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Clinical pharmacology of 4-demethoxydaunorubicin (DMDR)*

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Summary. DMDR, a daunorubicin derivative with a higher therapeutic index and lower cardiotoxicity than either the parent drug or doxorubicin, is active when given PO in experimental animals. We studied its pharmacokinetics in ten patients receiving DMDR IV or PO or IV and PO sequentially at 10–12.5 mg/m^2. DMDR and its metabolites were quantified by high-performance liquid chromatography and fluorometry. In nine patients who received DMDR IV the unchanged drug disappeared from the plasma biphasically with a mean terminal half-life of 27.0 ± 5.5 h, an apparent volume of distribution of 63.9 ± 12.6 l kg^{-1}, and a total clearance of 1.9 ± 0.4 l kg^{-1} h^{-1}. In 24 h only $5.1\% \pm 1.1\%$ of the dose was excreted in the urine. In comparison, in 19 studies the plasma half-life of DMDR given PO was 34.8 ± 6.7 h, $2.3\% \pm 1.3\%$ was excreted in the urine in 24 h, and the maximum plasma drug concentration was reached in about 1 h. The bioavailability of DMDR given PO was about 39% according to comparison of the areas under the plasma DMDR concentration versus time curves for the two routes, but 45% according to comparison of the 24-h cumulative urinary excretion rates. In one patient with severe liver dysfunction following oral administration, the plasma DMDR half-life was 56.8 h, more than twice the average length. By either route, the drug was quickly metabolized to one major metabolite, DMDR-ol. The plasma half-life of DMDR-ol was 72.5 ± 24.7 h, or 35.7 ± 7.4 when DMDR was administered IV or PO. In the plasma, DMDR-ol always exceeded DMDR in concentration. Moreover, the 24 h cumulative urinary excretion of DMDR-ol as a percentage of the dose of DMDR administered was 7.8 following IV and 7.4 following PO administration.

Introduction

The anthracycline antibiotics occupy a prominent niche in the armamentarium of cancer chemotherapeutic agents, especially doxorubicin, with its broad spectrum of activity against many malignancies. Unfortunately, their clinical applications are hampered by their potentially serious myocardial toxicity. Various attempts have been made to circumvent this most undesirable side effect; these include variations of dose and schedule, the synthesis of analogues, and the search for new anthracycline derivatives with improved therapeutic efficacy. 4-Demethoxydaunorubicin (DMDR), the daunorubicin derivative in which the 4-methoxy moiety has been replaced with hydrogen, is a promising product of the synthetic approaches [1]. In several experimental tumor models, DMDR is distinctly more potent than its parent drug [1, 4, 7]. Most important, in various species, it is significantly less prone to induce cardiac toxicity [5, 6, 9]. These properties together make DMDR an especially attractive candidate for clinical trials. Consequently, a phase I trial of DMDR was initiated at our institution. Concurrently, clinical pharmacological studies were also performed as an integral part of the trial. The results of these pharmacokinetic studies are reported herein.

Materials and methods

Patients. Adult patients who had histopathologically confirmed malignancy and had failed previously to respond to standard therapy, were eligible to participate in the present study. According to institutional policies, informed consent was obtained in every case. The lowest performance status of the patients was 3 on the Zubrod scale. The hematologic requirements of the eligible patients were: granulocyte counts, above 2000/μl; platelets, above 100000/μl; serum bilirubin concentration, under 1 mg/dl; and creatinine, under 0.5 mg/dl. Patients were excluded from the study if they had a history of significant cardiovascular disease, anthracycline exposure within the past year, or gastrointestinal problems that might interfere with drug absorption. Complete blood cell counts and clinical chemical evaluations were performed weekly. Electrocardiogram and ejection fraction studies were done before DMDR therapy. Additionally, an electrocardiogram was repeated before each course, and ejection fraction studies before every fourth course. DMDR was discontinued in patients who developed serious toxicity or progression of disease.

Drug. DMDR and 4-demethoxy-13-hydroxydaunorubicin (DMDR-ol) were supplied by Farmitalia Carlo Erba. The parenteral DMDR form was in 5-mg vials and was infused IV in 50 ml D_5W over 30 min. The oral preparation was in 5-mg and 10-mg capsules; the total oral dose was given in

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five daily doses. Both IV and PO treatments were administered in the morning. In the same patient DMDR administration PO alternated with treatment IV. If nausea or vomiting developed, an antiemetic was given PO 1 h before oral DMDR. Therapy was repeated every 21 days upon recovery from myelosuppression and other toxicities.

Pharmacokinetic studies. Pharmacokinetic studies were conducted for both routes of DMDR administration. In every study, blood was sampled at intervals from an indwelling heparin lock before drug administration and again at predetermined times thereafter. Specimens of 10 ml each were drawn from a vein in the opposite extremity, put in tubes containing heparin as an anticoagulant, and placed in ice until centrifugation at 12000 *g* for 10 min in a Sorvall RC2 centrifuge (DuPont Instruments, Wilmington, Del). The plasma was separated from the blood cells and kept frozen at 4 °C until analysis.

Urine was collected at 6-h intervals on the first day, and daily for another 3–6 days if possible.

All biological specimens were extracted by a modification of the procedure of Benjamin et al [2]. Briefly, a 2-ml sample was extracted twice, each time with 4 ml of an equivolume mixture of chloroform and isopropanol. The upper phase obtained by centrifugation at 49500 *g* for 20 min was combined, placed in a concentrator (Brinkman Instruments, Westbury, NY) in a stream of nitrogen. The residue was dissolved in 0.5 ml normal saline, sonicated, and analyzed for DMDR by high-performance liquid chromatography. Daunorubicin (100 ng/ml) in plasma was used as an internal standard. The extraction efficiencies for both plasma and urine were 80%–85%.

Chromatography. All analyses were performed with a Waters Associates (Milford, Mass) model 204 liquid chromatograph equipped with a Schoeffel model SF-970 fluorescent detector (Kratos Analytical Co., Westwood, NJ) and a Shimadzua (Kyoto, Japan) Chromatopac C-RIB Data Processor. Separation was achieved with an analytical reverse-phase μBondapak phenyl column (3.9 mm × 30 cm, Waters Associates). The solvent system consisted of citrate buffer (0.1 *M*, pH 4.5)-methanol-acetonitrile (2 : 1 : 1, v/v/v), and the flow rate was 1 ml/min. The fluorescence detector had the excitation wavelength set at 245 nm and the emission wavelength at 550 nm. The retention time of DMDR was 12.5 min, and that of DMDR-ol, 8 min. The lowest limit of detection was 0.5 ng DMDR/ml. Standard calibration curves were prepared daily with reference to control samples of each biological fluid. The coefficients of variation among assays were consistently 3.0% for plasma and 3.4% for urine. Control blanks were also checked daily to ensure the absence of interfering materials.

Pharmacokinetic analysis. Nonlinear regression analysis of our results was performed with the aid of the Prophet Program. Best fit was based on the open two compartment pharmacokinetic model.

Results

Response

Of the 13 patients taking part in this trial, the results from one patient could not be evaluated, because of an elevated serum bilirubin concentration at the onset of the clinical study (Table 1). Before DMDR, most patients had received one or two other chemotherapy regimens in addition to pelvic radiotherapy. In this population, no complete or partial response to DMDR was observed. However, the size of liver metastasis was decreased in a patient with ovarian cancer, as evidenced by computed tomography.

Table 1. Hematological toxicities of DMDR

No. of patients	Dose (mg/m^2)	No. of courses	Lowest granulocyte counts ($10^3/\mu l$)		Median platelet counts ($10^3/\mu l$)
			Median	Range	
Intravenous					
5	10	5	1.8	(1.0–1.9	219
6	12.5	7	0.8	(0.1–1.8)	153
Oral					
4	10 daily × 4	4	1.8	(0.5–3.1)	136
7	10 daily × 5	9	1.4	0.004–2.8)	198
1	10 daily × 6	1	0.9		218

Toxicities

Whether administered PO or IV, DMDR caused leukopenia that was dose-limiting (Table 1). The maximum tolerated dose of DMDR that could be given as a single IV administration appeared to be 12.5 mg/m^2 every 3 weeks. At this dosage the median lowest granulocyte count was 800 μl. The median number of days to the nadir was 15 (range: 13–23), and the median time to recovery to 1500 cells/μl or higher was 24 days (range: 21–26). Similarly, when given PO at 10 mg/m^2 daily for 5 days, DMDR elicited a median lowest granulocyte count of 1400/μl. The median number of days to neutropenia was 21 (range: 6–22), and the condition recovered by day 28 (range: 21–34). One patient with extensive lymphoma who received DMDR at 10 mg/m^2 daily for 6 days (Table 2) had late and prolonged granulocytopenia.

With either route of administration, thrombocytopenia was insignificant; no patient required platelet transfusions. Two courses resulted in the patients' platelet counts falling below 50000/μl; one was attributed to concurrent use of mitomycin C for control of hypercalcemia.

Nausea and vomiting were mild and infrequent in all cases. Only one patient developed total alopecia; in the others it could not be evaluated because of prior chemotherapy. Mild diarrhea occurred in one patient. Minimal

Table 2. Other toxicities of DMDR

No. of episodes (% of 26 evaluable courses [a])	
Nausea [b]	4 (15%)
Vomiting [b]	2 (8%)
Alopecia	1 (4%)
Diarrhea	1 (4%)
Extravasation	2 (2%)

[a] Range: 1–5 courses per patient
[b] Mild

extravasation typical of the anthracyclines was seen in two patients (Table 2), but skin grafting was not required. There was no acute cardiovascular toxicity.

Pharmacokinetics

The disappearance of DMDR from the plasma of the nine patients given this agent IV at a dose of 10–12.5 mg/m^2 was biphasic, with a mean initial half-life of 12.8 min and a terminal half-life of 27.0 h. A typical plasma clearance curve for DMDR is shown in Fig. 1; and the pharmacokinetic parameters are summarized in Table 3. The average apparent volume of distribution according to area under the curve was 63.9 l kg^{-1}, and the total clearance was 1.9 l kg^{-1} h^{-1}. In 24 h only 5.1% of the dose was excreted in the urine (Fig. 2). In 19 studies involving the same nine patients plus an additional one the plasma half-life of DMDR administered PO at 10 mg/m^2 was 34.8 h (Fig. 1, Table 3) and the maximum plasma drug concentration was reached at about 1 h. A comparison of the areas under the plasma drug concentration versus time curves following administration by the two routes indicated that the bioavailability to DMDR given PO was approximately 39%. Moreover, on average only 2.3% of the drug administered PO was excreted in the urine in 24 h, in contrast to 5.1% after IV administration. According to the excretion data, the average bioavailability of DMDR was therefore 45%.

From Table 3 it is apparent that escalation of the DMDR dose given IV from 10 to 12.5 mg m^{-2} was accompanied by an almost twofold increase of the plasma t½ and a decrease of similar magnitude in the total clearance of DMDR, while the apparent volumes of distribution and the 24-h cumulative excretion showed little or no change.

A comparison of the PO versus the IV studies in the same patients (Table 3) reveals that with the PO route the plasma half-life of DMDR was 7.7 times higher (about 30%), the apparent volume of distribution more than 6 times greater, and the total clearance nearly 5-fold faster. However, as alluded to above, the 24-h cumulative urinary excretion of DMDR given PO was less than one-half of that with IV administration.

In four of the five patients studied twice during the first PO course, the plasma t½ and the apparent volumes of distribution of DMDR increased from day 1 to day 5; in three of them the total clearance of the drug also became elevated. Moreover, the plasma DMDR concentrations in all these patients on day 5 were generally higher than those on day 1.

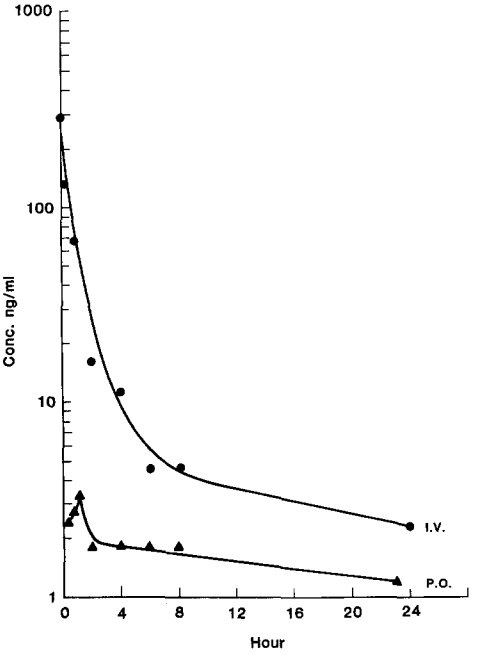

Fig. 1. Plasma disappearance of DMDR after 10 mg/m^2 of DMDR given IV or PO in a patient

One patient with severe liver dysfunction was examined four times during our studies: on day 1 of the first IV course with 12.5 mg m^{-2}, and subsequently on day 1 of the first PO course with 10 mg m^{-2} and again on days 1 and 5 of the second PO course. In this patient the plasma t½ of DMDR during the elimination phase progressively increased from 50.6 through 56.8 and 106.6, finally reaching 111.8 h. The apparent volume of distribution of DMDR correspondingly increased from 78.4 through 980.3, 607.3, to 787 .8 l kg^{-1}, whereas the total clearance changed from 1.1 through 11.9, 4.0, to 4.9 l kg^{-1} h^{-1}. On the other hand, the 24-h cumulative urinary excretion of DMDR remained constant despite the change of the route of administration from IV to PO; however, no urine specimens were available for drug analysis during the second PO course.

Table 3. Mean (± standard error) clinical pharmacokinetic parameters of DMDR

No. of studies	Dose (mg · m^{-2})	Route	Course	Day	t½ (β, h)	Va (l kg^{-1})	Cl l kg^{-1} h^{-1}	Urinary excretion (% in 24 h)
4	10	IV	1	1	18.4 ±2.5	63.5 ± 19.5	2.6 ±0.8	6.5 ±1.8
5	12.5	IV	1	1	33.9 ±8.9	64.3 ± 18.6	1.4 ±0.3	4.0 ±1.2
9		IV			27.0 ±5.5	63.9 ± 12.6	1.9 ±0.4	5.1 ±1.1
9	10	PO	1	1	25.0 ±4.7	243.6 ± 98.1	7.5 ±1.7	2.4 ±1.5
2	10	PO	2	1	69.6	490.1	6.0	ND[a]
6	10	PO	1	5	28.0 ±5.7	611.7 ±214.4	13.7 ±2.5	ND[a, b]
2	10	PO	2	5	64.0	480.4	6.2	ND
19		PO			34.8 ±6.7	410.7 ± 90.5	9.2 ±1.3	2.3 ±1.3[c]

[a] ND, not done
[b] In one of the studies 1.4% of the dose was excreted in the urine in 24 h
[c] Mean of nine studies

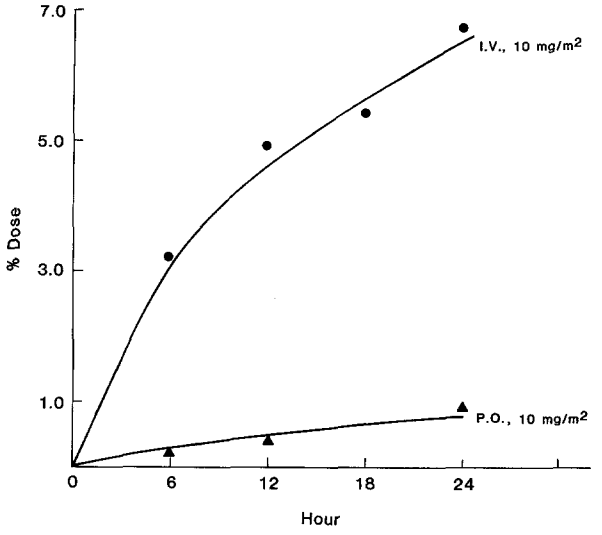

Fig. 2. Cumulative urinary excretion of DMDR after 10 mg/m^2 DMDR by the given IV or PO in a patient

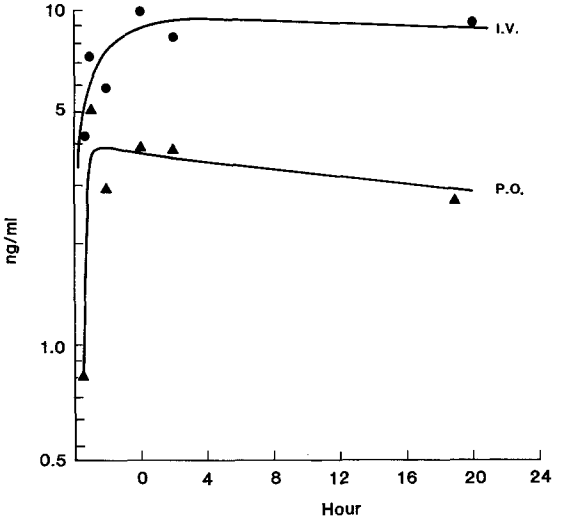

Fig. 3. Plasma disappearance of DMDR-ol after 10 mg/m^2 of DMDR given IV or PO in a patient

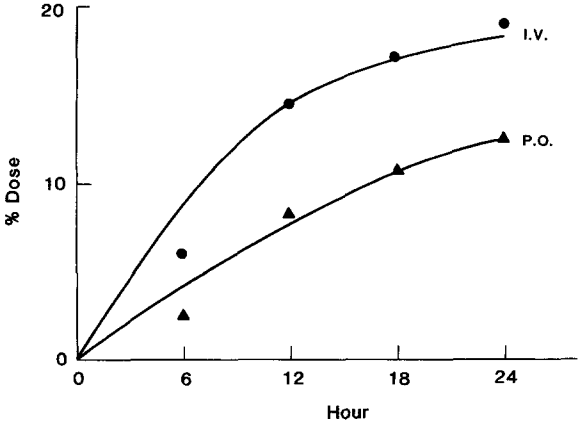

Fig. 4. Cumulative urinary excretion of DMDR-ol after 10 mg/m^2 of DMDR given IV or PO in a patient

Table 4. Plasma half-life and urinary excretion of DMDR-ol after DMDR administration

Patient No.	$t_{1/2}$, h		Urinary excretion (% of DMDR administered in 24 h)	
	IV	PO	IV	PO
1	44.2	25.0	Not done	
2	15.5	26.5	4.8	16.2
3	55.9	54.6	19.0	12.5
4	12.8	36.7	17.0	0.8
5	11.6	18.5	5.3	11.8
6	108.3	7.5	2.7	0.8
7	210.0	94.0	4.0	4.0
Mean ±SE	65.5 ±27.4	37.5 ±10.9	8.8 ±2.9	7.7 ±2.7

Table 5. Correlation between DMDR absorption and lowest granulocyte counts in patients

Patient No.	% Absorption	Lowest AGC × 10^{-3}
1	33.8	1.7
2	97.6	0.4
3	11.8	Normal
4	51.8	0.9
6	23.0	Normal
7	23.2	1.2
9	61.0	1.4
10[a]	7.2	0.8
Mean ±SE	38.7 ±10.7	0.8

[a] Severe liver dysfunction

With either route the drug was quickly metabolized to DMDR-ol. In seven patients the average plasma half-life of DMDR-ol was 65.5 h when DMDR was given IV, but 37.5 h when given PO (Fig. 3, Table 4). In plasma this metabolite almost always exceeded DMDR in concentration, regardless of the route of DMDR administration. In addition, the plasma concentrations of DMDR-ol were inevitably higher on day 5 than on day 1. Similarly, the cumulative urinary excretion of DMDR-ol also exceeded that of the parent drug. Furthermore, in the same patient the plasma half-life and the 24-h cumulative urinary excretion, of the metabolite varied widely with the route of administration, in a random fashion and with no consistent pattern.

The percentage of DMDR absorption apparently correlated with the lowest granulocyte count. Thus, when the absorption was complete the granulocyte counts were depressed to 400 μl^{-1}, whereas when the absorption was only 12%–23%, the granulocyte counts remained within the normal range (Table 5).

Discussion

Intellectually, it is instructive to compare DMDR with its parent agent, daunorubicin, with regard to its clinical toxicological manifestations and pharmacokinetics, remembering that structurally the only difference between them is the replacement of the methoxyl moiety in daunorubicin with a hydrogen in DMDR. Overall, this structural modifi-

cation does not appear to be critical: the two agents differ very little in their clinical toxicities and pharmacokinetics, and possibly in their apparent anticancer properties also.

By giving a single IV bolus every 3 weeks, we estimated the maximum tolerated DMDR dose to be 12.5 mg/m^2. At this dosage, the dose-limiting toxicity of the agent was leukopenia; thrombocytopenia was rare and insignificant (Table 1). In this regard, the hematological toxicity of daunorubicin is also neutropenia and not thrombocytopenia. However, with daunorubicin nausea, vomiting, anorexia, and fatigue were dose-limiting. In contrast, nausea and vomiting were mild and infrequent with DMDR. When it was given as an IV bolus once every 3 weeks, the maximum tolerated dose of DMDR was only one-sixth that of daunorubicin; clearly it is a more potent agent. At their maximum tolerated dosages no cardiac toxicity was elicited by either agent, but these studies were short. These observations are in agreement with those reported previously [3, 8].

Pharmacokinetically, DMDR closely resembles its parent agent as far as our results are concerned. The elimination half-life of DMDR after IV administration in nine patients was 27 h (Table 3), as against the 20.6 h reported for daunorubicin [12]. Although Daghestani et al [8] reported that DMDR disappeared from the plasma biphasically in only one of their five leukemic patients, but triphasically in the remaining four, they nevertheless noted that the average plasma half-life of DMDR during the final phase was 22.5 h, quite close to our result. In contrast, Pacciarini et al [10] reported that the plasma half-life of DMDR was 9.4 h in three patients after an IV dose of 15 mg m^{-2} and only 2.4 h in five patients after an oral dose of 45 mg m^{-2}. However, compared with our investigation theirs was quite limited in its scope.

Even more strikingly, the total clearance of DMDR in our studies was 1.9 l kg^{-1} h^{-1} or 71.4 l m^{-2} h^{-1}, assuming an average body weight of 65 kg and body surface area of 1.75 m^2 in our patients; in comparison, as estimated from the literature data [12], the total clearance of daunorubicin is 75 l m^{-2} h^{-1}.

Urinary excretion is a minor route of elimination of both DMDR and daunorubicin. In 24 h only about 5% of the IV-administered DMDR was excreted unchanged in the urine, with an additional 8% as DMDR-ol. Again, these properties of DMDR were remarkably similar to those described recently for daunorubicin [11]. Although earlier it was reported that the 5-day cumulative urinary excretion of daunorubicin and metabolites was 23% of the dose [2], the apparent discrepancies could be attributed to the facts that in our work the urine was collected for 24 h only and that no attempts were made to quantify other metabolites than DMDR-ol.

By comparison, of the principal DMDR metabolite with an authentic specimen, we established that it was the secondary alcohol, DMDR-ol, resulting from the in vivo reduction of the 9-hydroxyacetyl group in DMDR. This is entirely analogous to the biotransformation of daunorubicin to daunorubicinol. The average plasma half-life of DMDR-ol derived from IV DMDR was 65.5 h, more than twice that of DMDR itself. DMDR-ol not only persisted longer, but also consistently attained higher concentrations in the plasma than did the parent drug after the distribution phase. These findings were completely in accord with those communicated from other laboratories [3, 8, 10]. DMDR-ol is as active as DMDR against cultured cell lines and transplantable leukemias. However, daunorubicinol is not as active as daunorubicin. Therefore, consensus is that its prolonged retention must contribute significantly to the enhanced potency of DMDR.

After administration PO, DMDR was not completely bioavailable. Further, the plasma half-life of the DMDR-ol generated from the oral DMDR was only slightly longer than that of the latter (Tables 3 and 4). Thus, oral administration of DMDR offers little therapeutic advantage.

By and large, in any one patient all DMDR pharmacokinetic parameters were greater after oral administration, especially the apparent volume of distribution and the total clearance; the plasma half-life of the drug was only slightly lengthened, while the cumulative urinary excretion actually decreased (Table 3). A possible explanation is that when given by the oral route DMDR was subject to the first-pass effects, such as enhanced metabolic clearance, increased hepatobiliary excretion, and intensified deposition in the liver and other tissues.

Dose escalation of IV DMDR from 10 to 12.5 mg m^{-2} may have impeded the extrarenal clearance mechanisms of the drug, except tissue deposition, because the plasma half-life of DMDR was more than doubled and the total clearance was simultaneously halved, yet the volume of distribution showed no change. The urinary excretion was reduced slightly but this was probably not significant.

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