Determination of 5-Fluorouracil concentration in single pancreatic cancer cell based on capillary zone electrophoresis

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The pancreatic tumor cells for 5-FU (5-fluorouracil) determine existence or obtainability of granulation [1], 5-FU whether in pancreatic tumor cells to achieve effective drug concentration still unclear. Literature reported the same circle mark method only suitable for outside and outside cells of the interior chemotherapy drug concentration. For inside cells of the interior chemotherapy drug concentration, so far there is no satisfactory method. This study is used to purposefully develop the function of pancreatic tumor cells as the research object, established to establish microcapsules for the research object, established a set of microcapsules for the cells 5-FU concentration method.

1. Materials and Methods

1.1 Chemical Reagents

HEPES, 5-FU, HCCA, and Sigma Co. Ltd. BCA Protein Assay Kit, FACS (Florencyl, CAN) as Shanghai Cancer Institute of cancer tissue culture, bovine blood by Hangzhou Medical Science and Technology Development Co. Ltd. HPCE, Microfluidic Zone Electrophoresis (Microfluidic Zone Electrophoresis) (Agilent Co. Ltd) Germany Agilent Co. Ltd products.

1.2 Experimental Procedures

Each experimental group of the control group of the pancreatic cancer cell Panc-1 was sent by the University of Erfurt. DMEM was cultured with the 2×10⁷ cells after 24 h. In each 5-FU (100 mg·L⁻¹) culture, it was divided into 0.15, 0.30, and 0.60 mg·L⁻¹ of 3 groups for culture. Cell suspension was obtained by 200 μL for each group.

1.3 Determination of 5-FU Concentration

Capillary zone electrophoresis (CZE) was used to measure the 5-FU concentration in the sample. The components of the buffer solution were 25.0 mM sodium dihydrogen phosphate and 4.87 mM sodium hydroxide. The pH was 7.2. The temperature was 30 °C. The cell suspension was processed. The data were analyzed using analysis of variance (ANOVA) method.

1.4 Determination of Cell Viability

Cell viability was assessed using the MTT assay. The absorbance was measured at 570 nm. The results were calculated using the mean percentage of control.

2. Results

The concentration of 5-FU was measured in the control and treated groups. The results showed that the 5-FU concentration in the treated group was significantly lower than in the control group. The cell viability was also significantly decreased in the treated group.

3. Conclusion

The results of this study suggest that 5-FU has a significant inhibitory effect on the growth of pancreatic cancer cells. Further studies are needed to explore the mechanism of action of 5-FU and to develop more effective therapeutic strategies.

References

Tab 1 Data in time point (n=3)

<table>
<thead>
<tr>
<th>Exposure time/min of 5-FU vs IS</th>
<th>Area ratios /μg</th>
<th>5-FU contents μg</th>
<th>Optical densities</th>
<th>Protein contents/μg</th>
<th>Cell numbers ×10^4</th>
<th>5-FU content in cell/μg . cell^-1</th>
<th>5-FU concentrations in cell/mg . L^-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>0.12±0.04</td>
<td>2.79±0.45</td>
<td>1.52±0.02</td>
<td>3778±47.5</td>
<td>8.84±0.11</td>
<td>0.32±0.05</td>
<td>67.0±10.2</td>
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<td>30</td>
<td>0.20±0.02</td>
<td>3.57±0.24</td>
<td>1.55±0.01</td>
<td>3868±19.8</td>
<td>9.05±0.05</td>
<td>0.39±0.03</td>
<td>83.8±5.33</td>
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<td>60</td>
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<td>3.42±0.05</td>
<td>1.34±0.01</td>
<td>3349±12.3</td>
<td>7.83±0.03</td>
<td>0.44±0.01</td>
<td>92.9±1.60</td>
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<td>120</td>
<td>0.26±0.01</td>
<td>4.23±0.11</td>
<td>1.83±0.01</td>
<td>4569±31.3</td>
<td>10.70±0.07</td>
<td>0.397±0.01</td>
<td>84.3±2.33</td>
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<tr>
<td>240</td>
<td>0.13±0.01</td>
<td>2.92±0.07</td>
<td>1.26±0.01</td>
<td>3129±25.4</td>
<td>7.32±0.06</td>
<td>0.400±0.01</td>
<td>85.0±2.37</td>
</tr>
</tbody>
</table>

Fig 1 Electrophorograms of A: blank PANC-1 cell with theophylline (L.S. 0.14 mmol . L^-1); B, blank cell fortified with 5-FU (100 mg . L^-1) and theophylline; C, PANC-1 cells incubated for 15 min in a culture medium containing 5-FU (100 mg . L^-1)

Voltage used 25 kV, current 48.7 μA. Sample injection 2 s. Temperature 30°C

Fig 2 Intracellular concentration of 5-FU in PANC-1 cells vs exposure time to a medium containing 5-FU (100 mg . L^-1)

Each data point represents the mean±SD(n=3) and the solid lines were determined using non-linear regression. CZE analysis conditions were as in Fig 1

0.01). 平均样本蛋白含量范围为 1683.8±4555.6 μg, 所含细胞数为 3.92×10^6～10.66×10^6, 单细胞内 5-FU 含量为 0.2414～0.4428 μg (Tab 1).

PANC-1 细胞平均直径为 (20.79±1.58) μm, 平均体积为 (4704.2±1092.4) μm^3 (0.004704±0.0001092) nl. 单细胞内 5-FU 浓度为 53.99～92.86 mg . L^-1 (Tab 1).

PANC-1 细胞内 5-FU 浓度在 15 min 内上升较快，达 (66.99±10.16) mg . L^-1，为培养基中 5-FU 浓度的 67%, 30 min 为 (83.75±5.333) mg . L^-1。细胞内 5-FU 浓度于 60 min 达高峰 (92.86±1.604) mg . L^-1，为培养基中 5-FU 浓度的 93%，以后处于平台 (Fig 2)。

3 讨论

由于 PANC-1 细胞贴壁生长，收集细胞时用刮下法因部分细胞被破坏而不能准确计数；CZE 测定 5-FU 后，检测样本中细胞蛋白已无法再被检测。因此，本研究设计了平行样本。

PANC-1 细胞经 5-FU 的培养被温育后，细胞内 5-FU 浓度 15 min 内迅速升高 (67.0±10.16) mg . L^-1，30 min 达 (83.75±5.333) mg . L^-1，60 min 时达高峰浓度 (92.86±1.604) mg . L^-1，若扣除细胞核，60 min 时胞浆中 5-FU 浓度近似于培养液中浓度。此后 5-FU 浓度不再上升，药物曲线处于平台 (Fig 2)。这提示 5-FU 可进细胞内，细胞内 5-FU 浓度可达到细胞外水平，5-FU 主要以细胞内外浓度差为动力向细胞内转移，细胞膜对 5-FU 存在饱和现象。

本研究所建立的检测方法适合检测贴壁生长细胞的细胞内药物含量和浓度。细胞内 5-FU 浓度可用 mg . L^-1 表述，有利于细胞内 5-FU 浓度与培养液中浓度比较。该方法简便，样品无须萃取，进样量少，灵敏度高。

参考文献