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Cellular and plasma pharmacokinetics of weekly 20-mg 4'-epi-adriamycin bolus injection in patients with advanced breast carcinoma*

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Summary. Weekly low-dose injections of 20 mg 4'-epi-adriamycin (E-ADM) were given to 12 patients with advanced postmenopausal breast cancer for at least 8 weeks. In advance, all patients were given hormonal therapy and polychemotherapy not containing anthracyclines. E-ADM concentrations in plasma and urine and in blood and bone marrow cells were determined during 8 consecutive weeks. Plasma concentrations in the range of a few nanograms per milliliter were seen up to 72–96 h. Cellular concentrations appeared to be more than 100-fold the plasma concentrations, and were 190 ± 66 ng/10^9 cells on day 8, before the next injection was given. Nevertheless, no serious bone marrow toxicity was observed. In two patients with an increased plasma bilirubin concentration, cellular E-ADM concentrations were 20%–40% higher than those observed in the other patients. Plasma concentrations of E-ADM and 4'-epi-adriamycinol showed terminal half-lives 2–3 times longer and could be followed throughout the week. In three patients biopsies of skin metastases were examined. In two patients E-ADM could be demonstrated in the tumor tissue up to 7 days after the last injection. Although the number of patients investigated is too small to relate the drug kinetics to clinical response, it is of interest that the two patients with the highest cellular E-ADM concentrations responded better than the others.

Introduction

In breast carcinoma, adriamycin (ADM) is one of the most effective cytostatic agents in single-drug therapy [5, 6]. Unfortunately, cardiotoxicity of this drug given by bolus injection limits its use beyond a cumulative dose of 550 mg/m^2 body surface [5, 17, 18]. Therefore, other schedules have been developed, such as weekly low-dose (20 mg) ADM bolus injections [10, 13, 17] or continuous infusions over longer periods [4]. It has been claimed that these regimens are less cardiotoxic, while the therapeutic efficacy is maintained [4, 8]. Recently a new analogue, 4'-epi-adriamycin (E-ADM), has been introduced, which is reported to be equally potent and probably less cardiotoxic [7]. Jones and Mattsson reported low toxicity and a very high response rate of 51% in a phase II study with weekly low-dose E-ADM in pretreated postmenopausal patients with advanced breast carcinoma [11]. These results justified a multicenter controlled phase II study in which postmenopausal patients with advanced breast cancer were treated with 20 mg E-ADM weekly. This paper describes the pharmacokinetics in plasma and blood cells in 12 patients treated according to this protocol. Data on cellular E-ADM concentrations may elucidate part of the mechanism involved in the reported efficacy of this treatment schedule.

Patients and methods

Patients. Patients with advanced breast cancer with measurable parameters (UICC criteria) were eligible for this study. All patients had previously received chemotherapy not containing anthracyclines. The mean age of these patients was 60 years, ranging from 42 to 71 years of age. Ten patients had normal bilirubin values, two patients had impaired liver function with increased bilirubin values of 23 μmol/l (patient 11) and 112 μmol/l (patient 12) (normal value < 10 μmol/l). The kidney function was normal in all patients. All patients gave informed consent. The overall clinical results of this multicenter study will be reported separately.

Chemicals. E-ADM for clinical use and the pure analytic standards of E-ADM and 4'-epi-adriamycinol (E-ADMol) were kindly supplied by Farmitalia Carlo Erba (Milan, Italy). Other chemicals for extraction and chromatographic analysis were obtained from Merck (Darmstadt, FRG) and were of analytical grade.

Study design. E-ADM was administered weekly in a running infusion i. v. over 1–2 min for at least 8 weeks. The dose was 20 mg, irrespective of the body surface. Blood was collected before the next injection; during the 1st, 4th and 8th weeks also at 5 and 30 min and at 1, 2, 4, 24, 48, 96 and 168 h after the injection. In several patients bone marrow aspiration was performed before E-ADM administration on days 1, 22, and 50. Growth inhibition of bone marrow hematopoietic clonogenic cells was examined with the colony forming unit assay of granulocytes and macrophages (CFU-GM). The 24-h urines were collected for determination of E-ADM and its metabolites on days 7, 28, and 56. A 5-ml sample of this urine was stored at -20 °C until analysis.

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Venous blood was taken into heparinized polypropylene tubes and put on ice. Plasma was removed after 10 min centrifugation at 0 °C and 900 *g*. Erythrocytes in the pellet were lysed with ice-cold ammonium chloride [14]. After centrifugation at 0 °C the leukocytes were resuspended in ice-cold phosphate-buffered saline. The recovery of the leukocytes after the lysis step was 85%–90%. Plasma samples and cell suspensions were stored at −20 °C until analysis by high-pressure liquid chromatography. Bone marrow samples were collected in buffered acid citrate dextrose (ACD, formula A, pH 7.4). After washing in phosphate-buffered saline one part was used to culture CFU-GM. Red cells and normoblasts in the other part were lysed with ammonium chloride. The remaining white cells were resuspended and stored at −20 °C until analysis for E-ADM concentration.

Extraction procedure and high-pressure liquid chromatographic analysis. The method used has been described previously [14]. In short: After addition of adriamycinol as internal standard, 500 µl plasma or 250 µl sonicated cell suspension was extracted twice with chloroform/methanol 9:1 (v/v). After evaporation of the organic phase, the dry residue was dissolved in 750 µl chloroform/methanol/Tris buffer and 500-µl aliquots were injected onto a Lichrosorb 7 SI60 column (100 × 4.6 mm ID). With this extraction and chromotographic procedure no glucuronide metabolites can be detected. Although these metabolites are unique for E-ADM [1, 7, 16], they do not have cytotoxic activity (F Ganzina, Farmitalia Carlo Erba, personal communication, 1984). We therefore considered them of no importance for this study, and used the same method, as in our previous studies [14]. Measurement of peak height ratio of drug and internal standard was used for quantification of the drug concentration. Plasma and cell concentrations are expressed in nanograms per milliliter, where 10^9 cells are assumed to represent a volume of 1 ml. The recovery of the extraction was 80%–90%. The detection limit was 1 ng. The day-to-day variation for extraction from cells and plasma for the whole procedure was 9.0% and 8%, respectively ($n=18$).

Pharmacokinetic analysis. The E-ADM cellular and plasma concentration-versus-time data were fitted according to a two-compartment open model, and the pharmacokinetic data were calculated according to the conventional procedures [15].

Results

Pharmacokinetics

Cellular and plasma concentration data of E-ADM in ten patients with normal plasma bilirubin values are given in Fig. 1.

Plasma pharmacokinetics. The peak plasma concentrations at 5 min after the first bolus injection were 592 ± 112 ng/ml ($n=10$). The plasma disappearance curve was biphasic with half-life values of 9.6 ± 1.8 min and 18.2 ± 5.1 h, respectively. The main cytostatically active metabolite (E-ADMol) was observed during the first 3 days after injection. No significant accumulation of E-ADM was seen during 8 consecutive weeks. Plasma pharmacokinetic parameters (half-lives, AUC, clearance and distribution volume) for weeks 1, 4, and 8 are summarized in Table 1. No significant differences were noted in the subsequent weeks.

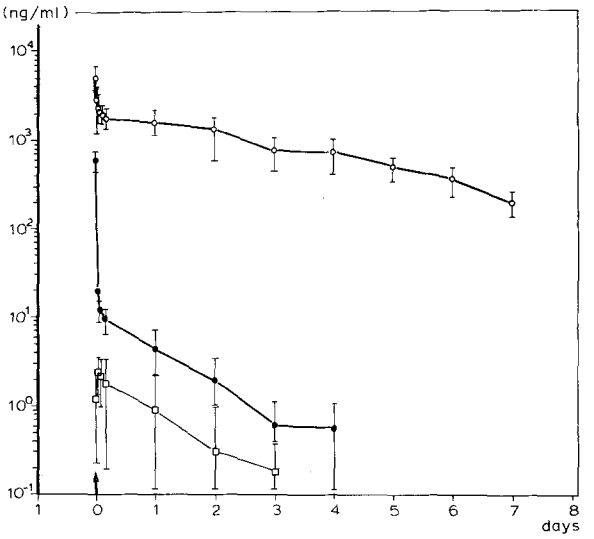

Fig. 1. Cellular and plasma concentration data of 4'-epi-adriamycin and 4'-epi-adriamycinol after 20-mg bolus injection during the 1st week in patients with normal bilirubin concentration. Means ± standard deviation are given ($n=10$ patients). ○, cellular 4'-epi-adriamycin; ●, plasma 4'-epi-adriamycin; □, plasma 4'-epi-adriamycinol; *arrow*, injection.

Cellular pharmacokinetics. Peak cellular (white blood cells) concentration at 5 min after the first bolus injection amounted to 4680 ± 1800 ng/10^9 cells ($n=10$). Cellular pharmacokinetics revealed a biphasic disappearance curve, with half-life values of 17.8 ± 8.3 min and 49.7 ± 10.8 h, respectively. The ratio of cellular to plasma concentration increased from 7.9 at 5 min after injection to 674 at 48 h and 3800 at 72 h. On day 7 the cellular concentration was still 190 ± 66 ng/10^9 cells ($n=104$). Cellular pharmacokinetic parameters are summarized in Table 1. During the treatment no significant cellular E-ADM accumulation was observed. In Fig. 2 an example of cellular and plasma concentration courses are given as measured over a period of 8 weeks in an individual patient.

Impaired liver function. In two patients with increased plasma bilirubin concentrations the plasma concentrations and cellular E-ADM concentrations had increased markedly (Fig. 3) compared with the values observed in the ten patients with normal plasma bilirubin values. The peak plasma (535 ± 160 ng/ml) and peak cellular (4160 ± 1920 ng/10^9 cells) concentrations at 5 min after bolus injection were in the same order as those observed in patients with normal plasma bilirubin. However, the plasma disappearance rates were lower, at 11.4 ± 4.8 min and 55.2 ± 11.2 h for the first and the second half-lives, respectively. Plasma concentrations of E-ADM and E-ADMol at the next injection (day 7) revealed a continuous exposure of cells to low plasma concentrations of 1.6–4.7 ng/ml E-ADM and 0–4.0 ng/ml E-ADMol, respectively. In addition, cellular concentrations had increased (20%–40%) compared with those observed in patients with a normal

Table 1. Plasma and cellular pharmacokinetic parameters of 20 mg 4'-epi-adriamycin weekly bolus injection in ten patients with normal plasma bilirubin values and two patients with increased plasma bilirubin concentrations

	T1/2α (h)	T1/2β (h)	A (ng/ml)	B (ng/ml)	AUC (μg h l^{-1})	Cl l/min	Vf l
Plasma pharmacokinetics							
	Normal bilirubin (n = 10 patients)						
Week 1	0.16±0.03	19.9±4.8	592±112	10.9±4.1	542±155	44±20	566±227
Week 4	0.14±0.02	14.9±3.2	557±211	10.2±1.9	515±195	57±13	618±215
Week 8	0.15±0.02	20.8±3.9	658±127	12.6±2.7	627± 59	37± 7	710± 95
Total	0.15±0.02	18.2±5.1	598±160	10.8±2.7	561±114	43±14	601±222
	Increased bilirubin (n = 2 patients)						
Total	0.19±0.08	55.2±11.2	535±160	19.6±2.5	902±50	16±3	1150±505

	T1/2α (h)	T1/2β (h)	A (ng/10^9 cells)	B (ng/10^9 cells)	AUC (μg h l^{-1})
Cellular pharmacokinetics					
	Normal bilirubin (n = 10 patients)				
Week 1	0.3±0.1	46.4±10.9	4680±1800	1370±170	152000±45000
Week 4	0.3±0.1	56.0± 8.8	5730±1500	1660±290	170000±31000
Week 8	0.4±0.1	46.2± 9.4	4950±1400	1860± 75	166000±46000
Total	0.3±0.1	49.7±10.8	5010±1700	1671±361	156000±33000
	Increased bilirubin (n = 2 patients)				
Total	0.2±0.2	64.4± 1.3	4160±1920	2055±360	206000±29000

T1/2α, T1/2β, first and second half-lives; A, B, extrapolated concentration constants; AUC, area under the curve; Cl, plasma clearance; Vf, volume of distribution

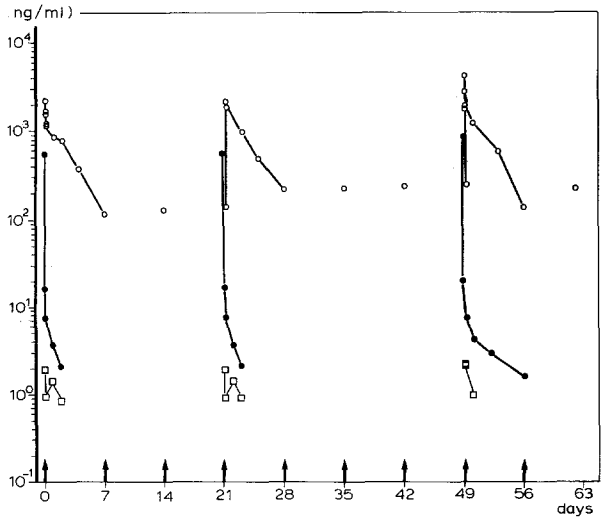

Fig. 2. Cellular and plasma concentrations of 4'-epi-adriamycin and 4'-epi-adriamycinol after weekly 20-mg bolus injection in a patient with normal bilirubin concentration during 8 consecutive weeks. Measured values are given. Symbols as for Fig. 1

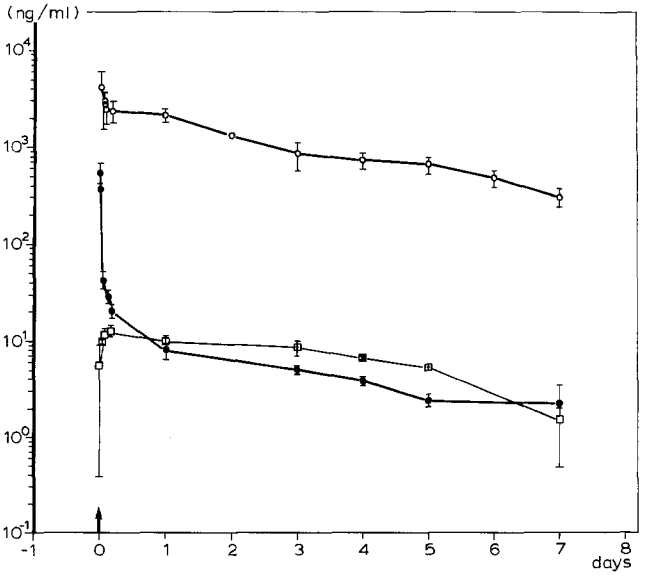

Fig. 3. Cellular and plasma concentrations of 4'-epi-adriamycin and 4'-epi-adriamycinol after weekly 20-mg bolus injection in two patients with increased bilirubin concentration. Means ± standard deviation are given (n = 2 patients). Symbols as for Fig. 1

liver function. In both patients the pretreatment jaundice improved during therapy. In five patients with liver metastases and increased alkaline phosphatase levels who nontheless had normal plasma bilirubin concentrations, plasma and cellular disappearance rates were in the normal range.

Urine E-ADM excretion. In the 24-h urine samples collected on day 7 of the 1st, 4th, and 8th weeks, only small amounts of E-ADM, E-ADMol, and some other metabolites were seen. These samples were not analyzed further. However, the presence of urinary E-ADM at the day of the next injection reflects a continuous presence of E-ADM in the plasma although these plasma concentrations are below the detection limit of 1 ng/ml.

Tumor cell E-ADM concentration

Skin metastases were removed surgically, from one patient in the 7th week 1 h after E-ADM injection, from a second patient in the 9th week just before the next injection, and from a third patient 2 weeks after the last injection. Histological examination in all cases confirmed the cutaneous localization of breast carcinoma. In samples of 13 and 30 mg tumor tissue the cellular E-ADM concentrations were determined. The tumor contained 1.68 μg E-ADM/g tissue in the first patient (blood leukocyte concentration at that time was 2.5 μg/g cells) and in the second patient 11 ng/g tumor tissue (blood leukocyte concentration at the time was 270 ng/g cells). For the third patient, no E-ADM was found 2 weeks after the last injection in tumor or nucleated blood cells.

Clonogenicity of hematopoietic progenitor cells

In four patients the clonogenicity in the CFU-GM of bone marrow cells was determined before treatment and at 4 and 8 weeks of treatment. Compared with the normal number of colonies observed in the control culture, in all patients the number of colonies was depressed before the start of the treatment: mean 39% (range 7%–80%). However, no significant decrease in clonogenicity was observed during the subsequent cultures at week 4: mean 60% (range 36%–96%) or at week 8: 47% (range 6%–50%) (Table 2). Cellular E-ADM concentrations in bone marrow cells at day 7 were 132 ± 123 ng/10^9 cells (range 0–310 ng/10^9 cells).

Pharmacokinetic data and clinical results

When the fixed dose of 20 mg per bolus injection was expressed as milligrams per square meter of body surface, the mean dose administered was 12.5 mg/m^2, ranging from 11.1 to 13.9 mg/m^2. No relation was observed with the clinical result and the administered dose expressed as milligrams of E-ADM per square meter.

Of the 12 patients described in this study, 9 could be evaluated for response of therapy. Two patients with increased bilirubin concentrations had partial remission lasting 4 and 6 months respectively, with improvement of liver function. Two others with normal bilirubin values had stable disease at 9+ and 12+ months. The area under the plasma concentration curve (AUCp) and the area under the cellular concentration curve (AUCc) for the two patients with the increased bilirubin (AUCp 902 ± 50 μg.h l^{-1} and AUCc 206000 ± 29000 μg hl^{-1} l^{-1}) were larger than the AUCs observed in the other ten patients (AUCp 561 ± 114 μg h l^{-1} and 156000 ± 33000 μg h l^{-1}). The AUCp and the AUCc of the two patients with stable disease did not differ significantly from the values obtained of those who failed to response.

Table 2. Percentage growth in the colony forming unit assay of hematopoietic cells before the start of treatment and before the 4th and 8th weeks of therapy

	% Growth (CFU-GM)[a]		
	Mean	Range	*n*
Before start	39	24–100	*n*
4 weeks	60	36– 96	3[b]
8 weeks	47	6– 50	4

[a] Compared with a normal control, which was set at 100%
[b] One culture infected

Discussion

E-ADM is advocated as superior to ADM in the treatment of breast cancer [7], since it is reported to be less myelotoxic [11] and less cardiotoxic at equimolar doses [7]. Besides bone marrow depression, alopecia, and gastrointestinal disturbances [19], a major side effect of anthracycline treatment is its cardiotoxicity, which is related to the total cumulative dose [13, 19] and most probably to the peak plasma concentration. This is lowered by reduction of the administered dose or by extension of the infusion time [8].

In the weekly low-dose regimen the peak plasma concentrations is lower than the one observed after the high-dose regimen; for instance, the peak plasma concentration after 75 mg bolus injection is 2- to 4-fold higher than that observed in this study [15]. This corresponds to observations with continuous infusions and may also be related to the absence of nausea and vomiting. It is remarkable that the continuous presence of E-ADM in blood and bone marrow cells was observed, although no overt bone marrow toxicity [11] and hardly any alopecia was noted [3, 11]. In several patients with low blood cell counts at the beginning of the therapy these values even improved.

The tissue concentrations reported at 2 h after injection of 10–20 mg/m^2 [9] were in the range of E-ADM concentrations in nucleated blood cells, as were the cellular concentrations detected in skin tumors. The prolonged presence of E-ADM in the tumor tissue (up to day 7) could point to one of the mechanisms involved in the observed response rate.

Although the number of patients investigated is too low to allow conclusions on the efficacy of this schedule, the preliminary clinical results so far are not impressive. This is in agreement with the experience of Castiglione et al. [3], who used even higher weekly dosages.

Increased bilirubin concentrations are known to be related to a reduced hepatic elimination [2]. We observed a prolonged terminal plasma half-life, but also higher cellular E-ADM concentrations in these patients. It is interesting that the only two patients who achieved PR had increased bilirubin concentrations, larger AUCc values, and plasma E-ADM and E-ADMol concentrations continuously above 1 ng/ml. This observation may justifiy an ex-

tension of the study, with a higher weekly dose or shorter intervals, provided that side effects remain low, since this is the main advantage of this weekly low-dose regimen. Since our data show a weak correlation between cellular pharmacokinetics of hematopoietic cells and therapeutic results, it may be of interest to measure tissue concentrations in relation to therapeutic results.

References

1. Broggini M, Colombo T, Martini D, Donelli MG (1980) Studies on the comparative distribution and biliary excretion of doxorubicin and 4'-epi-doxorubicin in mice and rats. Cancer Treat Rep 64: 897
2. Camaggi CM, Strocchi, Tamassia V, Martoni A, Giovanni M, Isfelice G, Canova N, Marraro D, Martini A, Pannuti F (1982) Pharmacokinetic studies of 4'-epi-doxorubicin in cancer patients with normal and impaired renal function and with hepatic metastasis. Cancer Treat Rep 66: 1819
3. Castiglione M, Goldhirsch A, Brunner K (1985) Weekly low-dose 4'-epi-adriamycin in advanced breast cancer: a phase II trial. ECCO 628 (abstract), p 163
4. Chlebowski RT, Paroly WS, Pugh RP (1980) Adriamycin given as weekly schedule without a loading course: clinically effective with reduced incidence of cardiotoxicity. Cancer Treat Rep 64: 47
5. Creech RH, Catalano RB, Shah MK (1980) An effective low-dose adriamycin regimen as secondary chemotherapy for metastatic breast cancer patients. Cancer 46: 433
6. Davis TE, Carbone PP (1978) Drug treatment of breast cancer. Drugs 16: 441
7. Ganzina F (1983) 4'-epi-doxorubicin, a new analogue of doxorubicin: a preliminary overview of preclinical and clinical data. Cancer Treat Rev 10: 1
8. Garnick MB, Weiss GR, Steele GD, Israel M, Schade D, Sack MJ, Frei E III (1983) Clinical evaluation of long-term, continuous-infusion doxorubicin. Cancer Treat Rep 67: 133
9. Italia C, Paglia L, Trabattoni A, Luchini S, Villas F, Beretta L, Marelli G, Natale N (1983) Distribution of 4'-epi-doxorubicin in human tissues. Br J Cancer 47: 545
10. Jaspers L, Possinger K, Schmid L, Wilmanns W (1985) A weekly administration schedule of doxorubicin in treatment of patients with advanced breast cancer. Cancer Chemother Pharmacol 14 [Suppl S135]: 139
11. Jones WG, Mattsson W (1984) Phase II study of weekly low-dose 4'-epi-doxorubicin in advanced postmenopausal breast cancer. Cancer Treat Rep 68: 675
12. Martini A, Moro E, Pacciarini MA, Tamassia V, Natale N, Piazza E (1984) Cross-over study of pharmacokinetics and haematological toxicity of 4'-epi-doxorubicin and doxorubicin in cancer patients. Int J Clin Pharmacol Res 4: 231
13. Mattsson W, Borgstron S, Landberg T (1982) A weekly schedule of low dose doxorubicin in treatment of advanced breast cancer. Clin Ther 5: 193
14. Speth PAJ, Linssen PCM, Boezeman JBM, Wessels JMC, Haanen C (1985) Quantitation of anthracyclines in human hematopoietic cell subpopulations by flow cytometry correlated with high-pressure liquid chromatography. Cytometry 6: 143
15. Wagner JG (ed) (1975) Fundamentals of clinical pharmacokinetics. Drug Intelligence Publications, Hamilton, Ill
16. Weenen H, Lankelma J, Penders PGM, McVie JG, Ten Bokkel Huinink WW, De Planque MM, Pinedo HM (1983) Pharmacokinetics of 4'-epi-doxorubicin in man. Invest New Drugs 1: 59
17. Weiss AJ, Manthel RW (1977) Experience with the use of adriamycin in combination with other anticancer agents using a weekly schedule, with particular reference to lack of cardiotoxicity. Cancer 40: 2046
18. Weiss AJ, Metter GE, Fletcher WS, Wilson WL, Grage TB, Ramirez G (1976) Studies on adriamycin using a weekly regimen demonstrating its clinical effectiveness and lack of cardiac toxicity. Cancer Treat Rep 60: 813

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