Chemical Stability of Artesunate Injection and Proposal for its Administration by Intravenous Infusion

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Abstract

Artesunate, the only artemisinin analogue that can be given intravenously, produces rapid parasite and fever clearance in falciparum malaria. A significant therapeutic problem is a high, late recrudescence rate, probably due to short half-lives of both artesunate and its active metabolite dihydroartemisinin relative to conventional dosing intervals. One method of extending the duration of action of artesunate could be to administer the drug by infusion rather than bolus injection, provided that it is chemically stable at ambient temperature.

Artesunate was found to be stable in 0·9% w/v sodium chloride at 9°C, 23°C and 36·5°C for 130, 10·6 and 1·6 h, respectively. Interpolating from an Arrhenius plot, artesunate should be stable for approximately 4 h at 30°C, a temperature representative of ambient conditions in tropical countries. Exposure to light did not affect the degradation rate. Single compartment pharmacokinetic modelling was used to evaluate potential differences in artesunate and dihydroartemisinin plasma concentrations following administration of artesunate by intravenous bolus or infusion. A bolus injection of artesunate at a dose of 4 mg kg^{-1} gives a peak concentration of 5·3 mg L^{-1}, falling to 0·005 mg L^{-1} at 5 h. The same dose infused over 4 h results in a peak concentration of 0·92 mg L^{-1}, falling to 0·005 mg L^{-1} at 8 h. Simultaneous modelling of dihydroartemisinin showed that while its peak plasma concentration was reduced by 27% and the peak delayed by 2·5 h following artesunate administration by infusion, substantially higher concentrations were maintained compared with those predicted after bolus artesunate.

These data indicate that artesunate can be administered as a high-dose intravenous infusion, thus avoiding high plasma concentrations. This strategy also has the potential to prolong the duration of antimalarial effect and reduce toxicity, and consequently improve clinical outcome in seriously ill patients.

Artemisinin and its semi-synthetic derivatives are emerging as front-line antimalarial agents (Hien & White 1993; White 1992, 1994a). However, because of difficulties in measuring the concentration of these drugs in blood, plasma and other biological fluids, their pharmacokinetics and pharmacodynamics have not been fully elucidated. Empirical dosage regimens have been developed from clinical studies that have assessed efficacy using simple measures such as parasite clearance time (White 1994b). In the case of artesunic acid (artesunate), the only currently available artemisinin derivative that can be given intravenously, regimens include a 2 mg kg^{-1} loading dose given by slow intravenous injection followed by a 1 mg kg^{-1} maintenance dose every 12 h (Hien 1994; Looareesuwan 1994), and a 1 mg kg^{-1} dose at 0, 4, 24 and 48 h (Li et al 1994).

Available pharmacokinetic data suggest that artesunate has a short half-life of 2–45 min (Yang et al 1985; Benakis et al 1993; Hien & White 1993; White 1994b). Its principal metabolite, dihydroartemisinin, is also a potent antimalarial with a reported half-life between twice (Benakis et al 1993) and twenty times (Na Bangchang et al 1994) that of the parent compound. However, this preliminary pharmacokinetic information has not yet been used to formulate rational dosage regimens. In addition, some of the current

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regimens are associated with a high recrudescence rate (Looareesuwan et al 1992; Hien & White 1993; Li et al 1994; White 1994b). As a result, initial treatment with artesunate is usually followed by administration of a quinoline drug (e.g. mefloquine) when the patient is able to take oral medication (Hien 1994; Li et al 1994; Looareesuwan 1994).

High recrudescence rates after artesunate could be due to a failure to maintain adequate drug and metabolite concentrations in the blood for a sufficient period of time (Ter Kuile et al 1993); that is, there is an inadequate area under the plasma concentration–time curve (AUC). Pharmacokinetic considerations suggest that administration of higher doses of artesunate by slow intravenous infusion may be a suitable method of achieving large AUCs of both parent drug and dihydroartemisinin, while reducing the peak artesunate plasma concentration achieved after conventional doses given by bolus injection. Whilst administration by intravenous infusion may be a desirable option, the drug must be chemically stable for the required period of time in a suitable vehicle and at ambient temperatures which, in tropical countries, may be relatively high. The aim of this study was to establish the chemical stability of artesunate injection in a small-volume infusion formulation and, using this stability data and available pharmacokinetic information, to formulate more appropriate dosage regimens than those currently in use.

Materials and Methods

Stability studies

Materials. Reference standards for artesunic acid and dihydroartemisinin were gifts from Colonel Brian Schuster (Walter Reed Army Institute of Research, Washington, DC, USA). Potassium dihydrogen orthophosphate (KH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4) and sodium bicarbonate were of analytical grade. Acetonitrile was of high-performance liquid chromatography (HPLC) grade and de-ionized water was used to prepare all buffers. Artesunate for injection was obtained from the Guilin No. 2 Pharmaceutical Factory (Guangxi, China). Sodium chloride 0·9% (saline) (w/v) injection ampoules were obtained from Astra Pharmaceuticals (North Ryde, NSW, Australia) and sodium chloride 0·9% (saline) injection 50-mL infusion bags (minibags) were obtained from Baxter Healthcare (Old Toongabbie, NSW, Australia).

Chromatography. Analysis by HPLC was carried out using a 590 Programmable Solvent Delivery pump with a Lambda-Max 481 LC spectrophotometer (Waters Associates, Milford, MA, USA). The detector was linked to a 3380A Integrator (Hewlett Packard, Avondale, PA, USA). Injections were made via a K65B Automated Sample Injector (ETP Kortec, Ermington, NSW, Australia). An Ultrasphere Octyl 5 μm, 4·6 mm × 25 cm HPLC column (Beckman Instruments, Fullerton, CA, USA) was used for separation of analytes. The mobile phase was 50% (v/v) acetonitrile in phosphate buffer 0·067 M pH 5·6 (KH_2PO_4 8·61 g L^{-1} and Na_2HPO_4 0·46 g L^{-1}), pumped at 1·5 mL min^{-1}. Detection was at 210 nm, using an attenuation of 0.05 absorbance units full scale. Retention times for artesunate, α-dihydroartemisinin and β-dihydroartemisinin were approximately 3·1, 4·6 and 6·4 min, respectively (Fig. 1). Peak-height measurements of artesunate were used to determine the concentration of solution relative to the original concentration. The within-day coefficient of variation for artesunate was 2% at 242 mg L^{-1} (n = 6), 4·3% at 97 mg L^{-1} (n = 6) and 6·1% at 49 mg L^{-1} (n = 4). The between-run coefficient of variation was 2·1% at 242 mg L^{-1} (n = 7), 5·3% at 97 mg L^{-1} (n = 9) and 4·4% at 49 mg L^{-1} (n = 9).

Stability study of artesunate stored in syringes. To determine the stability of artesunate for injection prepared according to the manufacturer's specification, 60 mg drug was dissolved in 1 mL 1% (w/v) sodium bicarbonate solution with sonication, diluted to 10 mL with saline injection, mixed by vortexing and filtered via a Millex OR 0·22-μm disposable sterilizing filter (Millipore, Bedford, MA, USA). Aliquots of 3 mL were transferred to 5-mL polypropylene syringes (Terumo Corporation, Tokyo, Japan) which were capped for the duration of the study. Experiments were conducted at 23 and 36·5°C in triplicate syringes which were protected from light. Samples (200 μL) were drawn at regular intervals over a 53-h period, diluted 1 in 80 and an aliquot transferred to polypropylene Eppendorf tubes which were stored at −70°C until analysed. Immediately before analysis, samples were thawed at room temperature (21°C) for 10 min and sonicated for 10 min. All samples from a single series were analysed in the same HPLC run. Aliquots

(80 μL) were injected onto the column and each sample was assayed in triplicate.

Stability study of artesunate in intravenous infusion bags. Artesunate (60 mg) was prepared for injection as described above. Aliquots of 5 mL were then transferred to 50-mL saline minibags. Experiments were conducted at 9, 23 and 36·5°C in duplicate bags protected from light. Samples (1 mL) were drawn at regular intervals over 56 h. Analysis of samples was as described above, except that aliquots of 20 μL were injected onto the column and each sample was assayed in duplicate.

Data analysis. Peak-height measurements were used to determine the proportion of original concentration for the sample (the peak height at zero time was taken to be 100%). The log of the mean percent of the original concentration was plotted against time and linear regression analysis was used to determine the degradation rate constant (SigmaPlot for Windows, Jandel Scientific, San Rafael, CA, USA). An Arrhenius plot (log of degradation rate constant vs the reciprocal of storage temperature in degrees Kelvin (Enever 1977)) was constructed to determine the stability at temperatures between 23 and 36·5°C by interpolation. Stability was expressed as the time to reach 90% of the original concentration t_{90}.

Pharmacokinetic modelling

Plasma concentration–time curves for artesunate and dihydroartemisinin following administration of artesunate by both intravenous bolus and infusion were simulated using a one-compartment open model for both drug and metabolite, rate constants and volumes of distribution reported in human subjects by Benakis et al (1993), and the iterative, non-linear least-squares curve-fitting programme PCNONLIN (Statistical Consultants Inc., KY, USA). For a bolus injection of artesunate, the plasma concentrations of artesunate (C_{arts}; mg L^{-1}) and dihydroartemisinin (C_{dqhs}; mg L^{-1}) are given by:

$$C_{arts} = (D/V_1) \times (e^{-k_1 t}) \qquad (1)$$

and

$$C_{dqhs} = [((Dk_1)/(V_2(k_2 - k_1))) \times (e^{-k_1 t} - e^{-k_2 t})] \\ \times (MW_{dqhs}/MW_{arts}) \qquad (2)$$

respectively, where D is the dose (mg) of artesunate administered at time t = 0 h, V_1 is the volume of distribution (0·76 L kg^{-1}) of artesunate, k_1 is the elimination rate constant (1·42 h^{-1}) for artesunate (assumed, given the rapid rate of biotransformation to dihydroartemisinin reported by

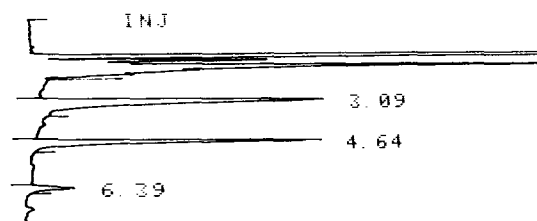Fig. 1. HPLC of artesunate (3·09 min), α-dihydroartemisinin (4·64 min) and β-dihydroartemisinin (6·39 min).

Benakis et al (1993), to be the same as the rate of formation of the metabolite), V_2 is the volume of distribution of dihydroartemisinin ($1.5 \, L \, kg^{-1}$), k_2 is the elimination rate constant for dihydroartemisinin ($0.44 \, h^{-1}$), MW_{dqhs} is the molecular weight of dihydroartemisinin (284) and MW_{arts} is the molecular weight of artesunate (384).

During a constant infusion of artesunate, the plasma concentrations of artesunate and dihydroartemisinin are given by:

$$C_{arts} = (R/k_1 V_1) \times (1 - e^{-k_1 t}) \quad (3)$$

and

$$C_{dqhs} = [R/(k_2 V_2(k_2 - k_1)) \times (k_2(1 - e^{-k_1 t})$$
$$-k_1(1 - e^{-k_2 t}))] \times (MW_{dqhs}/MW_{arts}) \quad (4)$$

and, after the infusion:

$$C_{arts} = C_1 e^{-k_1 t} \quad (5)$$

and

$$C_{dqhs} = [((C_1 V_1 k_1)/(V_2(k_2 - k_1))) \times (e^{-k_1 t} - e^{-k_2 t})$$
$$+ C_2 e^{-k_2 t}] \times (MW_{dqhs}/MW_{arts}) \quad (6)$$

where R is the rate of infusion ($mg \, kg^{-1} \, h^{-1}$) of artesunate, C_1 is the simulated plasma concentration ($mg \, L^{-1}$) of artesunate at the end of the infusion, and C_2 is the simulated plasma concentration ($mg \, L^{-1}$) of dihydroartemisinin at the end of the artesunate infusion.

Results

Stability studies

The preliminary studies in syringes showed t_{90} values of 12 h at 23°C and 1.8 h at 36°C. At the 9-h sampling time a precipitate was observed in the syringes and at the end of the experiment this precipitate was separated and freeze-dried. The melting point of the material was determined, and a portion was dissolved in HPLC mobile phase and chromatographed as outlined above. The precipitate had the same melting point (149-150°C) and chromatographed at the same retention time as the dihydroartemisinin reference standard.

The first-order degradation profile of artesunate in sodium chloride minibags is shown in Fig. 2. The calculated t_{90} values (Table 1) were consistent with the preliminary experiments. No precipitate was observed in the minibag studies; however, a peak in the chromatogram at the same retention time as dihydroartemisinin indicated that this was the major degradation product. A second peak (retention time = 4 min) also appeared as the artesunate degraded. This compound is as yet unidentified, but was not detectable before 4 h of storage at 23°C.

Saline injection was used as the diluent for most of our studies because the relatively low pH of 5% w/v glucose solutions might cause acid-catalysed hydrolysis of artesunate and increase the degradation rate. However, in one series of experiments in glucose 5% w/v minibags, protected from and exposed to light, we found the degradation rates to be equivalent to those from the sodium chloride solutions (data
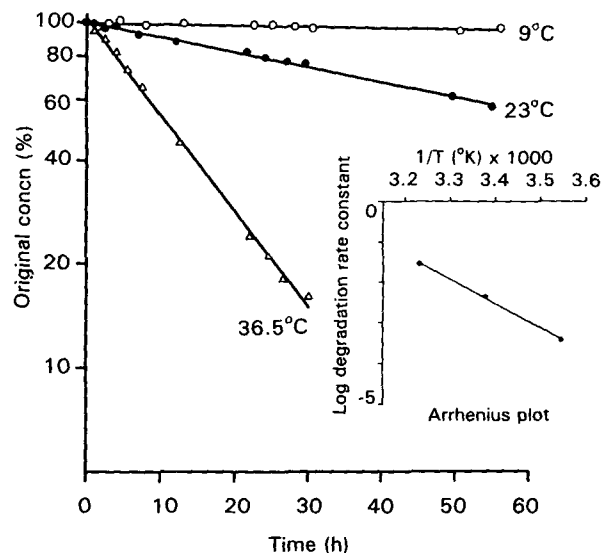

Fig. 2. First-order degradation profile of artesunate (30 mg) in 50 mL saline infusion bags at 9, 23 and 36.5°C. Each data point is the mean of duplicate assays of duplicate bags. The inset figure shows the Arrhenius plot derived from the degradation rate constants at the 3 different temperatures.

not shown). There was no difference between the stability of solutions protected from light and those exposed to standard artificial light in the laboratory.

Pharmacokinetic modelling

The simulated plasma concentration-time curves (Fig. 3) show that a bolus injection of 4 mg kg^{-1} artesunate results in a peak concentration of 5.3 mg L^{-1}, falling to less than 0.005 mg L^{-1} by 5 h. The peak concentration of dihydroartemisinin is 1.1 mg L^{-1} at 1.2 h post-dose, falling to 0.015 mg L^{-1} at 12 h. Administration of the same dose as a 4 h infusion results in a peak concentration of artesunate of approximately 0.92 mg L^{-1}, but the plasma concentration is maintained above 0.005 mg L^{-1} for almost 8 h. The predicted peak concentration of dihydroartemisinin is 0.8 mg L^{-1}, occurs 4.5 h after the start of the infusion, and falls to 0.04 mg L^{-1} at 12 h.

Discussion

Winstanley & Watkins (1992) have suggested that increasing the AUC can improve the antimalarial efficacy of quinine, and Ter Kuile et al (1993) have argued in favour of dosage regimens of artemisinin derivatives that maintain constant

Table 1. Stability data for artesunate (30 mg) in 50 mL saline injection infusion bags at 9, 23 and 36.5°C.

Temperature (°C)	t_{90}* (h)	Degradation rate constant (h^{-1})
9	130	3.6×10^{-4}
23	10.6	4.3×10^{-3}
30†	4	—
36.5	1.6	2.8×10^{-2}

*Time to reach 90% of original concentration. † Interpolated from Arrhenius plot (see inset in Fig. 2).
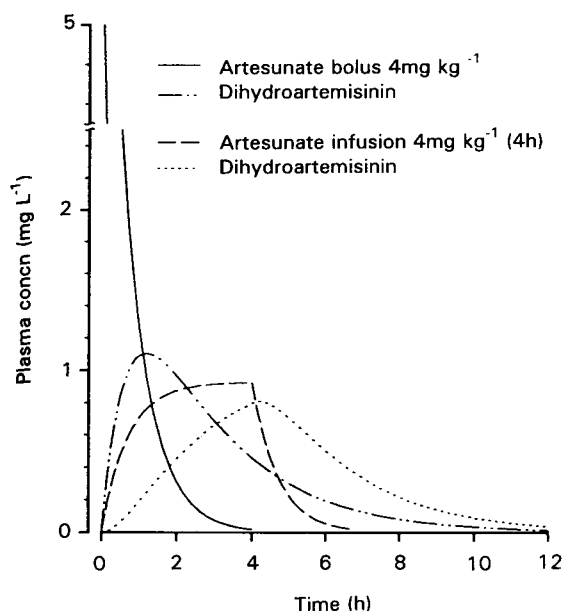
FIG. 3. Simulated plasma concentration–time curves for artesunate and dihydroartemisinin, comparing a $4\,mg\,kg^{-1}$ intravenous bolus injection of artesunate with a $4\,mg\,kg^{-1}$ intravenous infusion administered over $4\,h$. Pharmacokinetic descriptors for the simulation have been taken from the data of Benakis et al (1993) (artesunate elimination rate constant $= 1.42\,h^{-1}$ and volume of distribution $= 0.76\,L\,kg^{-1}$; dihydroartemisinin elimination rate constant $= 0.44\,h^{-1}$ and volume of distribution $= 1.5\,L\,kg^{-1}$).

blood concentrations. In the case of artesunate, artemether and arteether, where the active metabolite dihydroartemisinin also is a potent antimalarial agent, the contribution of the metabolite to the overall antimalarial efficacy ascribed to the parent drug must be considered when dosage regimens are developed.

Assuming that a greater AUC or a prolonged maintenance of constant blood concentrations of antimalarials will lead to a better therapeutic outcome, a high-dose intravenous infusion of artesunate may prove the best option for parenteral therapy. Our simulations show that an intravenous bolus artesunate dose of $4\,mg\,kg^{-1}$ gives a high peak plasma concentration of $5.3\,mg\,L^{-1}$ and an AUC of $3.7\,mg\,h\,L^{-1}$. However, administration of the same dose as a 4-h infusion substantially lowers the peak concentration to $0.92\,mg\,L^{-1}$ while leaving the AUC unchanged. By comparison, administration of artesunate as bolus doses of 1 and $2\,mg\,kg^{-1}$ would result in peak concentrations of 1.3 and $2.6\,mg\,L^{-1}$ and proportionally lower AUCs of 0.93 and $1.85\,mg\,h\,L^{-1}$, respectively. Thus, by administering artesunate as a high-dose infusion, a substantial AUC can be achieved whilst maintaining a relatively low peak concentration.

Although artesunate is effective against *Plasmodium berghei* in a rodent model (IC50 approximately $0.004\,mg\,L^{-1}$) its active metabolite, dihydroartemisinin, is at least four times more potent (Janse et al 1994). Dihydroartemisinin has similar in-vitro potency (EC50 approximately $0.001\,mg\,L^{-1}$) against *Plasmodium falciparum* (Alin et al 1992). Therefore, artesunate may be regarded as a pro-drug of dihydroartemisinin (White 1994b). The half-life of dihydroartemisinin in man has been variously reported as

$0.8\,h$ in volunteers (Yang et al 1985), $1.6\,h$ in patients (Benakis et al 1993), and 10.6 and $12.5\,h$ in healthy volunteers and patients, respectively (Na Bangchang et al 1994). We are unable to advance a logical pharmacokinetic explanation for the substantially longer half-lives reported in the latter study where dihydroartemisinin pharmacokinetic data were estimated following the oral administration of the pro-drug artemether. Even if the formation of dihydroartemisinin from artemether was rate limited, one would anticipate a half-life for dihydroartemisinin which was similar to that for artemether (3–$4\,h$). In the former two studies, dihydroartemisinin pharmacokinetic data were determined after administration of artesunate (the hemisuccinate ester of dihydroartemisinin), which is known to have a shorter half-life than dihydroartemisinin (Yang et al 1985; Benakis et al 1993; White 1994b). It is therefore unlikely that the rate of artesunate metabolism influences the terminal elimination half-life of dihydroartemisinin. Hence we chose to use the patient-derived pharmacokinetic data from Benakis et al (1993) in our simulations which show that a $4\,h$ infusion of artesunate will provide a substantial prolongation of plasma dihydroartemisinin concentrations compared with those following the same dose administered by bolus injection. To our knowledge there are currently no reports suggesting that maintenance of the plasma concentration of artesunate or dihydroartemisinin above specific (minimum) levels is desirable and direct extrapolation from in-vitro data may not be realistic (White 1992; Edwards 1994). However, Na Bangchang et al (1994) have suggested that plasma artemether concentrations should be maintained above $0.05\,mg\,L^{-1}$ for optimal therapeutic effect. Thus, while there are good pharmacokinetic reasons to prefer administration of artesunate by intravenous infusion rather than a bolus injection, the potential clinical benefits cannot be quantified at present.

Possible neurotoxicity associated with artemisinin analogues (Brewer et al 1994a,b; Wesche et al 1994) may provide additional support for the use of intravenous infusions, particularly when high-dose therapy is required. Dose-related neurotoxicity, manifesting initially as gait disturbances and loss of reflex responses and progressing to cardiopulmonary collapse and death, was reported in 5 of 6 beagle dogs that were given $20\,mg\,kg^{-1}\,day^{-1}$ arteether for 8 days (Brewer et al 1994a). Post-mortem examinations revealed neuronal degeneration and necrosis in the pons and medulla regions of the brain. Brewer et al (1994a) reported similar findings in rats administered arteether or artemether at doses of 25 and $50\,mg\,kg^{-1}\,day^{-1}$ and in dogs and rats treated with doses of artesunate of 15 and $30\,mg\,kg^{-1}\,day^{-1}$ for 14 days (Brewer et al 1993). Direct comparison of dose rates between animals and man requires appropriate allometric scaling data (Ings 1990) but to our knowledge these are not yet available for the artemisinin analogues.

In contrast to animal and in-vitro studies, there have been no reports of neurotoxicity in man. One reason for this lack of reported toxicity may be the difficulty in recognizing drug-induced neurological dysfunction in the clinical setting of severe malaria, together with an unknown pre-morbid neurological status (Brewer et al 1994a). However, residual

neurological deficits are uncommon in adult patients recovering from cerebral malaria (Warrell et al 1990) and there are no reports to suggest that this situation has changed since the introduction of artemisinin derivatives in Asian countries. Furthermore, most clinical studies of artesunate have been conducted with conservative doses of 1-2 mg kg^{-1} day^{-1} (Hien 1994; Li et al 1994; Looareesuwan 1994), which would avoid high plasma concentrations that might cause adverse effects. Nevertheless, Looareesuwan et al (1994) recently reported administration of high bolus doses of artesunate (approximately 4 mg kg^{-1}) every 12 h for 3 days with no adverse effects.

Administration of high doses of artesunate by intravenous infusion might be a desirable option if greater therapeutic efficacy or fewer adverse effects can be proven. Therefore, pharmaceutical stability of the drug in an intravenous infusion (minibag) formulation is an important prerequisite. Our study shows that high-dose artesunate infusions remain stable for 10 h in an air-conditioned environment (23°C) and for at least 4 h at temperatures (up to 30°C) that are consistent with ambient conditions in tropical countries. Furthermore, the in-vitro degradation is not affected by exposure to artificial light.

Artesunate is hydrolysed in aqueous solution to its less soluble but potent metabolite, dihydroartemisinin. When artesunate was prepared and stored in polypropylene syringes, precipitation of dihydroartemisinin occurred. However, in a more dilute minibag infusion formulation, dihydroartemisinin remained in solution. Despite the practical advantage of the degradation product being an active antimalarial, the limit of pharmaceutical stability must be regarded as the t_{90} for the parent drug.

Although the stability for artesunate in a minibag is not long by pharmaceutical standards, it is sufficient to allow administration of high doses by infusion, thus prolonging the duration of action and avoiding high plasma concentrations of the drug. The potential benefits of artesunate administered by intravenous infusion require evaluation by clinical studies which include comprehensive pharmacokinetic analysis and an objective assessment of both adverse effects and therapeutic outcomes.

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