DIFFERENTIAL UP-TAKE AND FLUORESCENCE OF CURCUMIN, A YELLOW PIGMENT FROM TURMERIC, IN NORMAL VS TUMOR CELLS

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Abstract

Using absorption and fluorescence spectroscopic methods, quantitative cellular uptake of curcumin, was calculated in two types of normal cells: spleen lymphocytes and NIH3T3 and two tumor cell lines: EL4 & MCF7. Both the uptake and fluorescence intensity of curcumin were significantly higher in tumor cells as compared to the normal cells. Using laser confocal microscopy, intracellular localization of curcumin was monitored and the results indicated that curcumin is located both in the cell membrane and in the nucleus.

Introduction

Curcumin is a polyphenolic yellow pigment isolated from the rhizomes of Curcuma longa (turmeric), a medicinal plant widely used in traditional Indian and Chinese medicine [1]. In spite of great advancement on the therapeutic research of curcumin, there are still very few studies reporting the methodologies to quantitatively estimate intracellular curcumin uptake and localization. In the present study, cellular uptake measurements of curcumin in four different cell types, two normal cells, viz., mouse spleen lymphocytes, NIH3T3 (mouse fibroblast cells), and two tumor cell lines, viz., EL4 (T cell lymphoma of murine origin) and MCF7 (breast cancer cells of human origin). Following absorption spectra of the cell lysate and the fluorescence spectra of cellular curcumin, quantitative uptake was calculated. Further, following curcumin fluorescence by confocal microscopy, its intracellular localization was monitored.
Materials and Method

Chemicals and equipment

Curcumin, 4 ’6-diamidino-2-phenylindol (DAPI), cell culture medium (RPMI 1640 & DMEM) were procured from the local agents. Mouse spleen lymphocytes were freshly isolated as given in reference [2] and EL4, MCF7 and NIH3T3 cell lines were obtained from National Centre for Cell Science (NCCS), India. JASCO V-530 spectrophotometer and Hitachi F-4010 fluorimeter were used, to record absorption and fluorescence spectra respectively. Solutions were prepared in nanopure water from a Millipore Milli-Q system.

Cell culture and uptake studies

Spleen, NIH3T3 & EL4 cell lines were cultured in suspension with RPMI medium and MCF7 cell line in DMEM medium. The treatment of cells with curcumin and then quantitative estimation of cellular curcumin for uptake studies was done as described in earlier reference [2].

Confocal microscopy and image analysis

Slides for confocal microscopy were prepared by fixing curcumin-loaded cells and then sealed with cover slip using mounting medium. Fluorescence imaging of cells was performed with an Olympus Fluoview 500 confocal laser-scanning microscope (Olympus, Tokyo, Japan) equipped with a multi-Argon laser for excitation at 458, 488 and 515 nm [3]. The images were acquired either with 20X objective or a 60X water immersion objective using the fluoview software (Olympus, Tokyo, Japan). The curcumin emission was collected using the barrier filter BA505. Fluorescence images of DAPI stained cells were excited using Coherent Mira 900F Titanium: Sapphire infrared laser (Coherent Verdi-V5, Santa Clara, CA, USA) tuned to 720 nm and the fluorescence in the range 400-470 was collected. At least five monochrome images were accumulated from five different microscopic fields of the same slide. The desired region on each fluorescent cell was selected and the mean fluorescence intensity/area for the region was determined and the average values of 15 different regions are presented in Table 1. To quantify the co-localization of fluorescent probes (curcumin and DAPI), images acquired separately for each of the probe were merged using the software.

Results

Uptake measurements

Cellular uptake of curcumin by the three cell lines using

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>λ&lt;sub&gt;ex&lt;/sub&gt; nm (λ&lt;sub&gt;em&lt;/sub&gt; = 420 nm)</th>
<th>Cellular uptake (pmoles/10&lt;sup&gt;6&lt;/sup&gt; cells)</th>
<th>Fl. Intensity/ pmoles of curcumin uptake (λ&lt;sub&gt;ex&lt;/sub&gt; = 420 nm)</th>
<th>Confocal imaging data Fl. Intensity / area of cell (λ&lt;sub&gt;ex&lt;/sub&gt; = 458 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen lymphocytes</td>
<td>504</td>
<td>23.2 ± 4.3</td>
<td>0.023</td>
<td>69 ± 12</td>
</tr>
<tr>
<td>EL4</td>
<td>500</td>
<td>34.5 ± 6.4</td>
<td>0.064</td>
<td>106 ± 23</td>
</tr>
<tr>
<td>NIH3T3</td>
<td>535</td>
<td>22.6 ± 3.5</td>
<td>0.007</td>
<td>220 ± 27</td>
</tr>
<tr>
<td>MCF7</td>
<td>520</td>
<td>44.2 ± 6.5</td>
<td>0.067</td>
<td>208 ± 66</td>
</tr>
</tbody>
</table>

Table 1: Cellular uptake, fluorescence spectral properties and intensity variations of curcumin in different cells
(EL4, NIH3T3 and MCF7) and spleen lymphocytes was calculated at different curcumin treatment concentrations of 10 and 20 nmoles/million/ml. After normalization to one nmoles/ml/million cells of curcumin treatment, cellular uptake was found to vary in the range of 20-40 p moles/million cells for different cells and the actual values are listed in Table 1. It can be seen that the tumor cells (EL4 and MCF7) showed higher uptake as compared to normal cells (NIH3T3 and lymphocytes).

**Fluorescence spectroscopic studies of intracellular curcumin**

Fig. 1 gives the fluorescence spectra of curcumin-loaded cells after excitation at 420 nm, along with the normalised fluorescence spectra given as inset of Fig. 1. The fluorescence spectrum of curcumin in lymphocytes and EL4 cells is sharp and the maximum is at 500 to 505 nm, while in MCF7 and NIH3T3 cells, the fluorescence spectrum is broad with maximum at 520 to 535 nm. The fluorescence intensity, when compared in these two sets of cells, showed significantly higher intensity in the two tumor cell lines. One of the reasons for such increase in fluorescence could be its increased uptake. To verify this, we calculated the fluorescence intensity to the same level of uptake and compared the fluorescence per unit uptake and the normalized results are listed in Table 1. It is clear that the fluorescence is at least 3-8 times more intense in the tumor cells than the normal cells. The shift in fluorescence spectra and intensity variations clearly indicate that curcumin experiences different hydrophobic environments inside these tumor cells and interacts with the cells differently.

**Fluorescence microscopy studies**

The above observed fluorescence changes, prompted us to understand its intracellular localization, by fluorescence imaging after exciting intracellular curcumin at 458 nm. Although, the absorption maximum of curcumin is at ~ 420 nm, at 458 nm, it has considerable absorption (45 to 70% of the maximum absorption in different cells). Figs. 2A and 2B give the confocal micrographs of lymphocytes and EL4 respectively and Figs. 3A and 3B for NIH3T3 and MCF7 cell lines along with their corresponding phase contrast images and a few magnified images. From these images, the average fluorescence intensity per unit area was calculated and the results are listed in Table 1.
The results supported our earlier observation that curcumin exhibits more fluorescence in both EL4 and MCF7 cells. Curcumin, being a lipophilic molecule is expected to be localized in the membrane. However, the images of EL4 and lymphocytes show fluorescence emission from the entire cells. Since in these cells, majority of the cell volume is occupied by the nucleus with very little cytoplasm, the emission could be from both the membrane and the nucleus.

In case of MCF7 and NIH3T3 cells, due to their distinct morphology, fluorescence images clearly indicate selective localization in the cell membrane and the nucleus. To further confirm its nuclear localization, these cells were stained with DAPI, a DNA selective fluorescent probe [4]. Fluorescence images of DAPI stained cells were excited using infrared laser tuned to 720 nm and the fluorescent emissions in the range 400-470 were collected. The cells were then subjected to dual staining for which cells treated with curcumin were thoroughly washed, stained with DAPI, and after fixation the images were recorded. The fluorescence from curcumin was pseudo-colored as green and that from DAPI as red.
provide confirmation, that tumor cells preferentially take up more curcumin. The fluorescence spectrum of curcumin in tumor cells is easy to detect and can be used as a marker to understand its interaction with different cellular proteins. The fluorescence imaging studies show localization in the cells and a surprising observation of its nuclear localization, this provides a chance to explore new avenues of research to understand its interactions with different nuclear factors and target proteins.

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References

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After graduating from the 41st batch of BARC training school, Dr. R. Pandey has been working at Radiation Biology & Health Sciences Division. She has made significant contribution towards the understanding of effects of low dose radiation on the immune system and has recently been awarded Ph. D. in Applied Biology from University of Mumbai for her work on the immunosuppressive activity of a novel tri-pyrrole pigment. Presently she is working on immunomodulation by natural products.

Dr. K. I. Priyadarshini joined Bhabha Atomic Research Centre in 1983. She is currently working on the elucidation of mechanisms of antioxidant action involving natural products and herbal extracts with potential application as radioprotectors, employing electron pulse radiolysis and in vitro biochemical studies. Dr Priyadarshini has co-authored more than 100 papers in peer reviewed international journals on radiation chemistry, photochemistry and radiation biology. She has been elected as the Fellow of the National Academy of Sciences, India and is a recipient of the Homi Bhabha Science & Technology Award.