Separation and Purification of Phosphatidylcholine in Swine Liver and Its Inhibition Effect on Proliferation of Rat Hepatoma Cells

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Abstract: Phosphatidylcholine in crude phospholipids from swine liver was separated and purified by using Al₂O₃ column chromatography with 95% alcohol as eluent. The purity was determined by thin layer chromatography on GF254 silica gel plate and with chloroform-methanol-water (65:25:4 v/v/v) as developing agent. The results showed that PC was completely separated from phosphatidyethanolamine by the elution with 95% alcohol and its purity and yield reached more than 90% and 80% with a elution volume of 225 mL and 87.6% and 87.3% with a elution volume of 425 mL respectively. The effect of the PC with different concentrations on the proliferation of rat hepatoma cell line CBRH-7919 was determined by microculture tetrazolium MTT assays in vitro and was compared with that of human leukemia cell line K562. Result shows that the PC derived from the liver inhibited the growth of CBRH-7919 cells significantly. It suggested that PC derived from animal liver might function as a specific inhibitor for hepatoma cells in a concentration dependent manner.

Key words: column chromatography; phosphatidylcholine; swine liver; biological activity; hepatoma cells
1.3.1.4 10 μL 100 μL GF254 4 2 180 60 70 10 min

1.4.1.5 PC [14 18] 10 0.25 0.82 100 10 9 1

1.3.2 70%

2.1.1 PC
在上述实验条件下，膜磷脂成分的洗脱顺序依次为

此，利用极性差异就可以实现各种磷脂组分的分离。

相似，所以对它们的分离往往成为实验成败的关键。

脱体积为

但此时其得率很小，仅为

率随着洗脱体积的增加而逐渐上升。如图

性介于两者之间。我们选择以

法来检测柱色谱的分离效果。如图

丝氨酸

膜磷脂中共包含

易于掌握和结果直观等特点，因此，我们选用

法具有设备简单、操作方便、分离条件

中含量较低，

经典的

位含有一个极性基团的磷酸酯，因

各主要成分得到较好的分离，

的纯度达

的得率为

和

的相

，达到了预期的分离目的。

的纯度和得率也分别达到

的浓度为

中的含量为

的色谱条件下，肝细胞膜磷脂

的纯度和得率变化不

时，产物

的纯度为

的结构非常

为固定相，

洗脱液，点样于

进行分离。在

洗柱，以

为洗脱剂的柱色谱法对

样于

醇洗脱过程中，实现了

果为

总洗脱体积为

图

薄层色谱法检测肝脏总磷脂及柱色谱洗脱液中的

。它们都是在

期

和

位含有一个极性基团的磷酸酯，因

为强极性，而

为非极性，

为

上为

种组分（斑点

见

等：肝源性磷脂酰胆碱的分离纯化及其对肝癌细胞抑制作用的评价

图

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Fig. 1  The effect of total elution volume on the purity and yield rate of PC

Conditions: mobile phase: 95% EtOH; flow rate: 2.5 mL/min; column temperature: 20 °C; detection wavelength: 203 nm; sample size: 5.3 mL.

1. purity; 2. yield rate.

TLC

PE

225 mL

90.6% PE

83.9% PE

225 ~ 425 mL

PC

80% PC

225 mL

90% PC

80% PC

425 mL

PC

87.6% PC

87.3% PC

2.2 TLC

PC

PE

TLC

PC

PE

TLC

PC

PE

TLC

PC

PE

Fig. 2 TLC determination of total phospholipids from swine liver and PC· PE components in eluates

TLC conditions: GF254 silica gel plate developed by the mixture of chloroform:methanol and H2O (65:25:4 v/v/v) at temperature of 20 °C and visualized under I2 vapor. A: B/C: a total phospholipids of the liver; the livers were subjected to Folch partition and the lower layer was applied to the TLC plate. A-b: standard marker of phosphatidylcholine (PC). A-c: standard marker of phosphatidylethanolamine (PE). B-b: condensed sample of 0 ~ 425 mL eluate with 95% alcohol as eluent. B-c: condensed sample of 0 ~ 225 mL eluate with 95% alcohol as eluent. C-b: condensed sample of 425 ~ 750 mL eluate with 95% alcohol as eluent. C-c: condensed sample of eluate with chloroform as eluent.
异性，这可能与肝脏中特殊的胞的生长具有明显的抑制作用，且存在时间性，我们观察了不同浓度的柱色谱法制备的肝源性合成路径及合成细胞做处理后，不同剂量的分子种类有关。说明肝源性后，不同剂量的分子种类有关。这个结果为今后开

结论

异性和广泛的生物功能为磷脂类抗癌与保健药物的研发提供了一定的实验依据和理论支持，同时也为

Table 3

<table>
<thead>
<tr>
<th>3 MTT</th>
<th>12 h</th>
<th>24 h</th>
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</thead>
<tbody>
<tr>
<td>PC</td>
<td>12 h</td>
<td>b</td>
</tr>
<tr>
<td>PC</td>
<td>24 h</td>
<td>b</td>
</tr>
</tbody>
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Fig. 3 Average inhibition rate with treatment of the chromatographic PC after a) 12 h and b) 24 h by MTT test in CBRH-7919 and K562 cells

1. Li Tao & Wang Tianzhi. Journal of Chinese Medicinal Materi-
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