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Cyclodextrin-grafted polysaccharides as supramolecular carrier systems for naproxen☆

Héctor L. Ramírez,[a] Aymara Valdivia,[a] Roberto Cao,[b,*] Juan J. Torres-Labandeira,[c] Alex Fragoso[b] and Reynaldo Villalonga[a,*]

[a]*Enzyme Technology Group, University of Matanzas, Matanzas 44740, Cuba*
[b]*Laboratory of Bioinorganic Chemistry, Havana University, Havana 10400, Cuba*
[c]*Faculty of Pharmacy, University of Santiago de Compostela, Campus Universitario Sur, 15782 Santiago de Compostela, Spain*

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Abstract—Dextran, mannan and carboxymethylcellulose, previously activated by periodate oxidation, were grafted with β-cyclodextrin moieties by reductive alkylation in the presence of sodium borohydride. These polymers were used as supramolecular carriers for naproxen, improving the 'in vivo' anti-inflammatory properties of this drug.
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Cyclodextrins (CDs) have been widely used in pharmaceutical application for increasing solubility and stability to low molecular weight drugs via supramolecular associations.[1–4] Such kind of interactions could be enhanced by adding hydrophilic polymers to the CD-drug formulations.[5–7] In the present work, we prepared new polymeric derivatives by attaching βCD moieties into the macromolecular chains of dextran (DEX, $MW = 7 \times 10^4$), carboxymethylcellulose (CMC, $MW = 3 \times 10^4$) and mannan (MAN, $MW = 2.7 \times 10^5$), and evaluated these modified polysaccharides as carriers for naproxen. Naproxen ((*S*)-(+)-6-methoxy-α-methyl-2-naphthaleneacetic acid, NAP) is a well-known commercial non-steroidal anti-inflammatory drug, but its pharmacological applications are currently limited by its deleterious effects on the gastrointestinal tract, low water solubility and short plasma half-life.[8] The main purpose of this research was to determine the effect of these novel polymeric carriers on the anti-inflammatory activity of NAP in vivo.

For preparing the CD-grafted polymers, mono-6-amino-6-deoxy-βCD was first obtained from the modification of mono-6-*O*-tosyl-βCD with concentrated ammonia, followed by chromatography on CM-Sephadex C-25.[9] The polysaccharides were further oxidized by dissolving 100 mg of polymers in 5 ml H_2O and treated with 400 mg *m*-$NaIO_4$ under continuous stirring at 4 °C in the dark for 2 h. The oxidation reactions were stopped by adding 100 μl of ethylene glycol and stirring for another 1 h, and further dialysed against distilled H_2O.[10] For introducing the βCD derivative into the polysaccharide chains, 20 mg of mono-6-amino-6-deoxy-βCD was added to 5 ml of the activated polymer solutions and then treated with 20 mg $NaBH_4$ for 4 h under continuous stirring (Scheme 1). The modified polymer solutions were further dialysed against distilled H_2O and finally lyophilized. The molecular weight of the polymers was determined by analytical GPC–HPLC on TESEK Hema-bio columns 40, 100, 300 and 1000 (4× 30 cm) calibrated with dextran standards. The degree of substitution was estimated by ^1H NMR spectrometry using a Bruker DRX-500 apparatus by calculating the integration ratio between the signals corresponding to the substituted C-6 of the CD residue (2.5–3.2 ppm) and those of the anomeric protons of the carrier (4.9–5.3 ppm). The stability constants (K_{st}) of the NAP:CD derivatives complexes were determined at 25 °C by fluorescence spectroscopy on a Perkin-Elmer LS 50B apparatus.

For determining the anti-inflammatory activity of all naproxen formulation, the carrageenan-induced paw

Keywords: Cyclodextrin; Supramolecular interactions; Naproxen; Anti-inflammatory; Drug delivery system.
☆ Patent pending on carriers.
* Corresponding authors. Tel.: +53 45 261251; fax: +53 45 253101 (R.V.); tel.: +53 7 8792145; fax: +53 7 8733502 (R.C.); e-mail addresses: cao@fq.uh.cu; reynaldo.villalonga@umcc.cu

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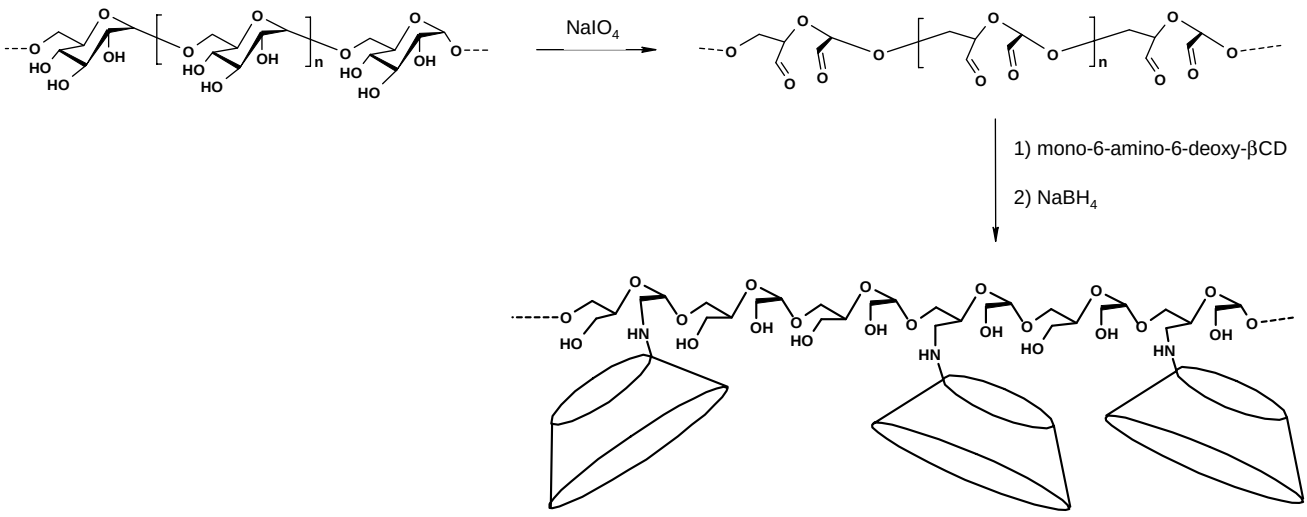

Scheme 1.

oedema test was employed.[11] Male Wistar rats (250–300 g, 10 animals per group, 3 groups per each drug formulation) were injected in the planar aponeurosis of the right hind paw with 100 μl of 1% carrageenan suspended in 0.9% NaCl. Naproxen formulations (4 mg NAP/kg, 20 mg polymer/kg), dissolved in phosphate-buffered saline, pH 7.4, were administered intraperitoneally after 1 h of the carrageenan injection. Physical mixtures of CD and the native polysaccharides were also tested in order to evaluate any effect not mediated by the macromolecular nature of the host carriers. Paw volume was measured by plethysmometry 4 h after sample administration. Oedema was determined by subtracting the volume of the control paw (only saline injected, without NAP or any sugar compound) from that of the treated paw. The inhibition of oedema was calculated in percentage with reference to the negative control group.

The average amount of CD attached to DEX, CMC and MAN was estimated as 135, 41 and 175 mol per mol of polysaccharide, respectively, according to ^{1}H NMR analysis. On the other hand, the molecular weight of these modified carriers was determined as $2.3 \pm 0.4 \times 10^5$ (DEX–CD), $7.7 \pm 0.2 \times 10^4$ (CMC–CD) and $4.7 \pm 0.4 \times 10^5$ (MAN–CD), respectively.

The inclusion complexes of NAP and the CD-grafted polymers were studied by fluorescence spectroscopy at 25 °C. The emission band of NAP (λ_{max} = 360 nm) increased when the amount of CD increased (data not shown). This indicates an increase in local hydrophobicity around the NAP molecules, as expected for the inclusion in the non-polar cavity of CD. This fluorescence behaviour has been previously reported for other related non-steroidal anti-inflammatory drugs in the presence of CDs.[12] Similar behaviour was observed with the CD-grafted polymers, demonstrating the formation of host–guest supramolecular aggregates between these molecules (Fig. 1).

The stability constants, K_{st}, for the complexes between NAP and DEX–CD, CMC–CD and MAN–CD were

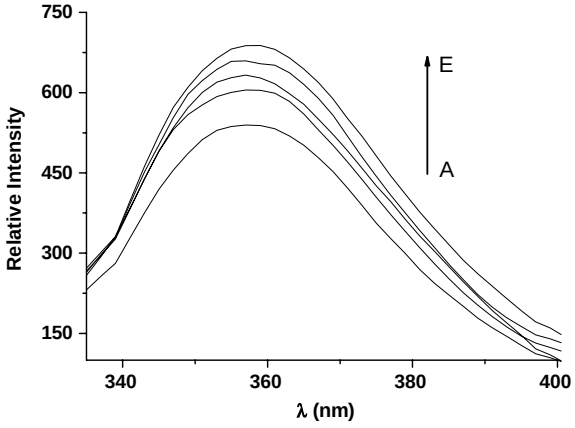

Figure 1. Fluorescence emission spectra of NAP at 25 °C in the presence of different concentrations of DEX–CD: (A) 0 μg/ml, (B) 1 μg/ml, (C) 2 μg/ml, (D) 3 μg/ml and (E) 5 μg/ml.

estimated as 2580, 1580 and 4050 M^{-1}, respectively. It is noticeable that CD forms more stable inclusion complexes with NAP after covalent attachment to neutral polysaccharides (K_{st} (NAP:CD) = 1830 M^{-1}). On the contrary, a lower value of K_{st} was observed for the CMC–CD derivatives. This fact could be explained by the electrostatic repulsion between the anionic drug and the negatively charged polysaccharides, mediated by Donnan effect, reducing then the amount of NAP in the microenvironment of the polymeric carrier.

As is illustrated in Figure 2, the anti-inflammatory activity of NAP was not significantly affected by the addition of CMC–CD polymer. On the contrary, the pharmacological activity of this drug was improved in the presence of the CD-grafted neutral polysaccharides. This increased anti-inflammatory activity was noticeably higher with MAN–CD polymer, resulting in a 2.3-fold reduction of oedema when comparing with the drug without carrier.

The improvement of the anti-inflammatory activity of NAP in the presence of the polymeric carriers is directly

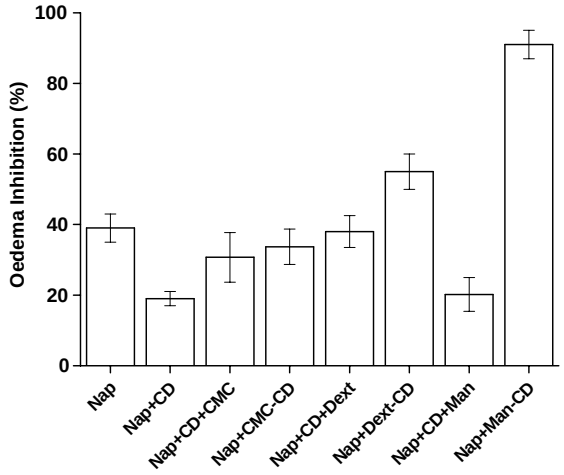

Figure 2. Anti-inflammatory activity of NAP formulations in carrageenan-induced paw oedema test. Doses: NAP = 4 mg/kg, polymers = 20 mg/kg.

mediated by the occurrence of supramolecular associations between the drug and the CD moieties attached to the polysaccharides. As can be seen in Figure 3, a linear relationship between the stability constant of the inclusion complexes of the drug and its pharmacological effect was observed. On the other hand, the anti-inflammatory activity of NAP also increases when the amount of βCD attached to the polymeric chains increases (Fig. 4).

It should be also noted that the solubility of all CD-polymer derivatives synthesized was higher than that of the corresponding to native CD. This fact could also contribute to the higher pharmacological activity of the NAP-carrier formulations, by increasing the solubility of the drug. In addition, the macromolecular nature of the carriers could allow to improve the pharmacokinetics of NAP by reducing its body clearance due to the increased hydrodynamic radius of the supramolecular adducts prepared thus reducing the glomerular filtration of the drug in the kidney.

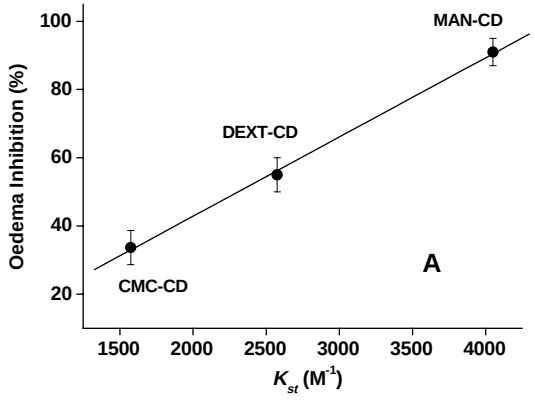

Figure 3. Influence of the stability constant of the NAP:CD-polymer complexes on the in vivo anti-inflammatory activity.

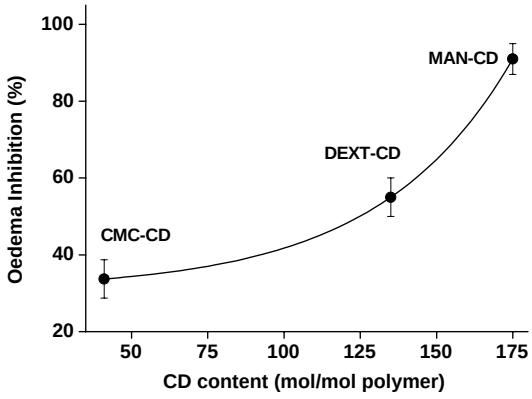

Figure 4. Influence of the CD content in the polymeric carriers on the in vivo anti-inflammatory activity of NAP formulations.

In conclusion, we have described the preparation of CD-modified DEX, CMC and MAN polymers and their use as carriers for NAP. The anti-inflammatory activity of NAP was noticeably improved in the presence of DEX–CD and MAN–CD. Experiments are in progress to generalize these drug delivery systems.

Acknowledgments

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