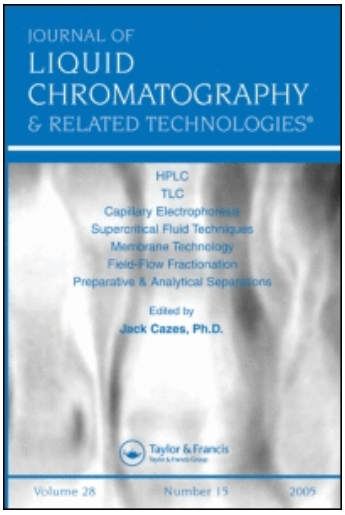

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HPLC/EC ASSAY OF THE DISTRIBUTION OF THE ANTIMALARIAL ARTEETHER INTO FAT AND MUSCLE TISSUE FOLLOWING INTRAVENOUS ADMINISTRATION

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ABSTRACT

An HPLC/electro-chemical method with an internal standard was developed for the analysis of arteether in fat and muscle tissue giving good recovery and accuracy with moderate precision. Following an intravenous administration of arteether (11.6 mg/Kg in a micro-emulsion), it was found that after 15 minutes the arteether concentration was 18.4±13.1 μg/g (15.8% of administered dose) in fat and 1.35±0.25 μg/g (5.8% of dose) was found in muscle tissue. The concentration of arteether in fat tissue initially dropped very rapidly ($t_{1/2}$ = 14 min), followed by a much slower rate of elimination ($t_{1/2}$ = 34 hr). Using the same dose, vehicle, and route of administration, arteether had been reported to be cleared from plasma at a higher rate ($t_{1/2}$ = 10 min).

INTRODUCTION

Arteether is a new semi-synthetic antimalarial drug derived from the natural product artemisinin (also known as Qinghaosu) that has been shown to be particularly effective against chloroquine-resistant parasites.[1,2] Artemether (the ethoxy group of arteether is replaced by a methoxy group) is a closely related antimalarial that has recently been introduced into commercial use in China in a dosage form

consisting of a simple solution of artemether in a vegetable oil vehicle intended for intramuscular injection. The plasma pharmacokinetics of artemisinin and artemether have been reported[3,4] and it has been generally found that the disappearance of artemether from plasma following I.M. administration is very slow. Following the intramuscular administration of arteether (vegetable oil vehicle) to dogs, the plasma concentration initially was found to fall fairly rapidly ($t_{1/2\alpha}$ = 0.84 hr), then much more slowly ($t_{1/2\beta}$ = 27.9 hr) as the drug was slowly released from the depot injection.[5,6] In our laboratories, it was found that when arteether was intravenously administered (in a fat micro-emulsion vehicle) to rats, that the concentration of the unchanged drug dropped very rapidly ($t_{1/2}$ = 10.0 ±0.6 min) during the 1.5 hour observation period, and that within this time period the concentration of the several metabolites exceeded the concentration of the parent compound.[7]

Since the tissue distribution of arteether had not previously been reported, one of the primary objectives of the present study was to develop an analytical method that would be suitable for fat and muscle tissue analysis. A second objective of the project was to gain some insight as to whether the very rapid fall ($t_{1/2}$ = 10.0 ±0.6 min) of arteether following the intravenous administration was the primarily the result of the rapid metabolism or whether the rapid fall might have been the result of the partitioning of unchanged arteether (which has an extremely high log P value) into fat depots.

EXPERIMENTAL

Arteether was synthesized from artemisinin using a previously reported procedure.[8] The arteether was given by intravenous administration of an oil/water micro-emulsion that was prepared within 24 hr of the animal dosing. The procedure used for the preparation was similar to a general procedure used for the extemporaneous preparation of oil soluble cancer-chemotherapeutic agents that are given by intravenous administration.[9] Under aseptic conditions, a 100.0 mg/mL solution of arteether in ethanol was slowly added dropwise (10 μL/min) to a vigorously stirred commercial fat emulsion (Liposyn II®, 20%, Abbott Laboratories) to give a final arteether concentration of 6.0 mg/mL. Male Wistar rats were anesthetized with sodium pentobarbital (60 mg/kg), then each animal was administered arteether (11.6 mg/kg in the micro-emulsion) by intravenous bolus injection into the femoral vein.

After sacrifice of the rat at the appropriate time interval, approximately 200 mg fat sample (or muscle tissue) was transferred to a teflon/glass homogenizer along with 20 µl of the internal standard (a 250 µg/ml solution of the propyl analog of arteether). Then, 1.2 ml of ethyl acetate:acetone (1:1) was added to the sample, the sample was homogenized, and then the sample was centrifuged. The supernatant was transferred to a conical vial and evaporated to a small volume under a stream of nitrogen in a water bath (< 50°), then adjusted to a final volume of approximately 250 µl (occasionally 200-500 µl). The extract was then transferred to the deoxygenation chamber of the HPLC injector.

The sample was degassed with argon for 1 min, then the sample was transferred directly into the 20 µl capacity loop of the HPLC injector by means of pressurization with argon gas using a closed system to avoid the introduction of atmospheric oxygen into the HPLC loop injector. The HPLC column consisted of a 4.7 mm x 11 cm a C-18 cartridge type of column (Whatman Particil ODS-3, 5 µ particle size) using a mobile phase of 70% (v/v) methanol in 0.1 M aqueous ammonium acetate at 1.0 ml/min. The HPLC mobile phase was deoxygenated by flowing argon gas (mobile phases continually refluxed in a distillation-condenser at 40° with 30 ml/min argon flow). The detector (LC-3A, Bioanalytical Systems, Inc.) was used in the reductive mode, with the potential of the mercury/gold electrode set to -0.9 volts. The analytical method was calibrated using an internal standard peak ratio method using simple solutions of arteether and the internal standard. The method was then validated using fat and muscle that had been spiked with known quantities of arteether.

RESULTS AND DISCUSSION

During the preliminary studies on the development of an analytical method that would be suitable for the assay of arteether (which is extremely lipophilic) in fat samples, a number of approaches to selectively remove arteether from the endogenous lipids were investigated. One approach that was successful was to use a small normal-phase silica gel column to selectively remove the arteether from the fat sample prior to analysis using the reversed-phase HPLC analytical column. However, it was later found that the concentration of arteether in the fat samples of dosed rats was actually high enough that such an elaborate sample work-up was not needed but might prove to be useful in the future if very low concentrations were

encountered. All of the results reported here utilized the simple homogenization, one-step extraction procedure described in the experimental section.

To validate the assay method, known quantities of arteether were added to fat samples then the standard assay was used (each sample was prepared and extracted in triplicate). To verify the long-term reproducibility of the method, additional samples were prepared, extracted, and assayed (in triplicate) on subsequent days. The results of these validation experiments (Table 1) showed that the extraction gave essentially quantitative recovery (107.6% ±7.4%) for the 15 μg/g samples. The amount found to be present (15.9 ±1.3 μg/g) using the internal standard calibration method was within the experimental error of the amount of arteether that had been added (15.0 μg/g) to the fat sample. When the fat samples were spiked at 60 μg/g the recovery and accuracy was also to be satisfactory. However with regard to the precision of the method at either 15 or 60 μg/g, small standard deviations (±2.6% for 15 μg/g and ±10.7% for 60 μg/g) were found for results obtained on any single day, but somewhat larger standard deviations (±8.7% for 15 μg/g and ±24.1% for 60 μg/g) were obtained for the variation of the results over several days.

The assay method was also evaluated by spiking muscle tissue with varying quantities of arteether, and then applying the standard assay method. The results of these experiments (Table 2) showed that the amount found to be present in the samples was equal to the amount that had been added to the sample within the standard deviation of the assay.

Previous studies for arteether using this route of administration and vehicle had revealed plasma kinetics that indicated that arteether might be extensively taken-up by tissue compartments within the first 15 minutes.[7] For this reason, our initial efforts at examining tissue levels of arteether focused on the 15 minute sampling time (Table 3). The HPLC/EC chromatograms of the 15 min fat samples (Fig.1, panel B) of the animals dosed with arteether were found to contained large amounts of arteether and the HPLC/EC chromatograms of the fat of negative control animals (Fig. 1, panel C) were found to be free of any significant interference. The concentration of arteether in the 15 min fat sample was found to be 18.4 μg/g (Table 3) and only 1.35 μg/g of arteether was found in muscle tissue. If one assumed that fat[10] comprised 10% of the total body weight and that muscle[11] comprised 50% of total body weight, those tissue concentrations corresponded to 15.8% of the initial dose as unchanged arteether in fat tissue and 5.8% of the dose as unchanged

TABLE 1. Determination of the Recovery, Accuracy, and Precision of the Assay for Fat Samples Spiked with Arteether.

Day	Amount added	Amount found[a]	% of added[b]	% Recovery[c]
1	15 μg/g	17.4 ±0.9 μg/g	116.0 ±6.0	106.4 ±25.6
2	15	14.9 ±0.3	99.3 ±2.0	115.5 ±18.3
3	15	15.4 ±0.1	102.7 ±0.6	100.9 ±4.4
average		15.9 ±1.3[d]	106.0 ±8.7[d]	107.6 ±7.4[d]
4	60	42.7 ±4.3	71.2 ±7.2	79.2 ±8.6
5	60	61.4 ±7.6	102.3 ±12.7	77.4 ±13.9
6	60	70.1 ±6.7	116.8 ±11.1	115.0 ±27.1
average		58.1 ±14.0[d]	96.8 ±23.3[d]	90.5 ±21.2[d]

a Three fat samples were separately extracted and analyzed each day. Each sample was spiked with the internal standard and the assay was calibrated using a simple solution of arteether and the internal standard.
b The amount of arteether found as a percentage of the amount that had been added to each of the three samples.
c The absolute recovery of arteether based on the size of the arteether peak alone without reference to the internal standard.
d Standard deviation of the variation between days.

TABLE 2. Determination of the Accuracy and Precision of the Assay for Muscle Tissue Samples Spiked with Arteether.

Sample	Amount added	Amount found	Percent found
1	1.84 μg	1.63 μg	88.6%
2	1.87	1.68	89.8
3	9.81	9.45	96.3
4	10.48	12.17	116.1
			97.8 ±12.7

arteether in muscle tissue. Using the same dose, route of administration, and vehicle; it had been previously reported[7] that at 15 min the plasma concentration of arteether was only 3.03 μg/ml (approx. 2% of dose) of unchanged arteether along with significant concentrations of 12 metabolites. Thus it would appear that even as early as 15 min following intravenous administration, a very large portion of the dose had undergone metabolic transformation, but of the unchanged drug that remains in the body at that time, the vast majority of the unchanged drug resided in fat tissue.

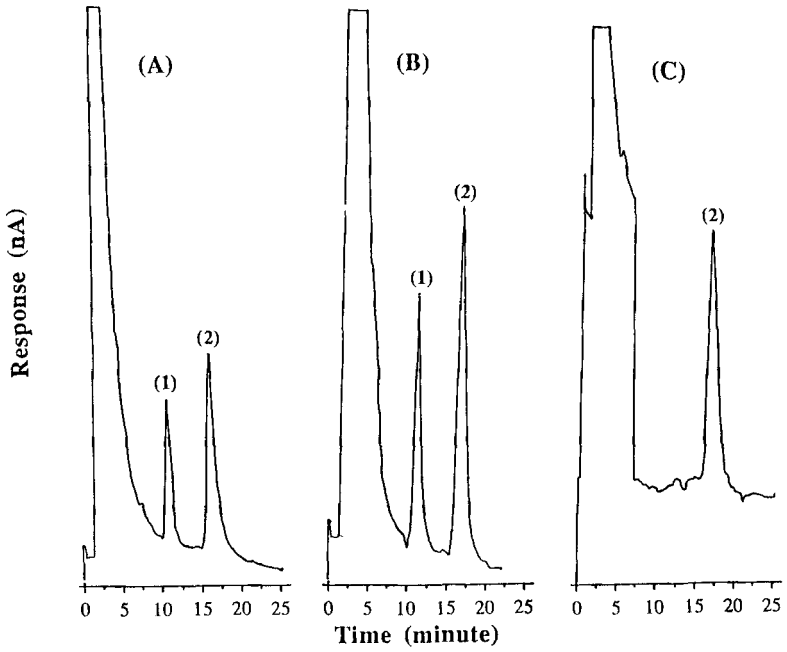

FIGURE 1: HPLC analysis of fat samples- A: fat sample spiked with arteether (1) and the internal standard (2), B: fat sample obtained 15 minutes after dosing found to contain 13 µg arteether per gram of fat, C: negative control fat sample containing only the internal standard.

TABLE 3. The distribution of Parent Compound into Fat and Muscle Tissue 15 Minutes after an Intravenous Injection of Arteether (11.6 mg/Kg).

		Arteether in tissue		Percent of Dose [a]	
Wt. of rat	dose	Fat	Muscle	Fat	Muscle
0.53 Kg	5.83 mg	6.8 µg/g	1.34 µg/g	5.8%	5.8%
0.49	5.39	32.7	1.60	28.2	6.9
0.41	4.51	15.9	1.12	13.7	4.8
0.48 Kg	5.24 mg	18.4±13.1	1.35±0.24 µg/g	15.8%	5.8 % avg.

a Conversions of µg/g in tissue to percent of dose values were based on the assumption that fat[10] comprised 10% of total body weight and muscle tissue[11] comprised 50% of total body weight.

TABLE 4. Kinetics of the Distribution of Arteether to Fat Tissue Following Intravenous Administration (11.6 mg/Kg).

0.25 hr	0.50 hr	1.0 hr	3.0 hr	5.0 hr	20.0 hr
13.63	4.76	2.56	2.50	1.98	
12.75	1.78	0.40	0.69	0.42	
1.70	2.82	3.06	1.83	1.83	1.03
2.46	5.48	3.41	3.99	4.00	2.14
avg. 7.63 µg/g	3.70 µg/g	2.36 µg/g	2.26 µg/g	2.06 µg/g	1.58 µg/g

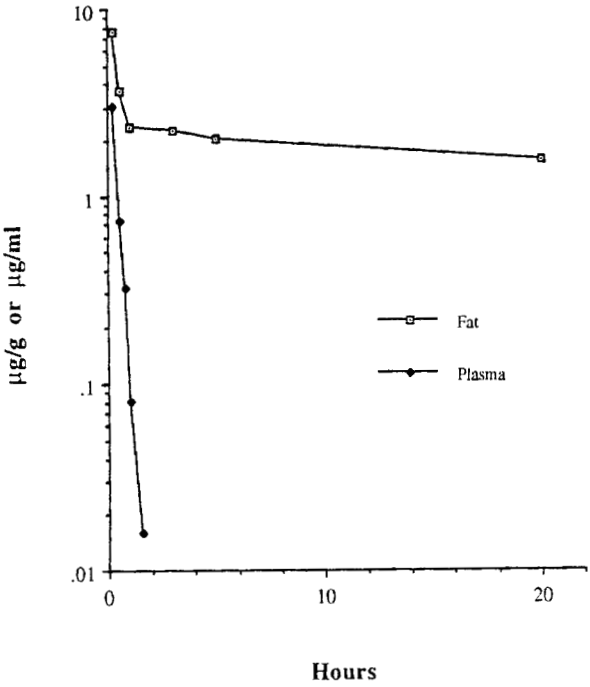

FIGURE 2: Concentration of unchanged arteether in plasma and fat tissue. The plasma analysis method and data had been previously published[7]

In an examination of the kinetics of the distribution of unchanged arteether in fat tissue (Table 4, Fig. 2), It was found that the concentration of arteether in fat dropped very quickly during the first half-hour ($t_{1/2}$ = 14 min, C_0 = 15.6 μg/g) followed by a much slower elimination over 20 hours ($t_{1/2}$ = 34 hr, C_0 = 2.4 μg/g). During the first 1.5 hr, the plasma concentration of unchanged arteether dropped very rapidly ($t_{1/2}$ = 10.0 ±0.6 min, Fig. 2). During the first 1.5 hr, it had been previously reported from these laboratories, that there were 12 different metabolites of arteether detected in plasma, several of which attained concentrations higher than arteether during that same time period.[7]

In summary, it would appear that when arteether was intravenously administered, that there was a very rapid uptake of the drug into fat (15.8% of dose at 15 min.), but that there was also a extremely rapid metabolic transformation during the first 60 minutes. By 60 minutes, the fat concentration had dropped to 2.36 μg/g (2.1% of dose) and to 1.58 μg/g (1.3% of dose) at 20 hr. In other words, a large portion of the unchanged arteether that remained at any one time period was found in fat, but because of rapid metabolic transformation, the concentration of arteether in both plasma and fat falls very rapidly during the first 60 minutes. After 60 minutes, only a very small fraction of the dose remained which was very slowly eliminated from fat over the 1 to 20 hr period.

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