

· 药学 ·

椒莪脂质体的制备及其质量标准的建立*

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摘要 目的:将椒莪油制成脂质体,优选制备工艺,建立质量标准。方法:采用薄膜超声法制备椒莪脂质体,通过正交实验优选处方和制备工艺,HPLC、GC 建立其质量标准。结果:最佳处方为卵磷脂:胆固醇 7:1,卵磷脂:油 3.5:1 ;HPLC 法测定脂质体中莪术油的含量,建立标准曲线,回归方程为 $Y=14958X+16795$, $r=0.9996$ 椒目仁油的测定方法同前文报道。得到的脂质体形态均一,包封率在 75%左右。结论:建立的制备工艺简单,便于操作 检测方法的精密性、回收率均符合要求。

关键词 莪术油 椒目仁油 脂质体 制备工艺

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Preparation of Jiao-E Liposome and Establishment of its Quality Standards*

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ABSTRACT Objective: Select liposome as the optimized formulations, choose the best preparation technology and establish its quality standards. Methods: Ultrasonic film was used to prepare the liposome, optimize the prescription and technology by orthogonal experiment, using HPLC, GC to establish its quality standards. Results: The best prescription is lecithin:cholesterol 7:1, lecithin:oil 3.5:1. The determination method of Curcuma oil is established by HPLC. And the determination method of Zanthoxylum seeds oil is established by GC as described before. The shape of obtained liposome is uniform, the encapsulation efficiency is about 75%. Conclusion: These methods established in this experiments are simple, stable, and reasonable.

Key words: Curcuma oil; Zanthoxylum seeds oil; Liposome; Preparation technology

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莪术油是从姜科植物莪术的干燥根茎中提取的挥发油,主要成分为多种倍半萜类,包括莪术醇、莪术酮、莪术双酮、莪术烯、呋喃二烯等多种成分^[1-2]。研究证明 莪术油具有抗肿瘤、抗病毒细菌、抗凝血和保肝等多种作用。其中,抗肿瘤是莪术油的重要作用。但莪术油毒性较大,有过敏、溶血等不良反应,且存在生物利用度差等缺点,在一定程度上限制了临床应用。

椒目^[3]为芸香科植物青椒或花椒的干燥成熟种子,是一种内外含油的油脂加工资源。其种仁含油 15%左右,不饱和脂肪酸约占总量的 90%(以 α - 亚麻酸为主)。 α - 亚麻酸为人体必需脂肪酸,具有益智、降血脂、降血压、抑制血小板凝聚、抗血栓形成、延缓衰老、抗过敏及抑制癌症的发生和转移等多种作用。

脂质体是将药物包封于类脂质双分子层中的一种超微型球状载体制剂,具有以下优点^[4] (1)保护被包裹的药物,有效控制药物释放 (2) 脂质体本身对人体无毒性和免疫抑制作用,进入体内后主要被网状内皮系统吞噬,能激活体内的自身免疫功能,并使药物主要在肝、脾、肺和骨髓等组织器官中累积,减少药物的治疗剂量和降低药物毒性,从而提高药物的治疗指数,减少毒副作用 (3)通过改变脂质体的大小和电荷,可以控制药

物在组织内的分布与在血液中的清除率等。目前脂质体作为一种抗癌、抗病毒药物的优良载体,成功用于多种抗癌药物。

前期对荷瘤小鼠的实验证实,莪术油与椒目仁油合用在有效发挥其抗癌作用的同时,毒副反应也明显降低(文章整理中)。由于该复方组成为油性成分,为了更好地发挥其疗效,便于给药,本实验设计将制该复方成脂质体剂型,优选制备工艺,建立质量标准。

1 材料

1.1 仪器

电子分析天平,德国 Sartorius 公司 旋转蒸发仪 RE-52A,上海亚荣生化仪器厂 ;DL-720A 超声波清洗机,上海之信仪器有限公司 高效液相色谱仪,美国 Waters 公司 ;TJ-25 低温高速离心机,美国 Beckman 公司 ;气相色谱仪,Agilent 4890D 透射电镜,JEM-2000EX。

1.2 试剂

莪术油,江西吉水县药用油厂,批号 20100328 椒目仁油,我所自提 羧牛儿酮对照品,中国药品生物制品检定所,批号 100185-200405 大豆卵磷脂,国药集团化学试剂有限公司,批

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号 20091218 胆固醇,天津市博迪化工有限公司,批号 201001
26 乙醚、甲醇等试剂均为分析纯。

2 方法与结果

2.1 脂质体制备及处方工艺筛选^[5]

2.1.1 溶剂选择 常用溶解脂质的有机溶剂为无水乙醇、乙醚、氯仿等,预实验结果显示乙醚、氯仿,溶解情况和成膜效果较

好,由于乙醚易挥发且毒性低,因此选用乙醚作为溶剂。

2.1.2 正交实验优选辣蓼脂质体的处方与制备工艺 选择对工艺影响较大的磷脂比、药脂比和超声时间为考察因素,根据预实验结果各选择三个水平,按 L₉(3⁴)正交表设计实验。以脂质体的包封率为考察指标,因素与水平见表 1,实验方案及结果见表 2。

表 1 辣蓼脂质体制备工艺的因素水平表

Table 1 The factor-level table of preparation of Jiao-E liposome

Factors	Levelslecithin:cholesterol (g/g)	Lecithin:oil (ml/g)	Volum of ether (ml)	Ultrasonic time (min)
	A	B	C	D
1	6:1	3:1	40	30
2	7:1	3.5:1	50	45
3	8:1	4:1	60	60

表 2 辣蓼脂质体的制备方案及结果

Table 2 The programs and results of Jiao-E liposome

No.	A	B	C	D	Encapsulation efficiency (%)
1	1	1	1	1	69.1
2	1	2	2	2	67.4
3	1	3	3	3	54.5
4	2	1	2	3	56.7
5	2	2	3	1	70.4
6	2	3	1	2	77.6
7	3	1	3	2	30.6
8	3	2	1	3	50.3
9	3	3	2	1	48.4
K1	180	146.4	187	175.6	
K2	304.7	188.1	162.5	185.6	
K3	129.3	170.5	155.5	161.5	
R	75.4	41.7	31.6	24.1	

由表 2 看出,最佳制备条件为:A2B2C1D2,即磷脂与胆固醇的比例为 7:1 药量与脂质的比例为 1:3.5,超声时间 45min,乙醚用量 40ml。

2.2 辣蓼脂质体质量标准的建立

2.2.1 辣蓼油含量测定方法的建立

色谱条件

色谱柱 X18 硅胶柱(4.6×250 mm,5 μm);流动相:乙腈-水,梯度洗脱(见表 3);检测波长 216nm;流速:1ml/min;进样量 5 μl;理论塔板数应不低于 5000。

表 3 流动相梯度洗脱

Table 3 The gradient of mobile phase

Time (min)	Acetonitrile:water
0~30	60:40
30~45	95:5

对照品溶液的制备

精密称取辣蓼酮对照品 4.03mg 至 25ml 容量瓶中,用无

水乙醇溶解并稀释至刻度,摇匀,得对照品储备溶液。精密量取空白脂质体 1.0ml 至 25ml 容量瓶中,用无水乙醇稀释至刻度,作为空白对照。

方法学考察

线性关系

精密称取辣蓼酮对照品 4.03mg 至 25ml 容量瓶中,精密量取 1、2、3、4、5、6ml 于 10ml 容量瓶中,用无水乙醇定容至刻度,在 216nm 测定峰面积,在上述色谱条件下,分别进样 5 μl,

表 4 辣蓼酮线性关系测定结果

Table 4 The Linear relationship of germacrone

Concentration (μg/ml)	Peak area
16.12	244638
32.24	505681
48.36	755653
64.48	991810
80.60	1210075
96.72	1465698

以峰面积值(Y)对浓度(X)进行回归,得标准曲线方程。结果见表4,牻牛儿酮标准曲线图,见图1。

牻牛儿酮在16~96 μ g/ml范围内,峰面积与浓度呈良好的线性关系,回归方程为 $Y=14958X+16795$ ($r=0.9996$)。

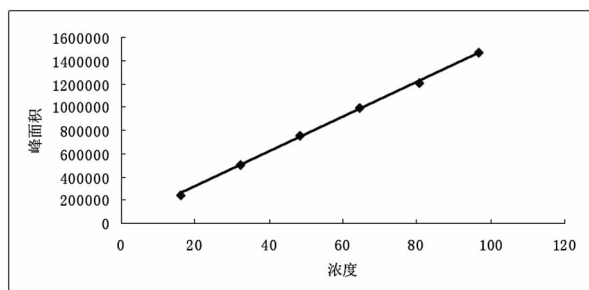


图1 牻牛儿酮的标准曲线

Fig. 1 The standard curve of germacrone

精密度实验

精密称取莪术油2.51mg至10ml容量瓶中,加无水乙醇溶解,定容至刻度,在20-25 $^{\circ}$ C放置1小时,在上述色谱条件下进样5 μ l,连续进样5次。结果分别为390215、386050、391373、390477、399756平均值为391574.2,计算RSD为1.28%。

稳定性实验

表5 莪术油稳定性测定结果

Table 5 The stability of curcuma oil

	Injection time (h)						Average	RSD%
	0	2	4	6	8	12		
Peak area	361861	368729	341036	354673	331142	344638	340471	1.4

表6 莪术油加样回收率实验结果

Table 6 The sample recovery test of curcuma oil

Curcuma oil (mg)	Contented germacrone (mg)	Added germacrone (mg)	Determination of germacrone (mg)	Recovery (%)	Average (%)	RSD (%)
2.86	0.2219	0.1569	0.3744318	97.18		
4.58	0.3554	0.1569	0.5055742	95.68	95.96	0.85
5.04	0.3906	0.1569	0.5400721	95.24		
5.39	0.4178	0.1569	0.5674528	95.35		
6.11	0.4730	0.1569	0.6242356	96.36		

表7 椒蒽脂质体包封率测定结果

Table 7 Encapsulation efficiency of Jiao-E liposome

No.	Encapsulation efficiency (%)	Average (%)
1	76.3	
2	75.0	75.3
3	74.5	

2.4 形态学考察

取椒蒽脂质体适量置铜网上,经磷钨酸负染后,于透射电镜下观察脂质体形态。透射电镜发现所测脂质体以小单室脂质体为主,圆形或椭圆形,外形圆整光滑,形态分布均一,见图2。

3 讨论

精密称取莪术油2.19mg至10ml容量瓶中,加无水乙醇溶解,定容至刻度,在20-25 $^{\circ}$ C放置1小时,分别在0、2、4、6、8、12、24h在上述色谱条件下进样5 μ l,测定峰面积。结果见表5。

加样回收率实验

分别称取莪术油一定量放入10ml容量瓶中,再称取定量牻牛儿酮对照品用无水乙醇溶解,定容至刻度分别进样5 μ l,测定峰面积,带入标准曲线得到实际测定浓度,计算回收率。结果见表6。

经以上考察确定的各分析方法,样品处理简单,专属性好,线性范围广,回收率和精密度均达到分析要求。可认为上述方法是测定脂质体中莪术油含量的较为理想的方法。

2.2.2 椒目仁油的含量测定 具体方法见前文报道^[6]。

2.3 包封率检测^[7-8]

取10ml脂质体,以12000rpm离心40min,收取上清液,乙醚萃取,收集醚层,挥干后用2.2的方法检测莪术油含量。按脂质体包封率的计算公式计算包封率,即包封率=(W总-W游)/W总 $\times$ 100%,载药量=(W总-W游)/W共聚物 $\times$ 100%。其中,W总为投入总药物量,W游为未包入脂质体的游离药物量,W共聚物为脂质体的总量。根据优化的处方制备椒蒽脂质体,测定其包封率,结果见表7。

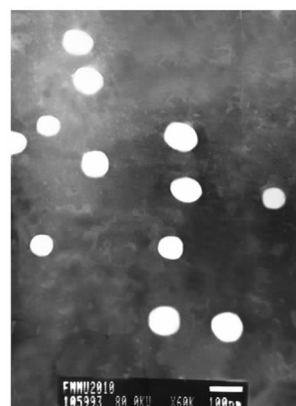


图2 椒蒽脂质体透射电镜照片($\times 12000$)

Figure 2 Picture of transmission electron microscopy ($\times 12000$)

脂质体的制备方法有多种,薄膜分散法具有操作简单,设

备易得的优点,适用于脂溶性较强的药物。本实验用正交试验法对制备工艺进行优化,确定条件为磷脂:胆固醇为 7:1,药量:脂质为 1:3.5,超声时间为 45min,乙醚量为 40ml。电镜下观察制得的脂质体外形圆整光滑,形态较为均一。

本实验采用高效液相色谱法测定椒莪脂质体中莪术油的含量,建立标准曲线,回归方程为 $y=14958x+16795$,线性关系良好($r=0.9996$),回收率 95.96%,RSD 为 0.85%。椒目仁油的检测方法同前文报道。结果显示该方法可用于椒莪脂质体的含量测定,具有简便易操作,稳定可靠的特点。

对于脂质体制剂而言,药物的包封率是指被包裹在脂质体中的药物占脂质体悬液中药物总量的比例,包封率是评价脂质体制剂质量好坏的最重要的指标,是其能否发挥较普通制剂高效、低毒特点关键。本实验采用高速离心法对椒莪脂质体和游离药物进行分离,以莪术油的含量为指标进行检测,测得该脂质体的包封率在 75.3%左右。

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