarubicin was evaluated in a phase I trial, based on preclinical studies that showed an advantage of pirarubicin over doxorubicin after locoregional hepatic administration. Pirarubicin was given to 9 patients with metastatic liver disease with intrapatient dose escalation. Of the 58 cycles evaluable for tolerance, no hepatobiliary or vascular toxicity was observed. The dose-limiting toxicity was granulocytopenia: the maximum administered doses ranged from 50 to 120 mg/m^2, suggesting variable rates of pirarubicin hepatic extraction between patients. Pharmacokinetic data obtained in 7 patients, in which a direct comparison of intravenous (i.v.) and i.a.h. administration was possible, indicated a median i.v. / i.a.h. ratio of 7.4 for the maximal plasma concentration, and a median ratio of 4 for the area under the plasma concentrations versus time curves, suggesting a high pirarubicin hepatic extraction. An unexpectedly high response rate was observed: two complete (colorectal carcinoma) and two partial responses. These data demonstrate that i.a.h. pirarubicin not only produced high locoregional concentrations and reduced systemic exposure, but can also achieve responses in metastatic liver disease of colorectal origin.

Key words: doxorubicin, pirarubicin, intra-arterial hepatic chemotherapy
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INTRODUCTION

INTRA-ARTERIAL HEPATIC (i.a.h.) chemotherapy is an investigational procedure for patients presenting isolated and unresectable hepatic metastases. The rationale for using the i.a.h. route is that it can increase local drug delivery to the tumour and reduce systemic toxicity because of lower drug concentrations outside the targeted organ [1–3]. In addition, drug administration via this route offers a specific advantage due to the dual vascularisation of the liver, which is predominantly portal in normal tissue, but mostly arterial in developed tumours [2–4]. A further advantage of the hepatic arterial route is the high capacity of the liver to metabolise numerous xenobiotics, thus reducing systemic exposure to the parent drug.

To date, clinical trials evaluating i.a.h. chemotherapy have mostly employed fluoropyrimidines, probably because of their activity in gastrointestinal cancers, and of their high hepatic extraction. However, hepatic and biliary toxicities may partly explain why a survival advantage has only been shown in one trial, in patients with metastases of colorectal origins [5–7]. Thus, to improve i.a.h. chemotherapy, other anticancer agents, fulfilling efficacy and toxicity criteria for locoregional administration need to be selected.

The rationale for the selection of drugs useful for i.a.h. chemotherapy can be based on a high local extraction rate, a high hepatic metabolism, and limited vascular, hepatic or biliary toxicities. Very few cytotoxic drugs fulfilling the above conditions for i.a.h. chemotherapy are under experimental and clinical investigation. In the anthracycline family (one of the most active classes of cytotoxic drugs used in systemic therapy) studies have been almost exclusively limited to doxorubicin. However, i.a.h. chemotherapy with doxorubicin does not lead to a clear therapeutic advantage over its intravenous (i.v.) administration [8, 9]. As a matter of fact, studies in both experimental models and man do not support a high doxorubicin hepatic extraction [10, 11]. For example, the administration of identical doses via i.a.h. or i.v. routes has shown a limited decrease in doxorubicin plasma levels after i.a.h. administration, but no reduction in systemic toxicities [8, 9, 12]. Also, the clinical evaluation of i.a.h. administration of the doxorubicin analogue, 4′-epi-doxorubicin, has not demonstrated any advantage over doxorubicin [13].

Because of the recognised efficacy and widespread use of anthracyclines in clinical cancer chemotherapy, it was, therefore, of interest to investigate an analogue with pharmacological properties that could offer better local selectivity over doxorubicin when administered by the i.a.h. route. Pirarubicin (4′-*O*-tetrahydropyranyl doxorubicin, THP-doxorubicin), a novel doxorubicin analogue with clinical antitumour activity similar

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to doxorubicin, but with less cardiotoxicity [14], was selected because its cellular uptake, *in vitro* and *in vivo*, is faster than that of doxorubicin [15, 16]. In addition, preclinical studies in the rabbit VX2 tumour model have demonstrated a clear advantage of pirarubicin over doxorubicin after i.a.h. administration, with a higher tumour delivery, a decrease in systemic exposure, and a greater antitumour efficacy [17].

Based on these encouraging preclinical data, a phase I trial was initiated to determine the maximum tolerated dose of pirarubicin when administered via the i.a.h. route. We report here the clinical and pharmacokinetic results of pirarubicin i.a.h. administration in patients with advanced liver metastases from digestive tumours.

PATIENTS AND METHODS

Patient selection

9 patients with histologically confirmed isolated, unresectable metastases from digestive tumours were entered in this study. Entry requirements included ECOG performance status <3, life expectancy of at least 12 weeks, absence of cytotoxic chemotherapy or radiotherapy for the last 3 weeks, normal blood counts, bilirubin <2 mg/dl, normal creatinine and prothrombin times, and absence of extrahepatic disease. Patients were categorised into three categories according to their liver involvement, estimated on pretherapeutic computed axial tomography (CAT) scans: less than 33% liver replacement, between 33 and 66%, and greater than 66%. All patients also underwent pretreatment liver angioscintigraphy. This test combined (i) the systemic injection of 99-colloidal rhenium sulphur to obtain a standard liver scintigram, (ii) a dynamic view of liver arterial perfusion by injection of technetium 99 pertechnetate, and (iii) static mapping of well-perfused territories after injection of technetium 99-labelled macroaggregated albumin. This allowed not only the verification of catheter permeability but also the evaluation of the type of tumour vascularity, and scoring into three categories (hypervascular, normovascular and hypovascular). Informed consent was obtained from all patients.

Drugs and chemicals

Pirarubicin (THP-doxorubicin) hydrochloride for patient administration, daunorubicin, doxorubicinol, doxorubicin and pirarubicinol were provided by Laboratorie Roger Bellon (Neuilly-sur-Seine, France). Solvents used for extraction and high performance liquid chromatography (HPLC) analyses were of HPLC grade.

Drug administration

Pirarubicin was infused over 40 min in 40 ml of a 5% dextrose solution, using a peristaltic pump (Vial Medical, Beckton-Dickinson, France). I.a.h. hepatic infusion was achieved via a subcutaneous access (Port-A-Cath®, Pharmacia, France) connected to a catheter which had been surgically implanted in the gastroduodenal artery. Pirarubicin was administered at a starting dose of 30 mg/m^2 (which is two-thirds the maximal i.v. tolerated dose) to the first patient. The following dose levels were 40 mg/m^2 for the next 3 patients, and 50 mg/m^2 for the others, if no toxicity was observed. In 7 of the 9 patients, the first i.a.h. administration was followed 3 weeks later by an i.v. administration at the same dose level and with the same schedule (40 min in 40 ml of a 5% dextrose solution), to allow a direct comparison in the same patient of the pharmacology of the drug using the two routes of administration. The pirarubicin i.a.h. injections were repeated every 3 weeks. A 10 mg/m^2 intrapatient dose escalation was allowed in the absence of myelosuppression greater than grade 2 (WHO), or in the absence of local toxicity in the previous i.a.h. administration. Pirarubicin administration was discontinued in case of hepatic or extrahepatic disease progression, local (arterial, hepatic or gastrointestinal) adverse effects, or serious systemic toxicity.

Patient follow-up

Patients were evaluated for systemic toxicity and for response according to WHO criteria. Blood counts were performed on days 7, 14 and 21, postinfusion. Local toxicity was monitored as follows: physical examination before each cycle (digestive endoscopy was conducted in case of abdominal pain); biochemical liver tests repeated on days 1, 3, 8 and 15 of each cycle; isotopic liver angioscintigraphy through the arterial access was perfomed every two cycles to ascertain the position of the catheter in the hepatic artery and verify its permeability.

Collection of blood samples

Heparinised systemic blood samples were collected prior to pirarubicin injection, 20 min after the initiation of the 40 min infusion, 1 min before the end of infusion, 0.5, 2, 5, 15 and 30 min and 1, 2, 4, 6 and 24 h thereafter. Blood samples were centrifuged (2000 *g*, 10 min) and the plasma samples were frozen at −20°C until HPLC analysis. Pharmacokinetic studies were performed during the first i.a.h. and i.v. cycles in 7 patients.

Plasma anthracycline concentration determination

Plasma concentrations were determined using reversed-phase HPLC. Daunorubicin was added as the internal standard. One millilitre of plasma was extracted on 100-mg octadecyl (C18) columns (1 ml Bakerbond spe®, Baker, Phillipsburg, New Jersey, U.S.A.), preconditioned with 1 ml of methanol, followed by 1 ml of water. After air drying, elution was accomplished with 1 ml of methanol/dichloromethane (1:1, v:v) following the addition of 200 μl of dimethylsulfoxide. The volume was then reduced to approximately 200 μl, under a nitrogen stream, before HPLC injection. This procedure allowed a 95% recovery of pirarubicin, doxorubicin, the internal standard and metabolites. The HPLC system consisted of a C18 column (Nucleosil C, 10 μm, 3.9 × 300 mm, SFCC, Neuilly-sur-Seine, France), a Wisp automatic injector (710B, Waters Associated, Milford, Massachusetts, U.S.A.), a 6000A pump (Waters Associated), and a fluorescence detector (Shoeffel FS 970) set at 251 nm (ex.) and 550 nm (em.). The mobile phase consisted of water (adjusted to pH 2.4 with phosphoric acid) and acetonitrile (68:32, v:v), at a flow rate of 1.75 ml/min. Under these conditions, the retention times of adriamycinol, doxorubicin, pirarubicinol, daunorubicin and pirarubicin were 3.6, 4.5, 5.9, 6.9 and 9.7 min, respectively.

Pharmacokinetic analysis

Plasma concentrations were best fitted to a three compartment model with first-order elimination, using a 40-min i.v. infusion input. Curve fitting was accomplished with the PC-NONLIN non-linear regression program (Statistical Consultants Inc., Lexington, Kentucky, U.S.A.), using a data weight of the reciprocal of the concentration. The total area under the plasma concentration versus time curve (AUC) was determined using the trapezoidal method. The other pharmacokinetic parameters were calculated according to standard methods [18].

Table 1. Patients' characteristics and pirarubicin starting doses

Patient number and diagnosis	Prior therapy (route)	PS	Per cent liver replacement	Starting dose (mg/m^2)
1. Fibrolamellar carcinoma of the liver	5FU–CDDP (i.a.h.)	0	>66	30
2. Colon adenocarcinoma	5FU–folinic acid (i.v.)	0	<33	40
3. Colon adenocarcinoma	5FU (i.a.h.)	2	>66	40
4. Anal epidermoid carcinoma	None	1	33–66	40
5. Cholangiocarcinoma	None	2	<33	50
6. Carcinoid tumour of pancreas	5FU–STZ (i.v.)	1	<33	50
7. Colon adenocarcinoma	None	2	<33	50
8. Gastric adenocarcinoma	5FU–CDDP (i.v.)	2	>66	50
9. Cholangiocarcinoma	5FU–ADM–CDDP (ADM, total dose = 300)	1	33–66	50

5-FU, 5-fluorouracil; CDDP, cisplatinum; STZ, streptozotocin; ADM, doxorubicin; i.a.h., intra-arterial hepatic; i.v., intravenous; PS, performance status.

RESULTS

Patients' characteristics

9 patients, who met standard eligibility criteria, were entered on this phase I trial, evaluating the administration of pirarubicin via the i.a.h. route. Pretreatment liver test abnormalities were restricted to alkaline phosphatases whose levels were 1.5 times above the upper normal limit in 5 patients. The other patient characteristics and the pirarubicin starting doses are presented in Table 1.

Toxicity

A total of 58 i.a.h. pirarubicin cycles were administered during this phase I study (Table 2). We did not observe any worsening of liver biochemical tests, arterial or catheter thrombosis, gastrointestinal ulcus or sclerosing cholangitis after any of the i.a.h. priarubicin injections. 3 patients (nos 1, 2 and 6) received more than 10 cycles and were followed for more than 2 years, and no late local toxicity such as sclerosing cholangitis was observed. In 1 case (patient 2), massive steatosis with hepatic cytolysis following 5-fluorouracil and cisplatinum i.a.h. chemotherapy was histologically documented before initiation of pirarubicin; in the same patient, after 11 months of i.a.h., pirarubicin, steatosis and cytolysis had markedly regressed on liver biopsy. In patient 7, a histological study of the liver after pirarubicin treatment did not show any sign of hepatic toxicity.

The absence of local toxicity allowed intrapatient dose escalation. The limiting toxicity was myelosupression with predominant granulocytopenia (Table 2). However, because of disease progression or refusal of further treatment in 5 patients, grade 3 granulocytopenia was reached in only 4 patients. Variations in maximum administered doses were observed between patients. Nevertheless, the maximum i.a.h. administered dose that each patient achieved was always superior to 50 mg/m^2, or to the maximal i.v. tolerated dose. Indeed, the i.a.h. mode of administration allowed the delivery of higher doses in most patients. Patient 1 had no myelosupression at 100 mg/m^2 i.a.h., and patient 6 managed to reach the i.a.h. dose level of 120 mg/m^2, whereas she experienced grade 2 granulocytopenia at 50 mg/m^2 given i.v. In 4 patients (nos 3, 4, 7 and 9), no haematological toxicity exceeding grade 2 occurred at 60 and 70 mg/m^2 doses after i.a.h. infusions, whereas the 50 mg/m^2 i.v. dose induced two episodes of grade 3, and one of grade 1 neutropenia. An

Table 2. Tolerance and response to i.a.h. administration of pirarubicin

Patient number	MAD (mg/m^2)	Number of cycles	Total dose (mg/m^2)	Neutropenia	Thrombocytopenia	Other toxicities (grade)	Response	Reason for removal from study
1	100	10	650	0	0	Alopecia (1) Nausea-vomiting (2)	PD	PD
2	80	15	1070	3	3	Alopecia (1) Nausea-vomiting (3)	CR (11 months)	Refusal
3	70	3	190	2	0	None	PD	PD
4	60	3	150	0	0	Alopecia (1)	PR (5 months)	Refusal
5	70	4	290	3	0	Nausea-vomiting (3)	PD	PD
6	120	15	1550	3	0	Nausea-vomiting (2)	PR (33 months)	Refusal
7	70	4	360	1	0	None	CR (14 months)	Surgical resection Histologically negative
8	50	1	100	3	3	None	PD	PD
9	70	3	220	2	0	Cardiomyopathy (2)	MR	Cardiac toxicity

MAD, maximum administered dose; PD, progressive disease; MR, minor response; PR, partial response; CR, complete response.

exception was patient 8, who developed grade 3 neutropenia and thrombocytopenia after a 50 mg/m^2 dose by i.a.h. infusion and was, therefore, unable to receive higher doses. This patient also developed grade 4 neutrophil and platelet toxicity with sepsis after the 50 mg/m^2 i.v. course.

A relationship between liver function tests and haematoxicity was explored: all patients who tolerated a dose escalation up to 80 mg/m^2 or more had normal liver pretherapeutic values; conversely, all patients with abnormal values experienced grade 2–3 neutropenia at maximum administered doses below or equal to 70 mg/m^2.

Other non-haematological toxicities observed in 4 patients included nausea, vomiting and alopecia (Table 2). One patient, the only one previously treated with doxorubicin, developed clinical left ventricular failure after cumulated doses of 300 and 220 mg/m^2 of doxorubicin and pirarubicin, respectively.

Responses

All patients had measurable hepatic disease. 2 patients achieved a complete response (CR) and 2 achieved a partial response (PR) (Table 2).

The complete responders had liver matastases of colorectal cancer origin. The liver nodules of the first CR (patient 2) disappeared on ultrasound scans. After 7 months, i.a.h. chemotherapy was interrupted at the patient's request. Three months later nodules were again detected on ultrasound scans, and the relapse was histologically confirmed.I.a.h. hepatic pirarubicin was resumed at 70–80 mg/m^2 every 4 weeks for 7 additional months, as long as disease remained stable and until evidence of new pregression. A total cumulated dose of 1070 mg/m^2 was administered. In the second complete responder (patient 7), resection of previously involved hepatic sgements was performed after achievement of normal liver CAT and ultrasound scans, and histopathological examination confirmed the complete response. The patient did not receive postoperative chemotherapy. An isolated hepatic recurrence was again diagnosed 9 months later and the patient underwent a second hepatic resection.

Two partial reponses were also documented in this phase I study (Table 2). One of these occurred in a patient with carcinoid metastases (patient 6) and was assessed after three cycles. Pirarubicin was interrupted after 16 cycles, although two residual nodules smaller than 1 cm remained. The patient was followed without treatment for 22 months until disease progression. The other partial response, was achieved in a patient with metastases from anal cancer (patient 4) and lasted 5 months.

3 of the 4 patients who responded to pirarubicin were previously refractory to 5-fluorouracil-based chemotherapy.

Pharmacokinetic evaluation of i.a.h. pirarubicin

Pharmacokinetics studies were performed at the starting doses in 7 patients after i.a.h. and i.v. administration of the same dose in the same patient. This allowed the quantification of the reduction in systemic exposure to pirarubicin after i.a.h. administration, and an estimation of the patient's hepatic extraction. Pirarubicin plasma profiles after i.v. or i.a.h. administration in patient 8 (who presented the lowest reduction in systemic exposure) and in patient 6 (who had the highest reduction in systemic exposure and received the highest i.a.h. dose) are depicted in Fig. 1.

Pirarubicin pharmacokinetic parameters presented in Table 3

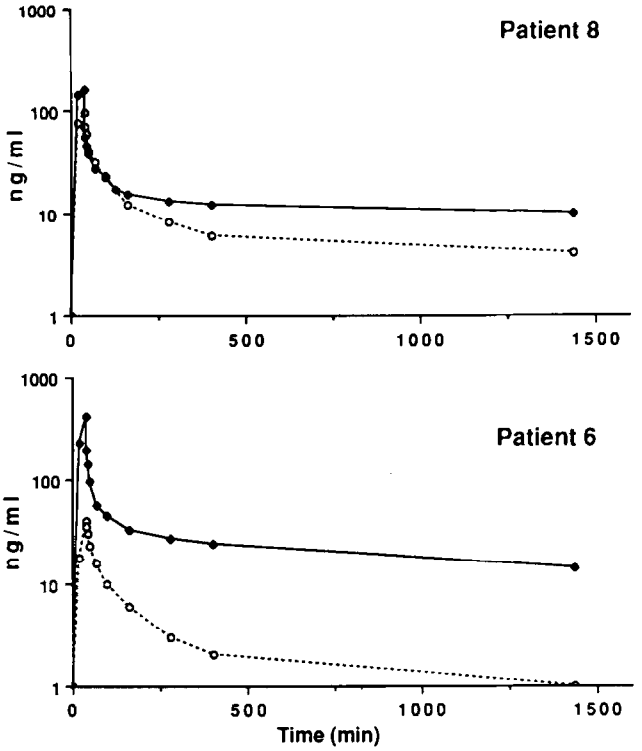

Fig. 1. Representative pirarubicin plasma concentrations after intravenous (closed diamonds) or intra-arterial hepatic (open circles) administration of a dose of 50 mg/m^2. Concentractions were determined by HPLC with fluorescence detection.

demonstrate that i.a.h. administration led to a significant decrease in both maximum concentration and AUC. The i.v. versus i.a.h. ratios for the maximal plasma concentrations varied from 1.6 to 10.6 (median 7.4). The minimum decrease in systemic exposure (AUC) using i.a.h. administration was 1.7-fold, and the maximum decrease was 8.4-fold (median 4), compared to i.v. administration (Table 3). A linear correlation

*Table 3. Pirarubicin maximum i.a.h. administered doses compared to i.v. and i.a.h. pharmacokinetic parameters**

	Patient number						
	6	1	2	7	9	5	8
Maximum i.a.h. administered dose	120	100	80	70	70	70	50
Maximal concentration (ng/ml)†							
i.v.	423	252	192	161	185	242	160
i.a.h.	40	32	71	52	25	20	99
i.v./i.a.h.‡	10.6	7.9	23.7	3.1	.7.4	12.1	1.6
Area under the plasma concentration curve (μg.min/ml)§							
i.v.	43	26	21	19	11	15	23
i.a.h.	5.1	5.6	4.8	5.0	3.9	5.4	13.5
i.v./i.a.h.‡	8.4	4.5	4.3	3.8	2.9	2.7	1.7

*The pharmocokinetic parameters were obtained after i.a.h. and i.v. administration of the starting dose. †Concentration at the end of infusion. ‡Ratios of i.v. and i.a.h. determinations. §Determined by the trapezoidal rule.

was observed between the maximum i.a.h. administered dose and the i.v. versus i.a.h. AUC ratios ($r=0.94, P<0.01$).

Patients with extensive liver involvement and poor performance status had the lowest i.v./i.a.h. AUC or maximal plasma concentrations ratios. In 3 patients, hepatic arterial angioscintigraphy allowed interesting comparisons with the pharmacokinetic data. Patients 6 and 1 had highly vascularised metastases and high i.v./i.a.h. AUC ratios, while patient 8 had a low i.v./i.a.h. AUC ratio and poorly vascularised metastases, with a marked shunt to pulmonary vascularisation, suggesting significant derivation of pirarubicin to the systemic blood flow before distribution to the liver. This was further corroborated by the fact that it was impossible to escalate the i.a.h. dose in this patient.

The study of pirarubicin metabolites did not show any significant difference in plasma levels after either i.a.h. or i.v. administration. Doxorubicin, which is an active metabolite of pirarubicin, was detected after i.a.h. and i.v. administrations, but the levels were too low to establish valid comparisons of AUC after i.v. or i.a.h. administration (data not shown).

DISCUSSION

This phase I study of i.a.h. pirarubicin administration demonstrated that this drug can be safely administered via the hepatic artery, without significant local toxicity after short infusions, but that systemic haematological toxicity is dose-limiting. The short infusion was chosen because of the potential risk of extravasation in the absence of an implanted pump, and it cannot be excluded that a longer infusion would not have induced local toxicity, as observed with fluoropyrimidines. The schedule of administration every 3 weeks was also convenient for outpatient drug administration.

An absence of local toxicity has also been found with doxorubicin [8–10]. However, i.a.h. doses of doxorubicin could not be escalated to levels superior to the maximum tolerated i.v. dose, and no important decrease in AUC or maximum concentrations could be obtained after i.a.h. compared to i.v. administration [8–10], suggesting that the hepatic extraction of the drug is limited at first passage. This does not seem to be the case with pirarubicin, since a dose escalation as high as twice the maximum tolerated i.v. dose was achieved with this i.a.h. mode of adminstration.

The better pirarubicin local extraction over that of doxorubicin, already demonstrated in an animal model [17], was also documented in this clinical study. *In contrast, experimental and clinical data resulting from the comparison of i.v. and i.a.h. infusions of doxorubicin do not attest to significant differences* [8, 10, 17]. In this trial, we have found that both the maximum plasma concentration and AUC decreased significantly after i.a.h. administration compared to i.v. injection. However, the AUC and maximum concentration i.v./i.a.h. ratios were variable between patients, and these variations may have been due to either the type of vascularisation of the tumours, to the existence of vascular shunts from the tumour to the lungs, or to the extent of tumour spread in the liver (which is inversely related to the amount of normal liver that can extract the drug). Although our population is relatively small and heterogeneous, it appears that patients who were able to tolerate a dose escalation, had well vascularised metastases or low liver replacement by the tumour.

Although the pharmacokinetic data correlated well with the haematological toxicity, these studies cannot be recommended on a routine basis to predict an initial, individualised dose. Based on this phase I study, the phase II recommended dose for pirarubicin i.a.h. administration every 3 weeks is 60 mg/m^2, in patients with good performance status and limited liver replacement (<30%), and 50 mg/m^2 in others. Dose escalation has to be adapted, according to haematological toxicity, before each cycle in order to achieve the highest local dose effect. Additional studies with pharmacokinetic monitoring during the infusion should determine if pirarubicin extraction is a saturable process, and if its local delivery can be further increased with protracted infusion.

An unexpected number of responses were observed in this heavily pretreated and poor prognosis patient population. The most remarkable responses were documented in 2 patients with liver metastases from colorectal adenocarcinomas, with histological confirmation of response in 1 case. These encouraging results deserve particular attention in a disease usually refractory to i.v. or i.a.h. doxorubicin, as well as to pirarubicin when administered by the i.v. route [19]. *It can be concluded that the local administration of pirarubicin could overcome a natural resistance of colorectal cancer to anthracyclines, since pirarubicin i.a.h. administration results in the delivery of higher doses than that achieved either when pirarubicin is administered via the i.v. route or with doxorubicin by i.a.h. infusion.*

Based on these promising results, a phase II study, exploring i.a.h. administration of pirarubicin in colorectal cancer patients with hepatic metastases, has been initiated [20].

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Inconsistent Expression of HLA-B Antigens on Peripheral Blood Lymphocytes of Stage I Melanoma Patients: an Indicator of Poor Prognosis

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The survival of stage I melanoma patients was evaluated and compared with the detectable expression of HLA antigens. Of 904 patients who were surgically treated, 219 were HLA typed on peripheral blood lymphocytes. Four consecutive HLA typings were considered necessary. Median follow-up was 8 years. Two main groups of patients were considered: (a) patients with consistent detectable expression of antigens; and (b) patients with inconsistent detectable expression of antigens. Patients with consistent HLA antigens detection had an 8-year survival rate of 87.7% compared with 49.2% of patients with an inconsistent rate ($P\ 10^{-7}$). Multivariate analysis of survival of the 182 HLA-typed patients who survived at least 24 months from surgery showed that two of the criteria had an independent impact on survival: tumour thickness (P 0.02) and HLA typing ($P\ 2 \times 10^{-5}$). Inconsistent detection of HLA antigens on peripheral blood lymphocytes during the first 24 months after surgery is an indicator of poor prognosis in stage I melanoma patients.

Key words: HLA typing, cutaneous melanoma, prognosis, stage I melanoma
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INTRODUCTION

THE PROGNOSIS of patients with cutaneous melanoma still confined to its site of origin (clinical stage I) is assessed on the histological characteristics of the primary tumour. Breslow's thickness and ulceration have been found to be the most important and reproducible prognostic criteria in a major series of publications [1–5]. Other histological features, such as regression [6–8], inflammatory reaction [9], mitotic activity [10] and lymphocytic infiltration [11], are still matters of discussion. Among host characteristics only sex was found to be significantly associated with survival [1].

In a previous study, we found that patients with melanoma showed a higher frequency of failure to express antigens of the HLA locus-B on peripheral blood lymphocytes before surgery compared with healthy donors [12, 13]. Therefore, we have investigated whether detection of HLA antigens is consistent with time, and verified whether a possible inconsistency is associated with survival of stage I melanoma patients.

PATIENTS AND METHODS

Patients

From 1980 to 1983 and between 1985 and 1987, 904 stage I melanoma patients were treated at the National Cancer Institute