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Pharmacokinetic Study of Mitomycin C with Emphasis on the Influence of Aging

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Mitomycin C (MMC) at a dose of 8 mg/m^2 was administered by short intravenous infusion to 14 cancer patients. All patients had normal renal, hepatic, cardiac and bone marrow functions. The plasma level of MMC, which was determined by high-performance liquid chromatography over a 24-h period after the infusion, fitted the 2-compartment model curve except for one patient. There was a significant correlation between the area under the time versus concentration curve (AUC) of MMC and the age of patients ($r=0.558$, $P<0.05$). Pharmacokinetic parameters in one of the older (a 69-year-old male) patients were extremely different from those of the other 13 patients. This patient experienced severe myelosuppression, probably due to the markedly increased AUC of MMC. Our results suggest that a patient's age has a significant influence on the pharmacokinetics of MMC in patients with normal major organ functions and that in some patients, MMC pharmacokinetics may be altered, possibly due to interpatient variation in the activation or metabolic pathway of MMC.

Key words: Mitomycin C — Pharmacokinetics — Influence of aging — Interpatient variation

Several factors such as renal or hepatic function, weight, sex, fat volume, albumin concentration and performance status may affect drug pharmacokinetics. Only a few reports have actually demonstrated the relationship between these factors and the pharmacokinetics or pharmacodynamics of anticancer agents.[1)] It is reasonable to assume that aging may be a major factor influencing drug pharmacokinetics. For the majority of drugs, the dosage and administration schedule employed are based upon information obtained in young adults, while on the contrary, phase I clinical trials of anticancer agents are usually performed in older patients.[2)] Nevertheless, the influence of aging in drug pharmacokinetic studies has been ignored.[2)] As increased numbers of older patients with malignancy are being treated by chemotherapy, more attention should be paid to the influence of aging on the pharmacological behavior of anticancer agents. Although mitomycin C (MMC) has been one of the major antineoplastic agents used in treatment of lung, gastric and other solid tumors for over 30 years,[3)] very little information on its pharmacokinetics and/or pharmacodynamics is available. In the present study we have analyzed the plasma pharmacokinetics of MMC injected as a bolus with special emphasis on the influence of aging.

PATIENTS AND METHODS

Patient eligibility Patients meeting the following criteria were eligible for this study: histological or cytological proof of malignant disease, a performance status (PS) of 0–2, on the Eastern Cooperative Oncology Group (ECOG) scale,[4)] recovery from all toxicities due to prior treatment and interval of at least 4 weeks after the last chemotherapy in patients with prior chemotherapy, a life expectancy of more than 8 weeks, total protein >6.0 g/dl, serum albumin >3.0 g/dl, white blood cell count $>3000/mm^3$, platelet count $>100,000/mm^3$ and hemoglobin >10 g/dl. Normal renal and hepatic function including normal biochemical values (bilirubin <1.2 mg/dl, GOT <40 U/liter, GPT <40 U/liter, creatinine <1.2 mg/dl and BUN <30 mg/dl), creatinine clearance (Ccr) >50 ml/min and indocyanine green (ICG) retention test at 15 min (R_{15}) of less than 15% were also required. Ccr was determined by the 24-h method. ICG at the dose of 0.5 mg/kg was injected as a bolus and a blood sample was collected 15 min after injection. R_{15} was calculated by means of the following equation:

$$R_{15}=C_{15}/1.00\times 100(\%)$$

where C_{15}=plasma ICG concentration at 15 min after the injection (mg/dl), and 1.00 is the theoretical ICG concentration at 0 min (mg/dl). Written informed consent was obtained from all patients.

Drug administration schedule Patients treated with combination chemotherapy including MMC were eligible for the present study. MMC at a dose of 8 mg/m^2 was dissolved in 20 ml of saline and given as a rapid intravenous infusion over one minute. In patients treated with combination chemotherapy, MMC was administered on day 1 and other drug(s) were administered on day 2 or later so that obtained pharmacokinetic values represent the pharmacokinetics of MMC given as a single agent at least until 24 h after bolus injection.

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Blood sampling Blood samples were collected in a heparin-containing syringe before and just after the end of the drug infusion and at 5, 10, 15 and 30 min and 1, 2, 6, 12 and 24 h after MMC infusion. Plasma was separated immediately by centrifugation at 800*g* for 15 min and then stored at −70°C until analysis.

Sample analysis The concentration of plasma MMC was determined by a high-performance liquid chromatographic (HPLC) method. The instrumentation consisted of a Hitachi 655A-12 pump operated at 1.0 ml/min (Tokyo), a Hitachi 655A-21 variable-wavelength detector (Tokyo) set at 360 nm and a Nucleosil 100-5 column (Kemuko, Tokyo), 4.5 × 250 mm. For extraction of MMC, 0.5 ml of plasma was mixed with 6 ml of ethyl acetate, and the mixture was shaken for 10 min, and centrifuged for 10 min at 3,000 rpm. The clear supernatant was transferred into a conical glass tube and evaporated to dryness at 40°C. The residue was dissolved in 1 ml chloroform-methanol (98:2) mixture, and analyzed by using the silica gel column and a mobile phase consisting of chloroform, methanol, and water (ratio, 90:10:0.15).

Pharmacokinetic and statistical analysis Pharmacokinetics of MMC was analyzed by using the computer programs PHACONET I and II[5] on an NEC 9801 personal computer (Nippon Electric Corporation Inc., Tokyo). Regression models were calculated by the method of least squares.

RESULTS

Fourteen patients were enrolled in this study. Twelve patients had non-small cell lung cancer, one patient cervical cancer and the other patient laryngeal squamous cell carcinoma. All patients received combined chemotherapeutic regimen(s) including MMC (Table I). No significant differences in performance status, body weight, Ccr, ICG test or albumin concentration were found according to age by using a regression analysis (data not shown). Plasma concentration-time curves for MMC administered as a rapid intravenous injection of 8 mg/m^2 over one minute to the 14 patients are shown in Fig. 1. The concentration-time curves fitted well with a 2-compartment model in 13 patients. The mean alpha and beta half-lives of MMC in these 13 patients were 9.9 (range 4.1 to 14.6) min and 6.9 (range 2.5 to 16.0) h, respectively. The concentration-time curve of the remaining subject (69 year-old male) fitted a one-compartment model. The half life of MMC in this patient was 4.3 h and pharmacokinetic parameters substantially different from those of the other patients were obtained (Table II). This individual was coadministered vindesin (VDS) on day 2 and experienced severe leukocytopenia (less than 500/mm^3 over 7 days). When data from this patient were excluded from the analysis, a significant correlation between age and the AUC of MMC was obtained in regression analysis (Fig. 2). The following equation for predicting AUC associated with administration of MMC at a dose of 8 mg/m^2 was derived:

$$\text{AUC } (\mu\text{g h/ml}) = 0.12 + 0.0036 \text{ Age (yr)}.$$

Table I. Characteristics of Patients

Number of patients	14
Evaluable courses	14
Median age (years) (range, 26 to 79)	55.6
Median performance status (range, 0 to 2)	1
Sex (M:F)	(7:7)
Tumor type (No. of patients)	
Lung (non-small cell)	12
Uterus	1
Larynx (squamous cell)	1
Chemotherapy regimen	
MVP regimen	12
MMC+VDS+254S	1
MMC+VDS	1

Abbreviations: MVP=mitomycin C (8 mg/m^2)+vindesine (3 mg/m^2)+*cis*-platinum (80 or 100 mg/m^2), VDS=vindesine (3 mg/m^2), MMC=mitomycin C (8 mg/m^2), 254S=(glycolato-O,O′)-diamine platinum (II) (100 mg/m^2)

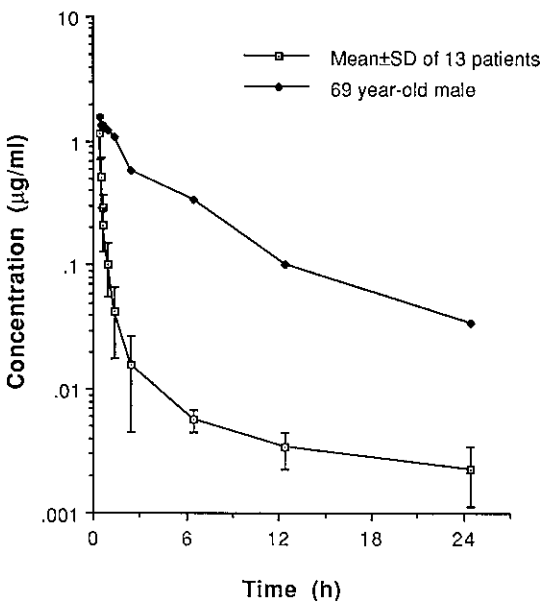

Fig. 1. The concentration versus time curve of MMC injected as a bolus in 13 patients fitted a two-compartment model curve. The curve for the remaining 69-year-old patient was extremely different from those of the other patients and fitted a one-compartment model.

Table II. Comparison of Pharmacokinetic Parameters of a Patient with Severe Myelosuppression and 13 Other Patients

	Ccr (ml/m)	ICG15′ (%)	AUC (μg h/ml)	CL (l/h)	PPC (μg/ml)
69-year-old M	65.3	8.5	6.58	1.64	1.40
Mean of the others	72.8	5.9	0.315	35.6	1.03

Abbreviations: Ccr=creatinine clearance, ICG=indocyanine green retention test (15 min), AUC=the area under the time versus concentration curve, CL=total body clearance, PPC=peak plasma concentration.

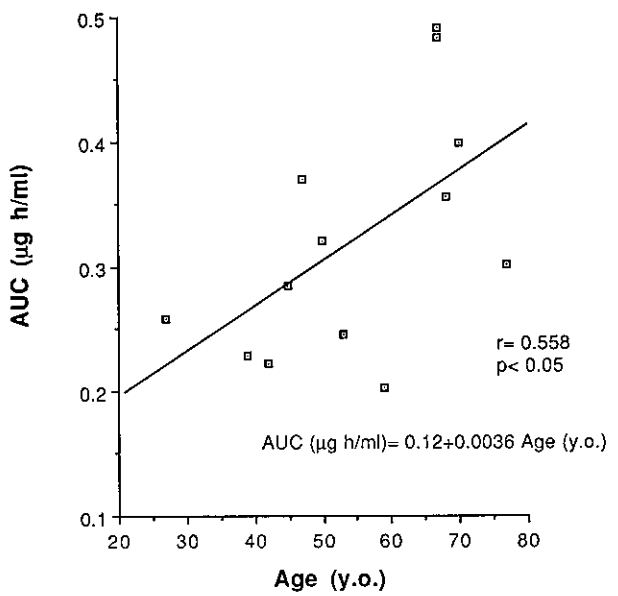

Fig. 2. The regression analysis revealed a significant correlation between the patients' age and the AUC of MMC injected as a bolus.

DISCUSSION

In recent years, the proportion of the elderly in the U.S. and Japanese populations has increased. It is necessary for clinicians to understand the influence of aging on the pharmacokinetics and pharmacodynamics of drugs. The influence of the aging on the absorption or excretion of drugs such as salicylate has been studied,[6] but there is little information as to whether the pharmacokinetics and/or pharmacodynamics of drugs used in cancer chemotherapy are affected by aging. The process of aging is considered to involve changes in the physiology and anatomy of the human body that may alter the absorption, distribution, biotransformation and excretion of drugs. Since the successful use of anticancer drugs requires control of plasma concentrations obtained within a narrow range, the influence of age on drug pharmacokinetics needs to be studied.[7]

MMC is regarded as the prototype bioreductive alkylating agent in clinical use. It had been difficult to analyze the pharmacokinetics of MMC precisely until an HPLC assay with a sensitivity of 1 ng MMC/ml plasma was developed in 1981.[8] Human pharmacokinetic studies of MMC have been reported by several authors,[9, 10] but little is known concerning its metabolism. Verweij *et al.* reported that the AUC of MMC in patients on combination chemotherapy was significantly lower than in patients treated with MMC alone.[11] In the present study, MMC was administered on day 1 and other agent(s) of combination chemotherapy were given on day 2 or later to avoid the influence of drug-drug interaction.

It is reasonable to consider that the biological functions tend to decrease in the elderly. We have tried to eliminate other variables which might affect the plasma concentration of MMC and examined the pharmacokinetics in patients with normal renal, hepatic and bone marrow functions. We found a significant correlation between age and the AUC in patients with normal organ functions. The result indicates that age itself is one of the variables which influence the pharmacokinetics of the anticancer agents. In phase I clinical trial, anticancer agents tend to be examined in older patients. Young patients whose drug clearance rate is higher than that of old patients may be able to accept higher dosages of anticancer agents than the dose recommended on the basis of phase I trials. We would have preferred to understand the pharmacodynamic consequences of aging upon MMC, but current clinical standards of anticancer treatment, which demand combination chemotherapy, confound such pharmacodynamic analysis.

One of the patients in the present study developed severe myelosuppression and MMC pharmacokinetic parameters in this patient were significantly different from those of the other patients. Although this patient received combination chemotherapy with MMC and VDS, it is reasonable to assume that the severe myelosuppression resulted from the markedly increased AUC, which was more than twenty times higher than the mean of the other patients. It was reported that the main excretion pathway of MMC is through the biliary duct,[12] but this patient's hepatic as well as other major organ functions were normal. These results support the hypothesis that individual variations in metabolism or activation pathway may cause pharmacokinetic changes. It is possible that such pharmacokinetic variations may be predictive of individual susceptibility to drug toxicity. Several pathways in MMC metabolism have been reported and NADPH:cytochrome P-450 reductase is thought to be one of the major metabolizing enzymes for MMC.[13, 14] It

is possible that genetic variations or polymorphism of enzymatic activity may cause the interpatient variability in pharmacokinetic behavior.

We are now developing a limited sampling model[15, 16] for MMC and will execute a large-scale clinical study to confirm the correlation between aging and pharmacokinetics and to explore the incidence of pharmacokinetic polymorphism of MMC.

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