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Synthesis and pharmacological evaluation of a new targeted drug carrier system: β-Cyclodextrin coupled to oxytocin

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Abstract—β-Cyclodextrin (β-CD) was monofunctionalized into its carboxylic derivative and then conjugated to the *N*-side of oxytocin (OT), a nonapeptide involved in human behavior and myometrium contraction. On isolated rat myometrium, this conjugate (β-CD-OT) partly preserves the contracting activity of OT (EC_{50} = 0.40 μM vs 1.7 nM). Moreover, the contraction induced frequency is also lowered by β-CD-OT. This novel hydrophilic targeted carrier could form a host–guest complex with prostaglandins and their derivatives used as labor inducers or with anticancer drugs used in cervix and endometrial cancer. This strategy can improve the solubility, the stability, and/or the biological activity of these drugs as well as reducing their side-effects.

Cyclodextrins (CDs) are water soluble cyclic oligosaccharides composed, for the most common species, of six (α-), seven (β-), or eight (γ-) α (1 → 4)-linked D-glucopyranose units. These units are arranged in a torus-shaped structure with hydrophilic outside and a hydrophobic cavity. Due to these structural features, CDs are able to form host–guest inclusion complexes with hydrophobic molecules possessing the appropriate size and shape. Initially, this property was used in the food and cosmetic industry where CDs were used as stabilizer for flavoring agents and labile fragrances, and to reduce unpleasant odor and taste.[1] In the same time CDs were used in pharmaceutical formulations generally to improve drug properties such as solubility, stability, and absorption.[2] As CDs are chiral entities, they are also used for asymmetric synthesis[3] and chiral separation in chemistry.[4] More recently, peptides were grafted on functionalized CDs to target a receptor or an enzyme involved in pathologies...[5] These conjugates can host a guest-drug which is then specifically driven to the target.

In the present report, we describe the functionalization of β-CD and its coupling to oxytocin (OT). The CNS release of OT, a very abundant neuropeptide, modulates social behavior, including maternal care and aggression, pair bonding, sexual behavior, social memory and support, and human trust, and downregulates stress responses, including anxiety.[6] The nonapeptide OT is also involved in various reproductive functions as uterotonic activity and milk-ejection during labor and lactation, respectively. All these activities are mediated through a seven transmembrane domains receptor belonging to the G protein-coupled receptors family.[7] Actually, OT is only approved as labor or abortive inducer.

Selective and mono-functionalization of β-CD is a major challenge due to the presence of seven identical 6-primary and 14 2,3-secondary hydroxyl functions of the glucose units. The synthetic pathway is depicted in Scheme 1. Under nitrogen, the 1-tosyl-1*H*-imidazole was prepared at room temperature by adding 1-tosyl chloride to a suspension of imidazole in CH_2Cl_2 (yield 88%).[8] Then, sodium hydroxide (1.1 N) and 1-tosylimidazole were added to an aqueous solution of β-CD **1**. After stirring for 1 h at room temperature, hydrochloric acid, 0.1 N, was added. The formed precipitate was collected and washed with water and acetone to afford the mono-6-deoxy-6-(4-tosyl)-β-cyclodextrin **2** (yield 33%).[8,9]

The monotosylated β-CD **2** was then converted to the mono-6-deoxy-6-formyl-β-cyclodextrin **3** through a Swern oxidation.[10] This formylation was performed un-

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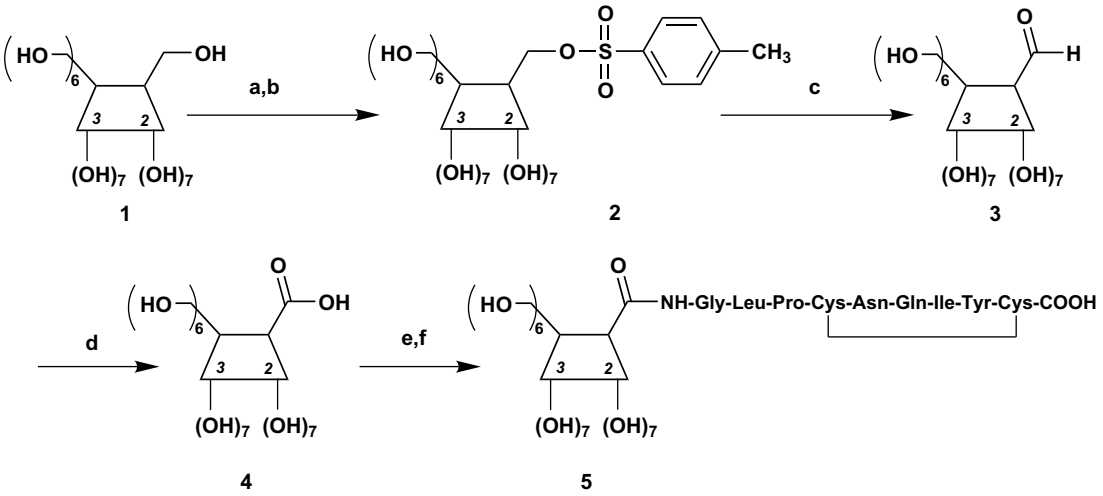

Scheme 1. Reagents and conditions: (a) 1-tosylimidazole, NaOH 1.1 N, 1 h, rt; (b) HCL 0.1 N; (c) DMSO, collidine, microwaves, 20 min, 200 °C; (d) phosphate buffer, pH 6.0, Br_2, 5 days; (e) DCC, HOBt, DMF, 0 °C, 1 h; (f) oxytocin, DMF, 48 h.

der microwaves by heating (200 °C, 20 min) the tosylate derivative in DMSO with collidine as a non-nucleophilic base (yield 60%).[11] The mono-6-deoxy-6-formyl-β-cyclodextrin **3** obtained was then oxidized to the corresponding carboxylic acid **4** by bromine at pH 6.0, which represent the optimized conditions.[12] Following this long reaction (5 days), preparative HPLC gave pure mono-6-deoxy-6-carboxy-β-cyclodextrin **4** (yield 20%).[13]

Finally, the β-cyclodextrinyl carboxylic acid **4** was activated by reaction with 1-hydroxybenzotriazole (HOBt) and dicyclohexylcarbodiimide (DCC) in DMF (0°, 1 h). This activated ester was then condensed with oxytocin in DMF (25 °C, 48 h). The mono-6-deoxy-6-oxytocinyl-β-cyclodextrin **5** (β-CD-OT) was then isolated by preparative HPLC (yield 30%).[14] The LC/MS analysis of the collected conjugate revealed the absence of uncoupled oxytocin as well as the absence of CD derivatives. Each synthesized molecule was purified by preparative LC/MS and characterized by analytical LC/MS, elemental analyses, and NMR spectroscopy, their structures being thus confirmed.

As OT is a highly potent contracting agent of uterine muscle, the contractile potency of the mono-6-deoxy-6-oxytocinyl-β-cyclodextrin was examined on myometrium isolated from rats pretreated with β-estradiol benzoate (500 μg kg^{-1}, ip) and compared with OT.[15] As shown in Figure 1, the OT concentration (EC_{50} = 1.7 nM) required to induce the half-maximal tension is 230-times lower than the conjugate β-CD-OT (EC_{50} = 0.40 μM).

The frequency of contractions induced by the conjugate β-CD-OT is also lower than that induced by OT alone. Indeed, the OT concentration which induces at least one contraction per minute is 1 nM while the required concentration of β-CD-OT is 0.6 μM (Fig. 2).

The β-CD-OT is a novel drug carrier system targeting specifically the OT receptor. This β-CD-OT conjugate is less potent than OT, but remains active at a submicromolar concentration. This conjugate has a double interest. First, it has been previously demonstrated that CDs could form a host–guest complex with several prostaglandins and derivatives, and then increase their stability, their solubilization and their half-life.[16] So the inclusion of approved uterotonic drugs such as dino-

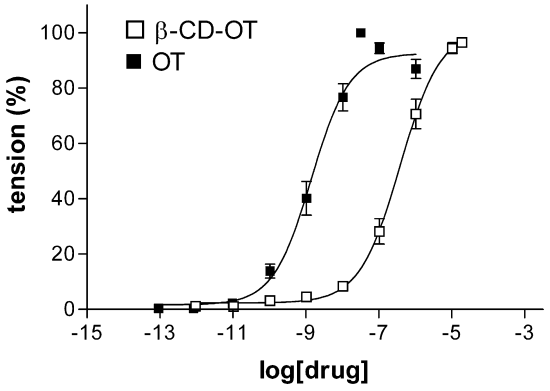

Figure 1. Isolated rat uterine contractile response to OT or β-CD-OT.

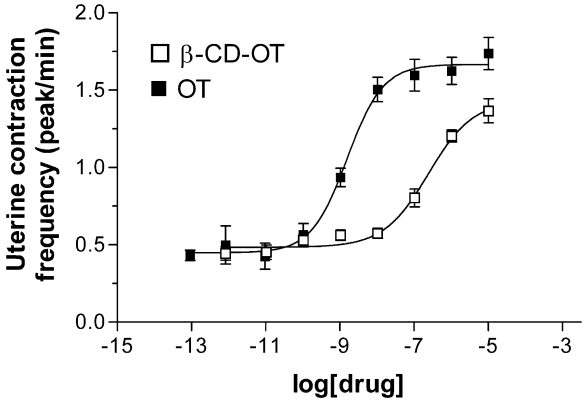

Figure 2. Frequency of uterine contractions induced by OT or β-CD-OT.

prostone (=prostaglandin E_2) or carboprost (=15-methyl $PGF_{2\alpha}$) could improve the stability and reduce the systemic side-effects of these drugs since they will be mainly driven to the uterus level, an OT-receptor rich tissue in case of pregnancy and delivery. The other interest of this conjugate is to form a host–guest complex with anticancer drugs used in the therapy of cervix cancer. This strategy could drive the anticancer to the uterus and so limit the side-effects of these anticancer drugs. Indeed, it has been reported that when paclitaxel, docetaxel, and doxorubicine, all used in endometrium and cervix cancer, form a complex with CD, their antitumoral activity is improved.[17] So, these drugs can probably be included as a guest in the OT-βCD carrier. By using this strategy, their solubility and their tumor concentration will be increased and as well as their toxicity will be lowered. As OT contains lipophilic amino acid residues, we examined its possibility to be inserted into the cavity of β-CD. It seems that this 'cup and ball' (well known as 'bilboquet') effect is unlikely. Indeed, the larger internal diameter of β-CD is only 6.5 Å[18] whereas the diameter of the cyclic sequence Cys-Asn-Gln-Ile-Tyr-Cys of OT is 11 Å.[19] Moreover, the distance between the C atom of the carbonyl linking oxytocin and the C-terminal atom of proline is 6 Å. As compared to the height of the torus-shaped β-CD (7.9 Å), this spacer is too short and the geometrical constraints are too strong to host OT. The great interest of this carrier has to be confirmed by the preparation of a drug/β-CD-OT complex.

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9. Mono-6-deoxy-6-(*p*-tosylsulfonyl)-β-cyclodextrin (**2**). In an E-flask equipped with a magnetic stirrer, sodium hydroxide (8.8 g; 220 mmol) was added to a suspension of β-CD (**1**, 25 g; 22 mmol) in distilled water. Then, 1-(*p*-toluenesulfonyl)imidazole) (5 g; 22.5 mmol) was added. After 1 h, hydrochloric acid, 0.1 N, was added dropwise to reach pH 5–6. The white formed precipitate was collected, washed with hot water (200 mL), with cold water (200 mL), with acetone (100 mL), and dried to give 9.23 g (yield = 33%) of the title compound **2**. ^{1}H NMR (400 MHz, DMSO-d_6)δ 2.44 (s, 3H), 3.20–3.69 (overlap with D_2O, 40 H), 4.17–4.22 (m, 1H), 4.33–4.40 (m, 2H), 4.45–4.49 (m, 2H), 4.53 (br s, 3H), 4.78 (br s, 2H), 4.85 (br s, 5H), 5.65–5.86 (m, 14H), 7.44 (d, 2H), 7.76 (d, 2H); *m/z* 1311 $[M+Na]^+$. Anal. Calcd for $C_{49}H_{76}O_{37}S{\cdot}3.5H_2O$: C, 43.23; H, 6.17. Found: C, 43.05; H, 5.94.
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11. Mono-6-deoxy-6-formyl-β-cyclodextrin (**3**). A microwave vial containing DMSO (10 mL) was filled with β-cyclodextrin monotosylate (**2**, 1.02 g; 0.78 mmol) and collidine (1 mL; 7.76 mmol). The vial was sealed with a teflon septum and irradiated at 2.450 GHz and 200 °C for 20 min (Biotage AB microwave oven, Uppsala). After heating, the vial was cooled, and its content added to acetone (200 mL). The formed precipitate was collected and dissolved in water (10 mL). This solution was added dropwise to ethanol (100 mL). The formed precipitate was collected, washed with ethanol, purified by preparative LC/MS (Econosphere-Alltech column, 250 cm × 22 mm; particle size 10 μm; mobile phase acetic acid 0.1%: CH_3CN (80:20 v/v); flow rate: 5 mL min^{-1}) to give 2.63 g (yield = 60%) of the titled compound **3**. ^{1}H NMR (400 MHz, DMSO-d_6) (note: this contains both the aldehyde and covalent hydrate form of the compound) δ 3.26–3.81 (overlap with D_2O, 40H), 4.16 (d, 1H), 4.38–4.45 (m, 6H), 4.79 (br s, 6H), 4.85–4.90 (d,1), 5.11 (t, 0.6H), 5.48 (d, 0.6H), 5.49 (d, 0.6H), 5.68–5.85 (m, 12H), 9.67 (s, 0.3); *m/z* 1133.1 $[M+H]^+$, 1155.3 $[M+Na]^+$. Anal. Calcd for $C_{42}H_{68}O_{35}{\cdot}4H_2O$: C, 41.86; H, 6.31. Found: C, 42.06; H, 6.02.
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13. Mono-6-deoxy-6-carboxy-β-cyclodextrin (**4**). Bromine (4 mL; 0.90 mmol) was added to a solution of 6-deoxy-6-formyl-β-cyclodextrin (**3**, 1.02 g; 0.90 mmol) in 0.1 M phosphate buffer (10 mL; pH 6.0). The solution was stirred for 5 days at room temperature in the dark. Then, excess of bromine was extracted by diethyl ether (4 × 20 mL). The aqueous phase was added to acetone (600 mL), and the formed precipitate was collected and purified by preparative LC/MS according to the above described procedure to give 0.21 g (yield = 20%) of the title compound **4**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.37–3.74 (overlap with D_2O, 40 H), 3.83 (d, 1H), 4.38–4.45 (m, 6H), 4.73–4.97 (m, 7H); 5.63–5.75 (m, 14H); *m/z* 1150.1 $[M+H]^+$, 171.2 $[M+Na]^+$. Anal. Calcd for $C_{42}H_{68}O_{36}{\cdot}4H_2O$: C, 41.31; H, 6.23. Found: C, 41.55; H, 6.11.
14. Mono-6-deoxy-6-oxytocinyl-β-cyclodextrin (**5**). 6-Deoxy-6-carboxy-β-cyclodextrin (**4**, 0.52 g, 0.45 mmol) was dissolved in DMF (3 mL) and the solution cooled to 0 °C. Then, HOBt (88 mg, 0.54 mmol) in DMF (2 mL) and DCC (111 mg, 0.54 mmol) in DMF (2 mL) were added. The solution was stirred and the temperature maintained at 0–4 °C. After 1 h, oxytocin (0.54 mg, 0.54 mmol) dissolved in DMF (5 mL) was added to the reactive mixture, which was stirred for 48 h at room temperature.

At the end of the reaction, 200 mL of acetone was added, the formed precipitate was collected, washed with acetone, dried, and purified by preparative LC/MS according to the above described procedure to give 0.21 g (yield = 30%) of the title compound **5**. ^{1}H NMR (DMSO-d_6) δ 0.77–0.84 (m, 12H, OT), 2.29–2.50 (overlap with DMSO, 14H, OT), 3.08–3.93 (overlap with D_2O, 41H (CD) + 11H (OT)), 4.38–4.45 (m, 6H), 4.53–4.70 (br m, 11H, OT), 4.73–4.97 (m, 7H, CD); 5.63–5.75 (m, 14H, CD), 5.91 (br s, 4H, OT), 7.10 (d, 2H, OT) 7.70 (d, 2H, OT), 8.46 (s, 8H, CONH); *m*/*z* 2138.1 $[M+H]^+$, 1070.1 $[M+2H]^{2+}$.

15. Female rats (Wistar, 200–250 g, Charles River Laboratories, Bruxelles, Belgium) are pretreated with β-estradiol benzoate (500 μg kg^{-1}, ip, Sigma, Brussels, Belgium) 24 h before the experiment. Rats were killed with pentobarbital (80 mg kg^{-1}, ip, Ceva Sante Animale, Brussels, Belgium) and myometrial tissue was removed and carefully trimmed of surrounding connective tissue. Uterine segments were placed longitudinally in a 20 mL tissue bath (EMKA Technologies, Paris, France) containing Krebs solution (mM: NaCl 118, KCl 5.4, $CaCl_2$ 2.5, $MgCl_2{\cdot}6\,H_2O$ 1.5, $NaHCO_3$ 25, NaH_2PO_4 1.2, glucose 10; pH 7.4) continuously bubbled with a mixture of O_2:CO_2 (95:5%) at 37 °C. Each uterine segment was placed under optimum resting tension of 1 g and allowed to equilibrate for 1 h before the experiment. During this equilibrium period, the buffer was renewed every 15 min before exposing the uterine segments to the contractile agent (OT or β-CD-OT). When a stable tension was obtained, cumulative increasing concentrations of OT or β-CD-OT (ranging from 10^{-13} to 10^{-5} M) were added to the bath until tension and frequency are stable. Contractile response of myometrial tissue segments was recorded isometrically with a force–displacement transducer IT1 (EMKA Technologies, Paris, France). The EC_{50} value of each drug was assessed for at least three concentration–response curves and corresponded to the concentration which elicited 50% the maximal tension. The EC_{50} values were calculated by nonlinear regression analysis (GraphPad Prism software). The results are expressed as means ± SEM, $n \geqslant 3$.
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