BACKGROUND

Our previous results suggest that cardiolipin, a specific phospholipid of mitochondria, could be implicated in the hypermetabolism associated to cancer cachexia. Although several methods exist to quantify phospholipids and especially cardiolipin, none are at the same time sufficiently sensitive, precise, rapid and low-cost.

VALIDATION OF HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY FOR THE QUANTIFICATION OF CARDIOLIPIN AND OTHER PHOSPHOLIPIDS IN MITOCHONDRIA

AIM OF THE STUDY

The aim of the study was to validate the High Performance Thin Layer Chromatography (HPTLC)-densitometry technique, according to the International Conference on Harmonization guidelines, to quantify phospholipids: sphingomyelin (SM), phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidylethanolamine (PE) and cardiolipin (CL). Furthermore, this method was applied to quantify 1) the enrichments of PE and CL in mitochondria via liposomes and 2) CL content in liver mitochondria from control rats and rats suffering of cancer cachexia. Validation of the method was performed by statistical analysis using one-way ANOVA (Prism 4 software).

METHOD VALIDATION

Samples were deposited on HPTLC plates using a Camag Linomat V sample applicator. Authentic lipids were deposited as mixtures of phosphatidylglycerol (PG), phosphatidylethanolamine (PE), sphingomyelin (SM), phosphatidylcholine (PC), phosphatidylglycerol (PG), cardiolipin (CL) and neutral lipids (NL, a mixture of triglycerides, cholesterol, cholesteryl esters and non-esterified fatty acids). After chromatographic separation, the densitometric image of phospholipid bands was obtained using a Reprostar Camag TLC scanner III. The amount of each phospholipid class was determined with the WinCats 1.4 software. The validation parameters evaluated were: linearity, accuracy, limit of detection (LOD), limit of quantification (LOQ), inter-day and intra-day assay precision, repeatability of measurement, and repeatability for sample application. Validation of the method was performed by statistical analysis using one-way ANOVA (Prism 4 software).

RESULTS

Validation results

<table>
<thead>
<tr>
<th>Classes</th>
<th>SM</th>
<th>PC</th>
<th>PG</th>
<th>PE</th>
<th>CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cochran test</td>
<td>0.8350</td>
<td>0.8947</td>
<td>0.6413</td>
<td>0.7543</td>
<td>0.8232</td>
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<td>Fischer test</td>
<td>3.4905</td>
<td>3.2389</td>
<td>3.8853</td>
<td>3.2389</td>
<td>4.0662</td>
</tr>
</tbody>
</table>

The Fischer test was used to test the homogeneity of the data. Moreover, the variation coefficient of repeatability (CVR) and reproducibility (CVR) were calculated. The linearity range was 0.4 to 8 µg. All statistical tests were significant to validate the method for all phospholipid classes.

APPLICATIONS

Quantification of CL by HPTLC-densitometry and comparison to probe NAO

Figure 5: HPTLC-densitometry was compared to the NAO (10'-nonyl acridine orange) technique to quantify cardiolipin in liver mitochondria from control and cancer rats. Our results showed that data were very similar as change between cancer and control was the same whatever the used technique (1.55 vs 1.53 for HPTLC-densitometry and NAO, respectively, by calculating their ratio cancer/control).

CONCLUSIONS

The HPTLC-densitometry method is a new, selective, precise, quantitative and low-cost method for the determination of cardiolipin and other phospholipids. Quantification is linear up to 8 µg. It will allow the determination of cardiolipin level, as well as other phospholipid classes in mitochondria in diverse pathologies where a default of phospholipids metabolism is present. For instance, our results have shown that CL content in liver mitochondria is increased by cancer cachexia.