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薄层色谱扫描测定蒿甲醚在大鼠体内的吸收与分布[1]

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Assessment of absorption and distribution of artemether in rats using a thin layer chromatography scanning technique[1]

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ABSTRACT TLC scanning technique was fonnd to have good specificity for studying the absorption and distribution of artemether in rats. Plasma or tissue homogenates 0.2-1.0 ml were placed in glass extraction tubes and water was added to make 1.0 ml. Each sample was extracted 3 times with 4 ml mixed organic solvent (*n*-pentane: dichloromethane = 1:1, vol:vol). The organic layers of 3 extractions were combined and evaporated. The residue was dissolved in 100-300 μl of ethylacetate and spotted on TLC plates. The chromatogram was developed in solvent system consisting of petroleum ether: chloroform: ethylacetate (4:2:1). The color developing agent was 0.25 g *p*-dimethylaminobenzaidehyde dissolved in a mixture of 2.25 ml 85% phosphoric acid, 47.6 ml of acetic acid and 20 ml of water.

Artemether fat emulsion was given intravenously at the dosage of 80 mg/kg. Groups of 5 rats were killed at 15, 30, 60 and 120 min after iv. The results showed that the peak tissue levels were obtained within 15 min, the drug disappeared from the blood very rapidly, and only 0.34 μg/ml was found in the plasma after 120 min. The higest level was found in brain which attained about 13.9 μg/g wet tissue 15 min after iv injection, moderate in heart, lung and skeletal muscle, whereas the levels in liver and kidney were low.

At 15, 30 and 60 min, the plasma drug concentrations were 18.5, 6.9 and 2.3 μg/ml, and the brain drug concentrations were 14.0, 8.8 and 3.4 μg/g wet tissue, respectively.

In vivo and *in vitro* experiments (Tab 2) indicated that artemether entered blood cells less than 15 min after iv injection. The ratio of blood cell/plasma artemether concentration was low, but gradually increased with time. Although the quantities of artemether trasported into the

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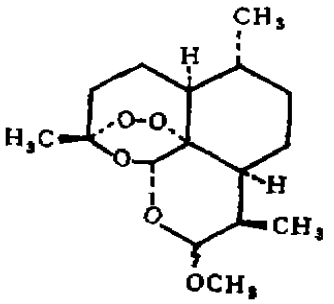
blood cells and tissues were not much, but the transported artemether were retained in blood cell and tissues longer than in plasma. It was also evident that artemether was shown to have a high affinity to blood cells and tissues, and to be able to cross the blood-brain barriers.

KEY WORDS artemether; thin layer chromatography; absorption; tissue distribution; antimalarials

提要 用薄层层析(TLC)扫描法定量专一性地测定生物样品中的蒿甲醚含量。大鼠快速 iv 蒿甲醚 80 mg/kg 能迅速分布在脑、心、肺、肝、肾和肌肉。其中以脑最高，肝、肾最低。蒿甲醚能较快进入红血球。球/浆中浓度比较低。球/浆中浓度比、组织/血浆中浓度比均随时相增加。

关键词 蒿甲醚；薄层色谱法；吸收；组织分布；抗疟药

蒿甲醚是我国研制的一种新型结构抗疟药[1]，它在大鼠、小鼠体内的分布与代谢已有用核素示踪法的研究报道[2]。但该法不能区分蒿甲醚及其代谢产物。而用 TLC 法可定量专一性对蒿甲醚的吸收和分布进行研究，对阐明药物作用机理和指导临床用药提供更好依据。



Artemether

MATERIALS

蒿甲醚由昆明制药厂生产，青蒿素由我所植化室供给，蒿甲醚、青蒿素分别用乙酸乙酯溶解，制成 100 μg/ml 的标准贮液。iv 给药时将蒿甲醚先用少量 N, N-二甲基乙酰胺溶解，然后再慢慢加入到脂肪乳剂中制成 80 mg 蒿甲醚/ml 脂肪乳剂。置 4 ℃保存。

Wistar 大鼠由中科院上海动物中心供给，♂，体重 142±SD 10 g，♀，158±10 g，各 10 只。

显色剂用对二甲氨基苯甲醛 0.25 g 溶于 2.92 ml 85%磷酸(AR)中，再加冰乙酸 47.6 ml 和 20 ml 水，混匀后 4 ℃保存。展开剂为石油醚(沸程 60—90℃)：氯仿：乙酸乙酯 = 4:2:1(vol:vol)。

岛津双波长色谱扫描仪 CS-910。实验条件线性化程序选 SX = 3，样品波长 λ_S 620 nm，参比波长 λ_R 735 nm，用反射法锯齿形扫描。

用本所中间工厂制备的 Silica gel 60 G (E Merck)薄层层析板。硅胶层厚度约 250 μm 均经 110℃活化 1 h 后使用。层析时待蒸气饱和上行展开，展开距离 16 cm，展开一次取出于通风柜内挥干，再上行展开一次。

METHODS AND RESULTS

给药剂量及匀浆制备 大鼠♀♂各半，iv 蒿甲醚 80 mg/kg，分别在药后 15，30，60，120 min 自腹主动脉放血(肝素抗凝)处死，立即取出脏器，置 0 ℃保存。全血离心(1000×g) 5 min，取出上层血浆，血球以一倍生理盐水洗涤，离心(1000×g)10 min 除去上层水相，重复洗 3 次。血球用 1.5 倍去离子水溶血。脏器洗净，吸干剪碎称重，以 1/15 mol/L Na_2HPO_4-KH_2PO_4 缓冲液 (pH 7.4) 制成 20% 的组织匀浆，4 ℃保存。

生物样品中蒿甲醚测定 取螺口试管。于测定管和标准管中分别加入内标物青蒿素 1.5 μg，在标准管中再加入 1.0 μg 标准品蒿甲醚及 1 ml 磷酸缓冲液，而在其他测定管中加入组织匀浆 1.0 ml 或血浆 0.2-1.0 ml 按 1:4 (vol:vol)加入混合提取液(正戊烷：二氯甲烷 1:1)。在 Votex-Genie 提取器上依次振摇 1min，1000×g 离心 10 min，取上层有机相，重复提取 3 次。合并有机相，在 45℃水浴上氮气吹干，各管分别加 0.02 ml 乙酸乙酯溶解后，用 0.5 mm 毛细管点样于薄层板上，经上行展开后，挥干，喷洒显色剂，80℃烘 1 h，在避光闭盒内放置 24 h 后，用双波长薄层扫描仪测定样品及标准品峰面积，按以下公式[3]计算生物样品蒿甲醚浓

度；$X_o = n \cdot M_2 Y / M_1 V$ 其中；X_o 为样品中蒿甲醚浓度(μg/ml 血浆，血球；μg/g 组织)；M_1，M_2 分别为标准管及测定管中蒿甲醚与青蒿素 TLC 扫描峰面积之比；Y 为标准管中所加蒿甲醚 μg；V 为取样容量 ml，n 为稀释倍数。

组织分布 大鼠(♀♂各一半)iv 蒿甲醚剂量后 15，30，60 min，分别处死，测定各组织蒿甲醚(Fig 1)。

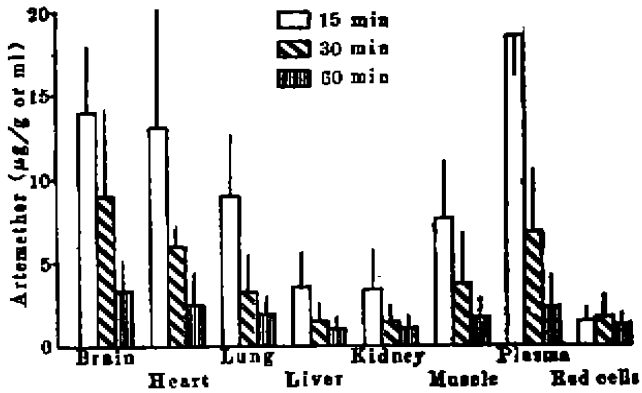


Fig 1. Distribution of artemether in tissue after iv 80 mg/kg in rats (6 rats/each time). $\bar{x} \pm SD$.

大鼠 iv 后 15 min，组织分布以脑最高；心、肺、肌肉次之；肝、肾最低。此时，各组织均已完成分布相水平，而很快进入消除相。从 Fig 1 可见，iv 后 30 及 60 min 各组织中蒿甲醚水平很快下降，血浆中水平下降尤为明显，在 iv 后 120 min 已下降到原水平的 2 %左右。组织浓度/血浆浓度比随时相而增加 (Tab 1)。

Tab 1. Tissue, blood cell/plasma concentration ratio at various times (6 rats/each time point).

Tissue	Ratio with plasma concentration 15	30	60 (min)
Blood cell	0.09	0.24	0.53
Brain	0.78	1.24	1.49
Heart	0.71	0.87	1.10
Lung	0.49	0.47	0.81
Liver	0.19	0.22	0.49
Kidney	0.18	0.21	0.45
Muscle	0.41	0.55	0.77

血球中分布 体内试验表明，在 iv 后 15 min 蒿甲醚已进入血球内达最高水平，而在 iv 后 1 h 内一直维持较高水平。同样在体外试验中，孵育 5 min 后，药物即进入红血球，并在 45 min 内一直维持原水平左右。但发现，体内试验，球/浆中浓度比随时相有增加趋势，而体外试验，此变化似乎不明显(Tab 2)。

Tab 2. Distribution of artemether in rats blood cell and plasma, $n = 6$.

Time (min)		Artemether concentration (μg/ml) 5	15	30	45	60
In vitro						
Blood cell	$\bar{x}$	1.43	1.38	1.48	1.36	—
	SD	0.70	0.50	0.68	0.34	
Plasma	$\bar{x}$	0.93	0.93	0.93	0.88	—
	SD	0.27	0.25	0.18	0.22	
Ratio		1.54.	1.48	1.57	1.54	—
In vivo						
Blood cell	$\bar{x}$	—	1.60	1.63	—	1.21
	SD		0.77	1.60		0.45
Plasma	$\bar{x}$	—	18.47	6.85	—	2.27
	SD		2.61	3.34		1.90
Ratio		—	0.09	0.24	—	0.53

DISCUSSION

本文蒿甲醚大鼠体内分布不同于以往核素示踪所获得组织分布是肝、肾最高，脑分布最低[2]。我们认为：核素示踪法测得是总放射性，未将原形药物和代谢物加以区分，故专一性不及用 TLC 定量好。

从 iv 后蒿甲醚的组织分布水平说明，该药能较快地分布到各组织，iv 后 15 min 脑内已达相当水平，说明蒿甲醚能透过血脑屏障[4]，这与蒿甲醚能治疗脑型疟的事实[5]相一致。组织/血浆中浓度比、血球/血浆中浓度比的时相变化表明(Tab 1)，蒿甲醚对组织具有更大亲和性，血球/血浆中浓度比在体内、体外试验结果不完全一样 (Tab 2)。我们分析：在体外实验，从药物代谢观点看，似可拟作一个"封闭"系统，此时血浆中药物浓度可基本保持恒定；而在体内试验，则完全是一种开放系统，血浆中药物不断通过体循环被带走。而实验表明，蒿甲醚对红血球又具有较大亲和性，从而造成血球/血浆中浓度比随时相而增加的现象。本实验中，蒿甲醚在红血球内绝对量与血浆比较仍然是较少的，这是因为大鼠是在正常生理条件下，要是在疟疾病理状态下，通常像蒿甲醚、氯喹等抗疟药应该在红血球内远比血浆高的多[6,7]。推测

可能在疟疾感染病理条件下，红血球对抗疟药的亲和性会增加，这种特殊富集作用可能是生物体一种自身保护反应措施。

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五种抗疟药对鸡疟原虫孢子增殖期作用的电镜观察[1]

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Electron microscopic observation of the sporogonic stage of *Plasmodium gallinaceum* after five antimalarials

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ABSTRACT Ultrastructural changes in *Plasmodium gallinaceum* oocysts and sporozoites were studies after 5 antimalarials (pyrimethamine, primaquine, artemisinine, 5-p-fluorobenzoxyl-primaquine citrate and nitroquine) were administered to *Aedes albopictus*. Obvious disfigurement, such as abnormal vacuoles of various sizes in the cytoplasma, thickened oocyst capsules and damaged sporozoite pellical membranes were found in many oocysts and sporozoites in the mosquitoes. When the grade of infection of sporozoites in the salivary glands of the 5 different groups of mosquitoes were compared with the control, the rank test ($H_c = 271$) showed a very significant variance ($P<0.01$).

KEY WORDS *Aedes*; *Plasmodium gallinaceum*; electron microscopy; primaquine; pyrimethamine; artemisinine; nitroquine

提要 用伯氨喹、乙胺嘧啶、青蒿素、5-对氟苯氧基伯喹柠檬酸盐及硝喹喂感染鸡疟原虫的阳性蚊。另外，以硝喹喂鸡后，再供蚊血餐。感染后 d 8 及 d 12 分别解剖蚊，电镜观察显示蚊体内部分或大部分卵囊及子孢子的胞质有空泡形成，卵囊被膜变厚，子孢子受损，胞质溶解或胞核浓缩变形，各喂药组蚊涎腺子孢子的感染度与对照组相比 $Hc = 271$, $P<0.01$。

关键词 伊蚊；鸡疟原虫；电子显微镜检查；伯氨

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