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Clinical pharmacokinetics of high-dose mitomycin C

Rudolf B. Schilcher, John D. Young, Voravit Ratanatharathorn, Chatchada Karanes, and Laurence H. Baker

Division of Oncology, Department of Internal Medicine, Wayne State University, School of Medicine, Detroit, MI 48201, USA

Summary. *The pharmacokinetic profile of high-dose mitomycin C was determined in blood plasma and urine of twelve patients with advanced malignancies in a program including autologous bone marrow transplantation. A total dose of 60 mg/m² was given, either as a single 60-min infusion or divided into infusions of 30 mg/m² on each of 2 days or 15 mg/m² on each of 4 days. One group was given 15-min infusions. Samples of blood plasma and urine were analyzed by high-performance liquid chromatography. Drug concentrations in plasma followed a biphasic pattern, with a terminal elimination half-life of 45 min. This half-life value and other parameters were unaffected by dose level, infusion time, and repeated doses. The lower peak plasma concentrations following 30 mg/m² given as 60-min infusions compared to the same dose given over 15 min may have accounted for a dramatic drop in the incidence of a severe hemorrhagic colitis. Mitomycin C was excreted in urine at about the same rate as it was eliminated from plasma, but a larger percentage of the dose appeared in urine after 15-min infusions than after 60-min infusions. The pharmacokinetic profile, together with clinical observations, suggests that the dose-limiting toxicity of mitomycin C may be related to peak drug levels, and that both these levels and the toxicity are lessened as the infusion time is increased.*

Introduction

The antibiotic mitomycin C (MMC) has shown dose- and schedule-dependent effectiveness in gastrointestinal, lung, and mesenchymal neoplasms. Effective dose schedules ranged from 50 μg/kg (2 mg/m²) daily over 6 days to single bolus injections of 20 mg/m² every 6 weeks, with mean total tolerated doses of 35.5–99.8 mg [1, 2]. Yet its clinical use has been limited by hematologic toxicity. With the concept of high-dose chemotherapy followed by autologous bone marrow transplantation (ABMT), an effective approach was established to ameliorate life-threatening myelosuppression and overcoming secondary resistance [3, 8]. However, these trials using ABMT were met with toxicities not seen at lower doses of the drug [12].

Since few clinical evaluations were accompanied by pharmacological studies, only limited data on pharmacokinetic behavior of high-dose MMC were available [14]. An early report of MMC pharmacokinetics led to the assumption of saturated metabolic pathways, because longer serum half-lives were observed at higher dose levels [7, 13]. Animal trials also supported the assumption of nonlinear kinetics. The recovery of MMC from the urine of rats increased from 13% to 35% with escalating dosage [15]. Although patients were treated with doses only up to 1.5 mg MMC/kg body weight (60 mg MMC/m²), while rats received 1–8 mg MMC/kg, there was concern that higher dose levels in patients might lead to disproportional increases in toxicity. Therefore, we were interested to obtain blood level data in patients given these untested doses of MMC followed by ABMT.

To study pharmacokinetic profiles in patients receiving MMC over a 15- or 60-min period rather than in a push injection it was desirable to detect concentrations as low as 10 ng/ml. A high-performance liquid chromatography (HPLC) assay reported in 1979 [10] could not be repeated in this laboratory, probably because the extraction efficiency was not consistent from day to day and in some samples was as low as 10%. A more recently reported assay used a nonionic resin for extraction, but had a sensitivity of 50 ng/ml [16]. A method described by den Hartigh [5] allowed monitoring of 1 ng MMC/ml plasma by means of a chloroform/isopropyl alcohol extraction procedure which proved to be superior to other methods. The latter procedure provides sufficient sensitivity to follow the elimination of MMC for several half-lives.

This paper reports on a pharmacological study accompanying a clinical phase I/II trial of high doses of MMC and ABMT, with particular emphasis on the detection of nonlinear pharmacokinetic profiles.

Materials and methods

Animal study. In a preliminary study, MMC was given as a single 3-min infusion of 3 mg/kg to two mongrel dogs each weighing about 33 kg. Polyethylene cannulas were placed in both femoral veins while the dogs were under halothane anaesthesia. After the infusion blood samples were collected from the contralateral vein, and urine specimens were obtained from a Foley catheter.

Patients and protocol. Twelve patients (median age 41 years, range 32–63 years) with histologically confirmed malignancies refractory to conventional therapy received high-dose MMC as a 15- or 60-min infusion followed by autologous bone marrow transplantation [12]. The drug was infused as a 60 mg/m² dose

Offprint requests to: R. B. Schilcher, Division of Oncology, Department of Internal Medicine, Wayne State University, P.O. Box 02188, Detroit, MI 48201, USA

on 1 day over 60 min, as a 30 mg/m^2 dose on each of 2 days over 15 or 60 min, or as a 15 mg/m^2 dose on each of 4 days over 60 min. Serial blood samples were obtained from twelve patients in 10-ml tubes containing either heparin or EDTA, before and at 1, 5, 10, 20, 30, 40, 50, 60, 90 min and 2, 3, 4, 5, and 6 h after the completion of MMC infusions. Blood specimens were immediately chilled on ice until plasma was separated by centrifugation at 2,800 rpm for 10 min in a Beckman TJ-6 centrifuge. Plasma samples were then stored at −18° C. Selected heparinized plasma samples and aliquots of urine were shipped in dry ice to Bristol Laboratories (Bristol-Myers, Syracuse, NY; Dr. D. van Harken) for microbiological assay [9]. The detection limit was 20 ng/ml vs 1 ng/ml for HPLC. Urine samples were collected for the intervals 0–2, 2–4, 4–6, and 6–8 h after treatment, and an aliquot was stored at −18° C.

Analytical methodology

Apparatus. The chromatographic system consisted of a Rheodyne Injector[1], a Perkin-Elmer LC-75 variable wavelength detector[2] set at 360 nm, a Waters 6000A pump[3] at 1.0 ml/min, a Linear strip chart recorder[1], and a Waters μ-Bondapak C_{18} column[3], 300 × 4.6 mm. The solvent system was 0.01 *M* phosphate buffer, pH 6.1/methanol (62/38 v/v).

Sample preparation. For extraction of MMC, 1.0 ml plasma was mixed with 10 ml chloroform/isopropyl alcohol (35/65 v/v), shaken for 1 min, and centrifuged for 5 min at 2,800 rpm. The clear supernatant was transferred into a conical glass tube and evaporated to dryness at 35° C under a stream of N_2. The residue was dissolved in 100 μl methanol, and a 20-μl aliquot was injected. Recovery of MMC was 79% from plasma, and the retention time was 6.1 min. Patient predose blood or urine samples did not contain interferences at the retention time of MMC. Standard curves for the drug dissolved in water or extracted from plasma were linear from 1 to 5,000 ng/ml, and peak heights from repeated injections of plasma or urine samples were within 10% of the mean. Urine samples were directly injected onto the column with a 100% recovery and a retention time of 6.1 min.

Data analysis. Blood plasma concentration data were weighted as reciprocals of the values and analyzed using the computer program AUTOAN (supplied by Dr. J.G. Wagner, Upjohn Center for Clinical Pharmacology, University of Michigan, Ann Arbor, MI). This program selected the most appropriate kinetic model, obtained preliminary estimates of rate constants and volumes of distribution, and fit the experimental results to model equations by using a nonlinear least-squares regression program. Terminal half-life values obtained from this analysis were then used to calculate the area under the plasma curve (AUC) from the last data point to infinity by dividing the last concentration by the rate constant. This area was then added to the area determined by the trapezoidal rule for data from time zero to the last data point for each patient. The total-body clearance and volume of distribution were calculated using these areas.

1 Obtained from Anspec Corp., Ann Arbor, MI 48107
2 Perkin-Elmer Corp., Norwalk, CT 06856
3 Waters Assoc., Milford, MA 01757

Results

Probe study in dogs

The blood plasma concentration versus time curve for a dog given 3 mg MMC/kg as a 3-min infusion is shown in Fig. 1. Immediately after the infusion the concentration was 23 μg/ml, and it declined in a biphasic manner thereafter. The alpha- and beta-half-life values were 3.5 and 50.9 min, respectively. The AUC from time zero to infinity was 9,564 (μg/l) h. The volume of distribution (V_B) and the total-body clearance (TBC) were 0.48 l/kg and 0.39 l/kg per h. In a second dog given the same dose, 28.2% of the dose was excreted in the urine as unchanged MMC within 8 h of administration. Severe intestinal bleeding was manifested after 48 h in both dogs and neither survived beyond 5 days. At necropsy the entire digestive tract showed hemorrhagic infiltrations and multiple ulcerations.

Single 1-h infusions

Plasma concentration data from seven patients given 15, 30, or 60 mg MMC/m^2 as a 1-h infusion are shown in Fig. 2. Table 1 shows the pharmacokinetic parameters calculated from these data. The peak concentrations as well as the AUCs were approximately proportional to the dose level. The V_B, TBC, and terminal half-life values were nearly constant as the dose was increased. For all seven patients the TBC was 33.9 ± 18.7 l/m^2 per h (mean ± SD), the V_B was 39.0 ± 22.0 l/m^2, and the terminal half-life was 45.2 ± 19.0 min.

Urinary excretion of MMC was measured in samples collected at 2-h intervals. The rate of excretion was plotted as a function of the mid-point of the collection interval (Fig. 3). The slopes of these curves, drawn by inspection, were approximately the same as the terminal slopes of the plasma curves shown in Fig. 2. The total percentage of the dose excreted in urine is shown in Table 2. No difference due to dose level was seen in excretion. The overall percentage of the dose excreted in urine for six patients given 1-h infusions was 3.5% ± 4.1%.

Single 15-min infusions

In two patients given 30 mg/m^2 as a 15-min infusion, plasma concentrations were determined both by microbiological assay and by HPLC assay (Fig. 4). To compare these assays the ratio of plasma concentrations determined by microbiological assay to those by HPLC was calculated for 24 pairs. This ratio was 0.90 ± 0.31. In four urine samples this comparison was also possible. In one sample the microbiological assay/HPLC ratio was 2.8, but in the remaining samples the average ratio was 1.00 ± 0.18.

A comparison of the plasma curves (HPLC data) for patients given 15-versus 60-min infusions of 30 mg MMC/m^2 (Fig. 4 vs Fig. 2) demonstrates that the longer infusion time lowered the peak plasma levels from 2,185 (2,550 and 1,820) to 792 ± 477 ng/ml. The shorter infusion time produced AUCs in these patients of 2,361 and 1,634 μg/l per h, as against 1,084 ± 710 μg/l per h in three patients given the same dose over the longer infusion time. Clinicially, all 15-min infusions were accompanied by severe protracted bowel toxicity in the form of hemorrhagic enterocolitis, which was confirmed by sigmoidoscopy and later by autopsy. This complication occurred in only one of nine patients who received an infusion over 60 min.

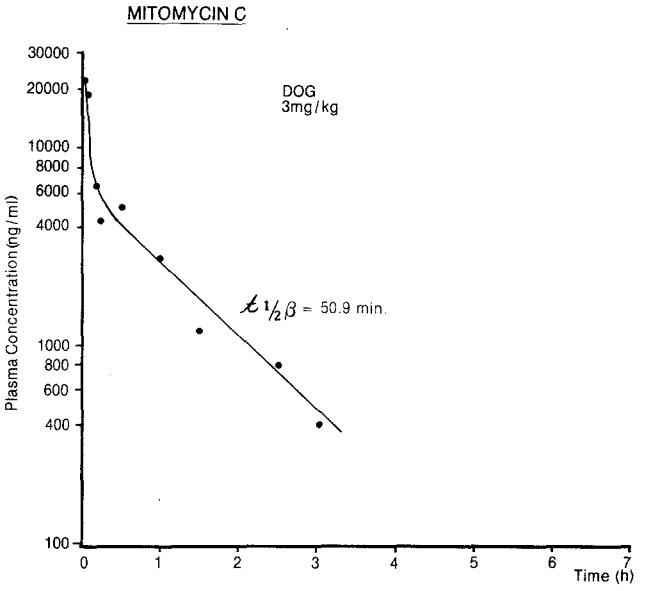

Fig. 1. Plasma concentration versus time profile of MMC (3 mg/kg) given as a 3 min IV infusion to a dog as part of an autologous bone marrow transplantation pilot study. Dose equaled about 73 mg MMC/m^2 BSA in patients [6]

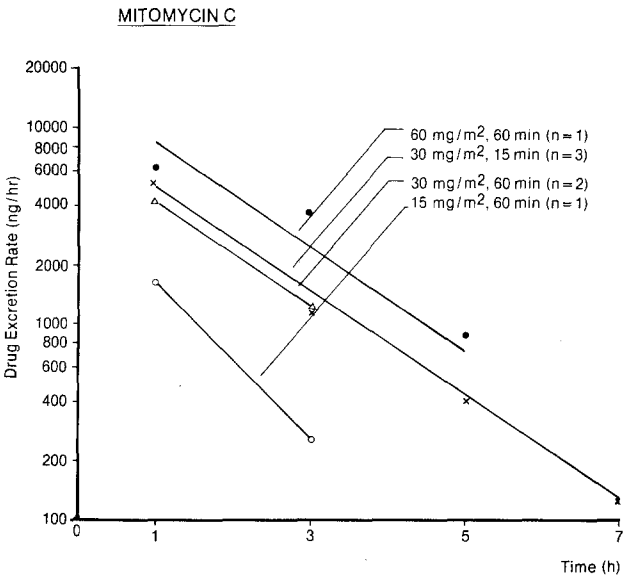

Fig. 3. Excretion of MMC in the urine of patients. Dose, infusion time and number of patients are shown in the Figure. The excretion rate was calculated for each 2 h collection interval and plotted at the midpoint. Lines were drawn by inspection

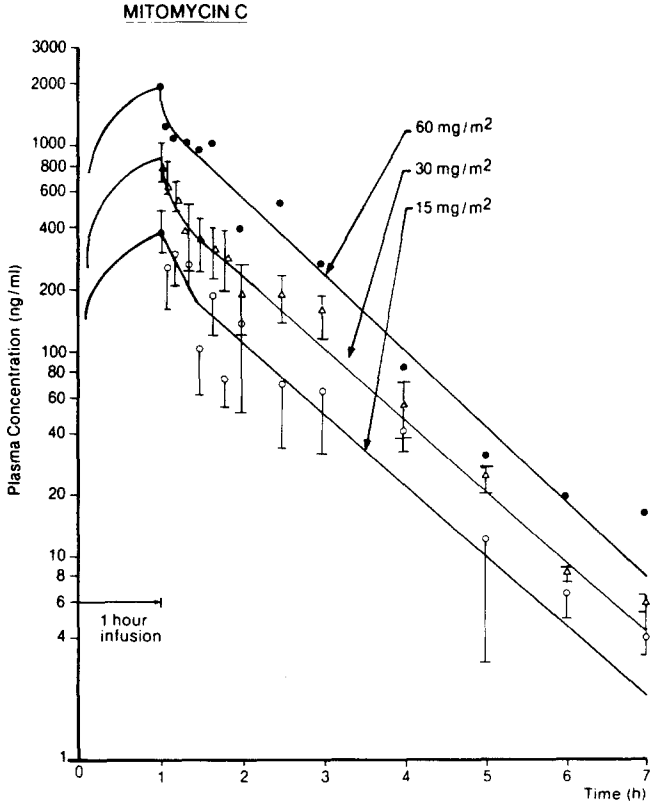

Fig. 2. Plasma concentration versus time profiles for MMC given as a 60 min IV infusion. Three patients received 15 mg/m^2, three patients 30 mg/m^2 and one patient 60 mg/m^2. Bars indicate mean ± standard error. Lines were drawn by inspection

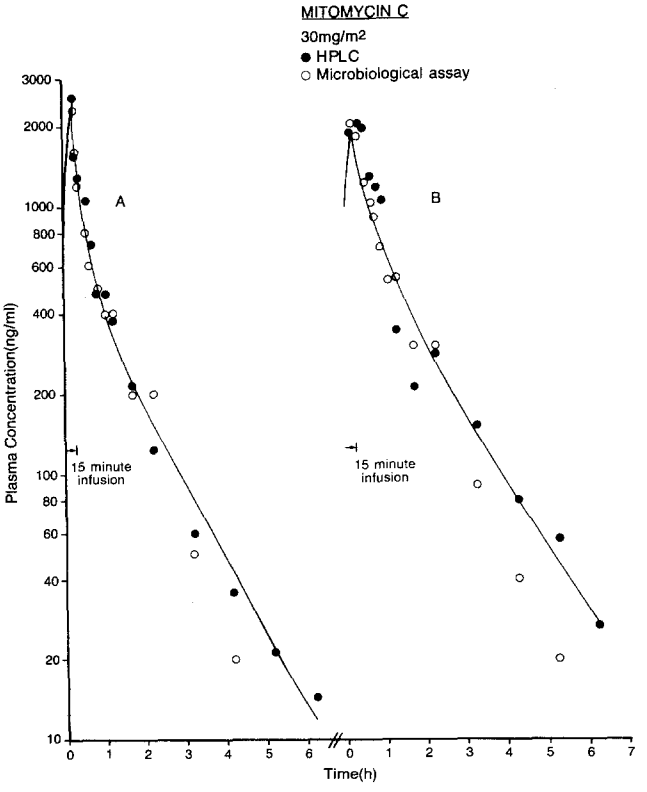

Fig. 4. Plasma concentrations of MMC measured by HPLC and microbiological assay in two patients (A, B) given 15 min infusions of 30 mg/m^2

Table 1. Pharmacokinetic parameters of high-dose mitomycin C in human plasma

	Infusion time 60 min		
	Dose		
	15 mg/m^2	30 mg/m^2	60 mg/m^2
No. of patients	3	3	1
Peak concentration[a] (ng/ml)	346 ± 151[f]	792 ± 477	1,965
AUC[b] (ng/ml)h	582 ± 397	1,082 ± 710	2,712
V_B[c] (L/m^2)	40.5 ± 24.8	41.8 ± 27.3	26.0
TBC[d] (L/m^2/h)	35.5 ± 23.0	36.3 ± 21.0	22.1
$t_{1/2}\beta$[e] (min)	48.6 ± 5.9	41.3 ± 26.5	48.9

[a] Concentration at the end of infusion
[b] Area under the concentration versus time curve; calculated for individual patients by trapezoidal rule
[c] Volume of distribution calculated from: V_B=dose/(AUC × β)
[d] Total body clearance calculated from: TBC = dose/AUC
[e] Terminal half-life; harmonic mean ± SD
[f] Mean ± SD

Table 2. Urinary excretion of mitomycin C in patients

	Infusion time			
	15 min	60 min		
	Dose			
	30 mg/m^2	15 mg/m^2	30 mg/m^2	60 mg/m^2
HPLC assay	20.4 ± 15.2[a] (3)	2.1 ± 1.8 (2)	4.5 ± 6.1 (3)	3.1 (1)
Microbiological assay	14.0 ± 4.4 (3)	–	–	–

[a] Mean ± SD (no. of patients). Values are percentages of the dose excreted as unchanged MMC in urine collected from 0 to 8 h

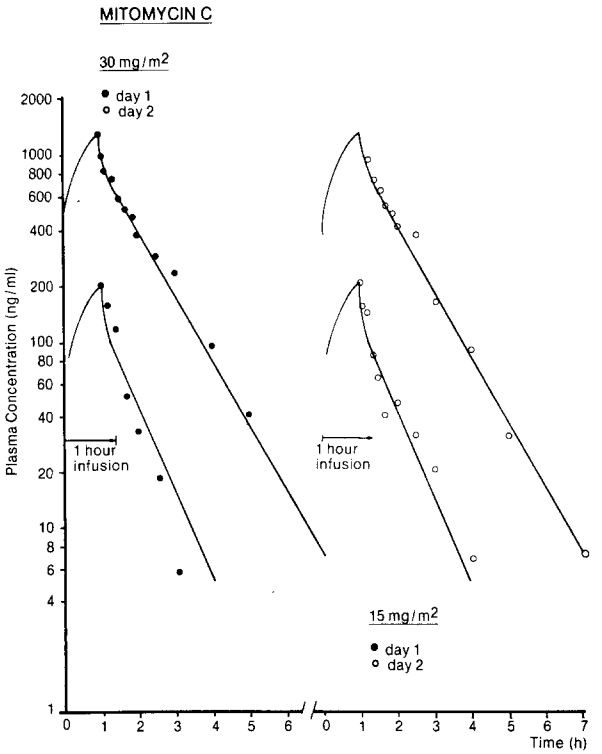

Fig. 5. Pharmacokinetic profiles of MMC (15 and 30 mg/m^2) in plasma of patients given 1 h IV infusions on 2 consecutive days

Urinary excretion of MMC in one patient given 30 mg/m^2 in a 15-min infusion can be compared with that following the same dose given in a 60-min infusion in Fig. 3. The slopes of these curves are approximately the same. However, the percentage of the dose excreted in the urine (Table 2) was greater in patients treated over the shorter infusion time, being 20.4% ± 15.2% versus 4.5% ± 6.1%.

Repeated daily infusions

Plasma curves for patients given consecutive daily doses of either 15 or 30 mg MMC/m^2 in 1-h infusions are shown in Fig. 5. The solid lines represent the best fit to the day 2 data drawn for both day 1 and day 2 to facilitate comparison. It is apparent that repeated infusions have little effect on the plasma curves in the same patient.

Discussion

The analytical data in this paper are in agreement with the work of other investigators who have described the high extraction efficiency and reproducibility of this method [5]. Since all samples were monitored at 360 nm, additional peaks of metabolites or decomposition products, which might have been identified at 254 nm [11], were not seen. Results obtained with HPLC and microbiological assays on the same plasma or urine samples (Fig. 4 and Table 2) suggest that the two methods provide equivalent values, although the superior

sensitivity of HPLC with UV detection allows measurement of MMC for longer times after dosage. Since MMC is eliminated biphasically, an accurate determination of the beta-phase half-life requires that blood samples be taken for 5 or 6 h after dosage. We believe that sample collection for less than four to five half-lives has led to erroneous reports of half-lives for MMC.

The major portion of available pharmacokinetic data of MMC originated from two publications in 1961 and 1971, and two reviews [2, 7, 13, 15]. Fujita had given 2–30 mg MMC (1.2–17.3 mg/m^2) as an injection of unspecified duration to five patients [7]. His observations were continued only to 120 min, and therefore most data were obtained during the distribution phase. The short sampling time would also explain why Reich, after recalculating the data, pointed out that too little information was available to determine kinetic parameters and estimated a small initial distribution volume of 9 l (5 l/m^2) [13]. In more recent articles, van Hazel and van Oosterom calculated kinetic data from a sampling time of less than 200 min [16, 17]. In two patients van Hazel found a TBC of 7.3 ml/min per kg (18 l/m^2 per h), and a TBC of 5.2 ml/min per kg (13 l/m^2 per h) in nine patients treated with MMC in combination chemotherapy. Because of the improved analytical sensitivity of the present method, plasma curves could be followed for up to seven half-lives of the drug. AUCs extrapolated from the last data point to infinity require an accurate terminal half-life value. Therefore, the results obtained in this study, with a TBC of 33.9 l/m^2 per h and a V_B of 39.0 l/m^2, and in similar studies conducted by den Hartigh and Pinedo [4, 11] reflect the elimination half-life and total-body clearance values of MMC more accurately.

On the basis of Fujita's observations in patients and Schwartz's findings in animals, both Crooke and Reich postulated in their reviews a dose-dependent metabolism and an excretion rate consistant with nonlinear kinetics [2, 7, 13, 15]. Other investigators concurred with this assumption but failed to provide evidence [4, 16]. In our study, patients received MMC over a four-fold dose range, with total amounts three times higher than Fujita's patients (17.3 mg/m^2 versus 60 mg/m^2). However, no evidence of saturation kinetics was found as doses and infusion times increased, other than a higher percentage of MMC excreted after 15-min infusions (2.1%–4.5% versus 20.4%). Recently, Pinedo also questioned Fujita's findings, since he was unable to confirm a nonlinear elimination process in the dose range up to 20 mg MMC/m^2 [11]. The earlier observations and the discrepancy in parameters might be a result of less sensitive assays, leading to shorter detection times and calculations of a one-compartment model composed primarily of the distribution phase. Our data also suggest that infusion times may be an important factor in the disposition and bowel toxicity of high-dose MMC, although most authors do not report infusion times precisely.

In conclusion, no evidence of saturated metabolic pathways was found on the basis of blood curves of patients receiving high-dose mitomycin C followed by autologous bone marrow transplantation; moreover, elimination kinetics were linear even with repeated doses. Extending infusion time from 15 to 60 min both lowered peak plasma levels and apparently diminished toxic side-effects [12].

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