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Metabolism of epirubicin to glucuronides: relationship to the pharmacodynamics of the drug

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Summary. In pharmacokinetic studies of epirubicin, we observed that its main metabolite, epirubicin glucuronide, presented a marked interpatient variation. It was even possible to separate the patients into two groups: those with a high epirubicin glucuronide : epirubicin plasma ratio and those with a low ratio, with few patients in between. We retrospectively analyzed the clinical files of 48 patients who had been subjected to a pharmacokinetic study of epirubicin. We observed that those with a low epirubicin glucuronide : epirubicin ratio had significantly lower plasma levels of fibrinogen and α2-globulins, suggesting that a reduced glucuronidation of epirubicin could be associated with hepatocellular insufficiency. These patients also had significantly lower percentages of change in granulocytes after therapy and responded better to the course of treatment studied. We cannot presently propose a hypothesis to explain these observations.

Introduction

Much effort is presently being invested in the establishment of relationships between the behavior of drugs in humans (pharmacokinetics) and the response of patients to these drugs (pharmacodynamics). Few anticancer agents have been investigated in such studies, and almost nothing is presently known about anthracyclines [3]. Some sparse results in the literature have shown the possible relationship between plasma levels, total area under the curve (AUC), or early-phase exposure to doxorubicin or epirubicin and the clinical results achieved [6, 9, 10]; however, these studies did not allow therapeutic drug monitoring.

The metabolism of these drugs has also been studied with the aim of establishing a relationship between the metabolic profile and the outcome of the treatment. Greene et al. [5] showed that daunorubicin reductase activity in white blood cells was higher in leukemic patients who responded to therapy, and Gessner et al. [4] observed that daunorubicin aglycones were present in higher quantities in the plasma of non-responding leukemic patients. These results have not subsequently been confirmed.

Epirubicin is characterized by a unique metabolic pathway leading to a 4′-*O*-glucuronide [14]. This conjugation is possible because of the equatorial position of the hydroxyl in the 4′position, whereas this substituent is in an axial position in doxorubicin. This pathway has been shown to be limited to humans as no epirubicin glucuronides have been detected in laboratory animals that are usually treated with epirubicin [7]. We have previously reported the pharmacokinetics of epirubicin in nine breast cancer patients [11] and in ten subjects with Hodkgin's disease [13]. The quantitative importance of epirubicin glucuronide in plasma was emphasized in these reports; thereafter, we studied the early-phase kinetics (0–4 h) of epirubicin in 18 lymphoma patients and the complete pharmacokinetics (0–48 h) of the drug in 11 subjects with soft-tissue sarcoma (in preparation). When we compared the relative importance of epirubicin glucuronide in plasma in these 48 patients, we became aware of the wide dispersion of the AUC value for this metabolite relative to that of the parent compound. We therefore decided to analyze carefully the clinical files of these patients, both before and after treatment, to see if clinical biological features could underlie the differences observed in the metabolism of epirubicin.

Patients and methods

Patients

The patients included in this retrospective study had been subjected to a pharmacokinetic analysis of epirubicin associated with the development of this new anthracycline. In all, 9 breast cancer patients had entered a comparative study on doxorubicin [11], 10 patients with Hodgkin's disease received repetitive injections of doxorubicin [13], 18 patients with non-Hodgkin's lymphoma underwent a short-term pharmacokinetic analysis (0–4 h), and 11 subjects with soft-tissue sarcoma had also been

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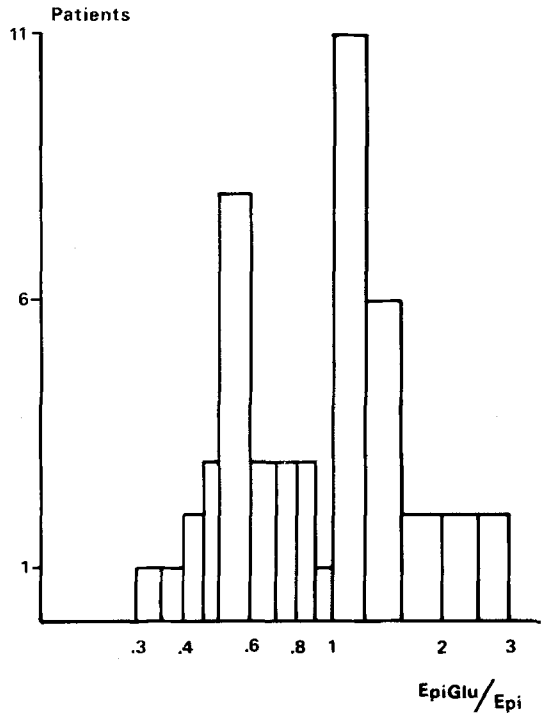

Fig. 1. Distribution of patients as a function of the AUC (0–4) ratio for epirubicin glucuronide (*EpiGlu*) : epirubicin (*Epi*)

studied. When the diagnosis in the patients' files was reconsidered, it appeared that one patient in the Hodgkin's disease group had non-Hodgkin's lymphoma and that three patients in the group with non-Hodgkin's lymphoma had Hodgkin's disease, Ewing's sarcoma, and myeloma, respectively. The treatments associated with epirubicin included 5-fluorouracil and cyclophosphamide for breast cancer; bleomycin, vinblastine and prednisone for Hodgkin's disease; cyclophosphamide, vincristine and prednisolone for non-Hodgkin's lymphoma; and cisplatin and eldisine for sarcoma. The dose of epirubicin was 50 mg/m^2 for breast cancer, 25 or 35 mg/m^2 for Hodgkin's disease, 60 mg/m^2 for non-Hodgkin's lymphoma, and 100 ml/m^2 for sarcoma. Most patients had renal and hepatic functions within the usual range of normality; four subjects had elevated transaminase and/or alkaline phosphatase levels. All but three patients had not previously received anthracyclines before this study. All were studied at the first course of epirubicin treatment; the patients with Hodgkin's disease were also evaluated at the second and third courses of therapy, and the results obtained were compared only with those obtained during the first course.

The performance status and the tolerance of and response to the treatment were evaluated according to WHO criteria [15]. The following biological determinations were performed using Boehringer-Mannheim reagent kits on a Hitachi-705 autoanalyzer: urea, creatinine, bilirubin, gamma-glutamyl-transpeptidase (γGT), glutamate aspartate transaminase (SGPT), alkaline phosphatase and total proteins. Prothrombin time and fibrinogen were evaluated with Diagnostica Stago reagent kits. The distribution of serum protein classes was evaluated after electrophoresis on Titan III Helena cellulose acetate plates. Blood cell counts and hemoglobin measurements were performed on a Coultronics counter. Percentages of change could be evaluated only in 22 patients who underwent blood cell counts every week after treatment; the percentage of change was evaluated as:

$$\frac{\text{pre-therapeutic count} - \text{14th day post-therapeutic count}}{\text{pre-therapeutic count}} \times 100.$$

Pharmacokinetics and metabolism

Blood sampling was performed after a bolus injection of epirubicin, which lasted 3–10 min in most cases. The usual sampling times were: 5, 10, 20 and 40 min and 1, 2, 4, 8, 24, 32, and 48 h. The last 4 samples were not drawn from the 18 non-Hodgkin's lymphoma patients. Extraction and analysis of epirubicin and its metabolites were performed as previously described in detail [11, 13]. The relative importance of epirubicin glucuronide could be assessed by evaluation of the ratio of the areas under the curve from 0 to 4 h (AUC 0–4) for epirubicin glucuronide: epirubicin. Using this parameter, it was possible to separate the patients into two equivalent groups according to the relative importance of epirubicin glucuronide in plasma. The characteristics of the patients from the two respective groups were compared using parametric or non-parametric tests according to the features to be compared.

Table 1. Distribution of some relevant pretherapeutic biological parameters in two groups of patients

	Group I R >>0.9	Group II R <0.9	*P* value
Bilirubin (μmol/l)	7.04	9.22	= 0.065
Fibrinogen (g/l)	5.7	3.79	<0.001
Albumin (g/l)	34.6	37.4	NS
α-1 globulins (g/l)	2.44	1.73	= 0.067
α-2 globulin (g/l)	9.59	6.43	<0.001

Groups were defined as described in the text: group I patients showed an AUC (0–4) ratio (R) for epirubicin glucuronide : epirubicin of >0.9; that of group II subjects was <0.9. Only the parameters that differed between the two groups are presented. All other usual biological parameters did not show a significant difference between the groups (urea, creatinine, transaminase, alkaline phosphatase, total proteins, β-and γ-globulins). NS, Not significant

Results

The ratio of the AUC (0–4) was chosen as the reference value because it was evaluable in all patients and was less subject to punctual variations than the 1- or 2-h concentration ratios, which were also evaluated. From a theoretical point of view, the ratio of the total areas under the curve from 0 to 48 h for epirubicin glucuronide: epirubicin (AUC 0–48) would have been the best parameter to evaluate. However, it could not be computed in 18 patients, and the low levels of glucuronides in plasma (1–5 ng/ml) were not accurately determined in our laboratory before 1984; a subsequent underestimation of the AUC (0–48) in several patients made this parameter unsuitable for analysis. The correlation between AUC (0–4) ratios and 1- or 2-h concentration ratios was highly significant ($r = 0.717$, $P < 0.001$; $r = 0.837$, $P < 0.001$). When the AUC (0–4) ratios were plotted as histograms, it was evident that two groups of patients could be defined with no overlapping between them: for the first group (24 patients), the AUC (0–4) ratio was >0.9; that of the second group (24 patients) was <0.9. Only four patients showed an AUC (0–4) ratio of between 0.8 and 1, which justified the existence of two populations, whose distribution was not far from log-normal (Fig. 1). The two groups remained almost unchanged when they were founded based on the 1- or 2-h concentration ratio, in which cases the limit between the two groups was a ratio for epirubicin glucuronide : epirubicin of 3.5. The tables present the groups as defined by the AUC (0–4) ratios; all significant differences were also observed when the groups were defined according to the concentration ratios.

Table 2. Distribution of patients according to the tolerance and tumor response to the course of treatment studied

	Group I R >>0.9	Group II R <0.9	*P* value
Mean dose received (mg/m^2)	69 ($n = 9$)	91 ($n = 13$)	0.0052
Change in hematologic parameters (%):			
Leukocytes	56.4	30.3	0.06
Granulocytes	66.8	30.4	0.02
Hemoglobin	3	5.9	NS
Platelets	7.5	10.8	NS
Response to treatment			
Responders	7	13	
Non-responders	16	7	<0.05
Not evaluable	1	4	

Groups of patients were defined as in Table 1. NS, Not significant

Table 3. Distribution of patients according to the relative importance of epirubicin metabolites as compared with epirubicin

	Group I R >>0.9	Group II R <0.9	*P* value
Epirubicin AUC (0–4) (ng ml^{-1} h)	561	503	NS
Epirubicin glucuronide : epirubicin	1.409	0.602	<0.001
Epirubicinol : epirubicin	0.204	0.118	= 0.035
Epirubicinol glucuronide : epirubicin	0.279	0.156	<0.001

Groups of patients were defined as in Table 1. NS, Not significant

When the distribution of patients was calculated according to age, sex, tumor type, and previous treatment, no difference appeared between the two groups. Relevant pretherapeutic data are shown in Table 1. In each group, two patients had frankly abnormal liver tests as determined from enzyme activities. Mean fibrinogen and α_2-globulin levels appeared to be the only parameters that were significantly different between the two groups, suggesting that hepatocellular insufficiency could have been responsible for the lower levels of metabolites in plasma. A slightly higher mean bilirubin value was also observed in patients with low AUC ratios, although no bilirubinemia exceeding 20 µmol/l was seen. There was no difference in treatment modalities between the two groups. During the first course of chemotherapy, which was the only one studied, no difference in tolerance appeared to exist between the two groups of patients. A more careful analysis of the percentage of change in hematologic parameters (leucocytes, granulocytes, hemoglobin, platelets) was possible in 22 patients. Although a distortion of the delivered dose occurred between these two reduced groups, it is remarkable that patients with a low AUC (0–4) ratio, who received a mean dose of 90 mg/m^2 showed a significantly lower percentage of change in granulocytes than did those with a high AUC (0–4) ratio, who were given a mean dose of 68 mg/m^2 epirubicin (Table 2). Finally, the tumor response to the course of treatment studied could be evaluated in 43 cases (Table 2). We observed that 30% of patients with a high AUC (0–4) ratio were responders as were 65% of those with a low ratio ($P < 0.05$). This significance was maintained when the groups were defined with according to the 1- and 2-h concentration ratios.

In ten patients, three successive pharmacokinetic studies were performed at 15-day intervals. Seven patients showed the same metabolite: unchanged drug ratio, and three subjects moved from the high-ratio group to the low-ratio group at the second course of treatment. No change in the distribution of these patients among the two groups occurred between the second and the third courses of treatment.

It is noteworthy that the other epirubicin metabolites found in plasma followed the same pattern as epirubicin glucuronide. In the group with high AUC (0–4) ratios for epirubicin glucuronide : epirubicin, the AUC (0–4) ratios for epirubicinol : epirubicin and for epirubicinol glucuronide : epirubicin were significantly higher than the corresponding ratios in the group with low AUC (0–4) ratios for epirubicin glucuronide : epirubicin (Table 3).

Discussion

Very few pharmacokinetic studies of anthracyclines have shown a relationship between the parameters and the patients' clinical features. Many other factors may play a key role in the determination of tumor response to a drug; among these, drug distribution, elimination, and metabolism is exceptionally important enough to be significantly correlated to the response. The results observed with anthracyclines [6, 9, 10], although statistically significant, might barely be reproducible by other authors. In contrast, dose-dependent toxicities such as hematologic toxicity may prove to be well predicted by pharmacokinetic parameters. The percentage of change in white blood cell counts has been shown to be dependent on the AUC of several drugs, including menogaril, a new anthracycline antibiotic [1].

We describe a relationship observed between epirubicin metabolism and clinical features of the patients treated with the drug. This was a retrospective study using the files of the patients. It could be interesting to develop a prospective study designed to confirm these findings. The first observation was the distribution of patients into two groups according to the quantitative importance of the metabolism of epirubicin to glucuronide. Genetic polymorphism of drug metabolism has been described for acetylation or hydroxylation; however, drug conjugation to glucuronic acid has never been shown to be subject to genetic variation.

Patients with low glucuronide : epirubicin ratios had significantly lower levels of some plasma proteins synthesized in the liver. Although these patients showed no other sign of hepatic disturbance, we think that this could be due to hepatocellular insufficiency. Swirsky et al. [12] recently reported that two patients with hepatocellular insufficiency showed the lowest ratios of epirubicin glucuronide : epirubicin (0.33 and 0.44 vs 1.67, 2.05, and 2.06). These two patients had no detectable levels of epirubicinol

glucuronide. It therefore seems that a decrease in the synthetic activities of the liver cells could be accompanied by a decrease in the glucuronidation capacity of the liver.

It is difficult, however, to hypothesize about the findings that both the hematologic tolerance and the response to treatment were improved in patients with a low epirubicin glucuronide : epirubicin ratio. It has been shown that epirubicin glucuronides have no cytotoxic activity; they cannot enter blood cells (personal unpublished results). A lower production of epirubicin glucuronides might be responsible for better availability of the active drug; however, low plasma levels of epirubicin glucuronides did not correlate with high AUC values for epirubicin. We therefore have no explanation for these findings in the present study, and we would like to compare these results with those reported by other authors who have investigated epirubicin pharmacokinetics [2, 8].

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