BIODEGRADABLE NANOPARTICLES LOADED WITH \textit{Bauhinia monandra} LECTIN

J. S. Rodrigues$^1$, R. Gref$^2$, L. C. B. Coelho$^1$, G. Ponchel$^2$ and N. S. Santos-Magalhães$^1$

$^1$Universidade Federal de Pernambuco, Doutorado Ciencias Biologicas, Recife/PE, Brazil
$^2$Université Paris Sud, Faculté de Pharmacie, UMR CNRS 8612, Châtenay Malabry, France
e-mail address of presenting autor: jaque_rodrigues@hotmail.com

Introduction

Lectins are carbohydrate-binding proteins widely used in biochemical, immunochemical, and histochemical studies. They are recognition proteins of ubiquitous distribution in nature, which are involved, in numerous cellular processes through their characteristic structure and interaction mechanisms [1]. These characteristics have attracted the interest of pharmaceutical scientists for oral delivery, due to their good resistance to acidic pH and to the presence of binding sites along the gastrointestinal tract.

The genus \textit{Bauhinia} (Fabaceae) contains a number of ornamental species, which are well distributed in Brazilian cities; they have been used as forage, as human food and in popular medicine for the treatment of diabetes and as a diuretic. As it is the case with lectins, BmoLL showed a primary monosaccharide specificity, however, it did not recognize glycoproteins. [1]

An extensive research work was carried on to develop nanoparticulate systems able to encapsulate water-soluble compounds, such as proteins and vaccines. Protein-loaded poly (lactide-co-glycolide) (PLGA) nanospheres were developed and characterized [2,3]. Stealth poly (lactide)(PLA) - polyethylene glycol (PEG) nanoparticles were proposed as protein carriers for nasal administration [4]. Human serum albumin was encapsulated within PEG-coated nanospheres [5].

However, all the preparation methods, based on a double emulsion, procedure involve the use of organic solvents and sonication steps, which can be deleterious for the biological activity of the entrapped molecules. The aim of this study was to optimize the entrapment of BmoLL lectin in biodegradable nanoparticles and to prove its usefulness as a targeting carrier.

The effects of various parameters involved in the fabrication of biodegradable nanospheres were evaluated with regard to the hemagglutinating activity of the lectin of Bauhinia monandra (BmoLL).

Experimental Methods

- **Hemagglutination assay**
  
The evaluation of the hemagglutinating activities (HA) was performed with glutaraldehyde-treated rabbit erythrocytes [6].

- **Effect of the exposition to ultrasounds and to Ultra-Turrax on the activity of BmoLL lectin**
  
  250 \textmu l of protein solution of lectin BmoLL – 41 \textmu g/ml (citrate phosphate buffer, pH 6,5)- were added to 2 ml of distilled water and sonicated or agitated for 30 to 60 seconds.

- **Effect of organic solvent on BmoLL lectin**
  
  100 \textmu l of an solution of lectin BmoLL, BmoLL – 41 \textmu g/ml (citrate phosphate buffer, pH 6,5)-, 2ml of organic solvent (methylene chloride or acetone), and 4ml de distilled water were mixed together. The organic solvent was evaporated by stirring.

- **Preparation of the nanospheres**

  Nanospheres were prepared by a double emulsion method [3], using different polymers (PLA, PLGA and polycaprolactone-PCL) and sodium cholate (SC) or polyvinyl alcohol (PVA).

- **Nanospheres characterization**

  The size distribution was determined in distilled water at 20°C using a N4 Plus Coulter. The amount of BmoLL lectin entrapped in the nanoparticles was determined by an assay of the supernatant after two ultra centrifugations (25000rpm / 15 minutes and 40,000 rpm/30minutes), using the Lowry - Peterson method [7].
Results and Discussion

No difference was observed in the haemagglutinating activity of the BmoLL lectin after three sonication steps of 30 and 60 sec, or after ultra-turrax. Moreover, mixing aqueous solutions of lectin with organic solvents such as dichloromethane or acetone, followed by solvent evaporation, didn’t affect the haemagglutinating activity, showing the good stability of this lectin under the various experimental conditions used for the manufacture of the nanospheres.

We optimized the parameters involved in the preparation of the nanospheres (ratio organic to aqueous phase, polymer concentration, type of surfactant) in order to obtain monodisperse particles with a diameter lower than 200 nm. The diameter of the nanospheres prepared using SC was three times lower than the one obtained using PVA as a surfactant.

Finally, nanospheres with a main diameter around 150 nm were obtained whatever the nature or the molecular weight of the polymer used (PCL 2000g/mole, PLGA 50/50 21000g/mole, PLGA 75/25 26000g/mole and PLA 42000g/mole) [Table 1].

The highest rate of encapsulation of the lectin BmoLL was obtained by using the copolymer PLGA 50/50 (68.5% ± 5%), whereas the nanospheres prepared with PCL 2000 g/mole presented the lowest encapsulation rate (40%±8%) [Table 1].

Thus, loading of about 1.5μg of protein in the polymers were obtained. Moreover, the hemagglutinating activity of BmoLL lectin was maintained.

Table 1

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Mean Diameter (nm±SD)</th>
<th>Polydispersity Index</th>
<th>Encapsulation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL (2000)</td>
<td>143±47</td>
<td>0.22</td>
<td>40±8</td>
</tr>
<tr>
<td>PLGA50/50</td>
<td>155±49</td>
<td>0.19</td>
<td>69±5</td>
</tr>
<tr>
<td>PLGA75/25</td>
<td>163±47</td>
<td>0.14</td>
<td>58±8</td>
</tr>
<tr>
<td>PLA50(42000)</td>
<td>153±51</td>
<td>0.09</td>
<td>58±5</td>
</tr>
</tbody>
</table>

Conclusions

This study shows that the BmoLL lectin could be efficiently encapsulated into nanospheres of about 150 nm and that the process of preparation of the nanospheres didn’t modify the haemagglutinating activity of the BmoLL lectin.

Studies are underway to determine the release profile of the lectin in various media and to investigate the interaction of these particles with Caco-2 cells in culture.

Acknowledgments

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References