Table 1. Water-holding capacity (g^{-1}) of unwoven absorbent dressing. Measurements in triplicate, with lowest, mean and highest shown.

	Dressing		
	C (pure cotton)	F (cotton & viscose)	J (cotton & viscose)
Wire basket with BP drainage method	22·70, 23·97, 24·96	19·63, 20·42, 21·23	20·75, 21·42, 22·12
—average sinking time (s)	4	42	3
Wire basket with more complete drainage	18·52, 19·57, 20·44	16·85, 17·07, 17·43	16·75, 17·30, 17·86
Plastic tubing method	13·19, 13·35, 13·52	12·69, 12·81, 12·89	12·32, 12·42, 12·52
—average sinking time (s)	12	34	9

BP = British Pharmacopoeia 1988.

requirements in plastic tubing go down to 13 and 12 g g^{-1}, respectively. Even a poor dressing, defined as such by excessive sinking time (British Pharmacopoeia Appendix XX L1), can hold a considerable amount of water, and only $\pm 10\%$ of the required weight will be involved in any discrimination. In fact the sinking times both in basket or tubing clearly reveal dressing F as inferior. A good dressing sinks in the basket in less than half the British Pharmacopoeia requirement of "not more than ten seconds". In the tubing, not more than 25 s can be expected. F failed our picric acid evaluation procedure (Betts et al 1988), as well as these sinking requirements. However, it holds about the same amount of water as J, under different tests here.

Although dressings C and J normally comply with Pharmacopoeial requirements, on a wet, humid day they both failed the water-holding basket test. With 5 g dressing involved, variation due to atmospheric change is detectable. This was not observed with the plastic tubing test, which involves less than a gram of dressing.

It is suggested that the water-holding capacity of official unwoven dressings should be evaluated by both a plastic tubing method, and a basket method modified to allow more complete drainage than at present. Values obtained need to be related to various dressings classified as good or bad. The actual significance of water-holding needs consideration.

The optimum plastic tube length and weight of dressing tested were recommended by Renea McDonagh and Elizabeth Rust in work with picric acid evaluation. Using their method Elizabeth Rust failed dressing F.

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Letter to the Editor

Interaction of arteether with the red blood cell in-vitro and its possible importance in the interpretation of plasma concentrations in-vivo

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Blood or plasma level monitoring of drugs is routinely used, often without proper consideration as to its meaning, to assess compliance with, or in the design of, therapeutic dosage regimens. Recently, this apparently more rational approach has been directed at the treatment of malaria (Panisko & Keystone 1990). Among the more promising new antimalarials is qinghaosu or artemisinin, the antimalarial principle isolated from the wormwood *Artemisia annua* L., and its derivatives arteether and artemether, which are respectively ethyl and methyl ethers of dihydroartemisinin, a reduction product of artemisinin with greater antimalarial activity than qinghaosu itself (Klayman 1985). Artemether has been widely studied in the Peoples Republic of China, and in 1987 was registered in that country as an antimalarial (World Health Organization 1990). Arteether has been selected for development by the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (Brossi et al 1988). Aside from the Chinese literature, there is little information on the clinical pharmacology of artemether and the human pharmacokinetics of arteether are unknown. The availability of novel analytical methodology (Idowu et al 1989; Melendez et al 1991) has prompted research in this area by western scientists but data available thus far are restricted to observations in plasma, whereas the concentrations within blood or the erythrocyte may be equally important. While attempting to adapt the analytical method of Idowu et al (1989) for use in the determination of arteether in whole blood, it became apparent that there were significant losses of this analyte when whole blood to which arteether had been added was stored at either room temperature (21°C) or 4°C or particularly after storage at −20°C, despite attempts to minimize adsorption to glassware. We hypothesized that these observations might be a result of drug decomposition or sequestration of arteether with the blood, particularly the

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Table 1. Recovery of radioactivity following incubation of [^{14}C-ethyl]arteether in plasma, erythrocyte ghosts and haemoglobin for 24 h at 37°C.

Sample (n = 5)	^{14}C added (μCi) (mean ± s.d.)	^{14}C measured (μCi) (mean ± s.d.)	Recovery (%)
Haemoglobin (0·12 g mL^{-1})	$7{\cdot}8 \times 10^{-3}$	$7{\cdot}2 \pm 1{\cdot}3 \times 10^{-3}$	92·3
Plasma	$7{\cdot}8 \times 10^{-3}$	$7{\cdot}5 \pm 0{\cdot}7 \times 10^{-3}$	96·1
Water	$7{\cdot}8 \times 10^{-3}$	$7{\cdot}5 \pm 0{\cdot}7 \times 10^{-3}$	96·1
Erythrocyte ghosts	$7{\cdot}8 \times 10^{-3}$	$5{\cdot}9 \pm 1{\cdot}3 \times 10^{-3}$	75·6
Solubilized erythrocyte ghosts	$1{\cdot}31 \times 10^{-2}$	$7{\cdot}2 \pm 1{\cdot}6 \times 10^{-3}$	55·0
Non-sonicated erythrocyte ghosts	$1{\cdot}15 \times 10^{-2}$	$0{\cdot}85 \pm 0{\cdot}15 \times 10^{-2}$	73·9
Sonicated erythrocyte ghosts	$1{\cdot}15 \times 10^{-2}$	$1{\cdot}11 \pm 0{\cdot}40 \times 10^{-2}$	96·5

erythrocytes, or binding to a component of the red cell membrane. The experiments reported here were designed to address some of these issues and establish their possible relevance to the routine measurement of these compounds in biological fluids in-vivo.

Initially, incubations were performed by adding arteether (Sapec, Lugano, Switzerland) as a solution in methanol to whole human blood (1·0 mL) contained in a series of glass culture tubes to achieve a final concentration of 1·0 mg L^{-1}. Glassware was treated with dichlorodimethylsilane (5% v/v in toluene) to minimize adsorption to the glass. The tubes were each placed in a freezer at −20°C and samples taken immediately and at 0·5, 1, 2, 4, 8 and 24 h subsequently for analysis by HPLC (Idowu et al 1989). Concentrations of arteether in whole blood (initially 1·0 μg L^{-1}) declined steadily at −20°C, such that at 24 h concentrations (n = 5; mean ± s.d.) measured by HPLC (Idowu et al 1989) were 502 ± 106 μg L^{-1}. Using radiolabelled [^{14}C-ethyl]arteether (sp. act. 7·79 mCi mmol^{-1}; radiochemical purity 99%; 0·02 μCi; Walter Reed Army Institute of Research, Washington DC, USA) the recovery of radioactivity was assessed following incubation with red cell ghosts prepared according to Steck & Kant (1974), for 24 h at 37°C with and without mechanical disruption, using an ultrasonic processor (Heat Systems, Farningdale, New York, USA) and measurement of radioactivity by liquid scintillation spectrometry. A similar experiment was performed in the presence of a tissue solubiliser (NCS; Amersham International, UK), measuring recovery of radioactivity.

The results, shown in Table 1, demonstrate that recovery of radioactivity from aqueous solution and plasma is essentially quantitative but drug related material (~25%) remains bound to erythrocyte ghosts. Sonication, but not solubilization, of the erythrocyte membranes produces quantitative recovery of radioactivity indicating this percentage of ^{14}C to be bound tightly to the membrane. More dramatically, however, analysis by HPLC of material present in incubations with ghosts showed a tenfold reduction in recoverable arteether (1·00 ± 0·08; n = 5 to 0·07 ± 0·03 mg L^{-1}; n = 10) whereas recovery of the drug from incubations with haemoglobin was quantitative (0·91 ± 0·03 n = 5 vs 0·91 ± 0·06 mg L^{-1}; n = 10). Finally, to establish the role of the peroxide linkage, a common feature of the molecular structures of qinghaosu and its derivatives, and assessment of the recovery of arteether after incubation with erythrocyte ghosts at 37°C was made in the presence of *t*-butyl hydroperoxide (4×10^{-3} M) added immediately before, or 24 h following arteether (1·0 mg L^{-1}). Arteether concentrations were measured by HPLC (Idowu et al 1989). Recovery of arteether was quantitative (0·91 ± 0·10 mg L^{-1}) following prior incubation with peroxide, but the drug could not be detected if peroxide was added 24 h after arteether, suggesting that a covalent linkage between some component of the erythrocyte membrane and the peroxide bridge is central to the interaction between the membrane and the drug molecule.

We conclude that the nature of the interaction between arteether and the components of the blood, while not entirely clear from these studies, appears to involve the red cell membrane and the peroxide moiety of the sesquiterpene lactone. The consequences of such an interaction, aside from the difficulty of measuring whole blood concentrations of arteether in-vivo, are that interpretation of plasma concentration vs time profiles now becomes difficult and relating such concentrations to the pharmacological response when a proportion of the drug remains strongly and variably bound in this way is highly problematic, particularly if it is this bound fraction that reflects the antimalarial effect of the drug. Further, application of standard pharmacokinetic principles, notably the association of absorption and disposition processes with rate constants derived from measurements made in plasma are inappropriate. Experiments are in progress to establish the biochemical basis of this unique observation and its pharmacodynamic implications, notably its relation to possible drug related toxicity and, importantly, the antimalarial action of this group of drugs which currently is unknown. Meanwhile, we would caution against the potentially dangerous exercise of relating directly the measured plasma concentration of arteether or artemether to an observed pharmacologically-mediated event.

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