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Prednisolone succinate–glucosamine conjugate: Synthesis, characterization and *in vitro* cellular uptake by kidney cell lines

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Abstract

Prednisolone succinate–glucosamine (PSG) conjugate, a prodrug for prednisolone, was synthesized and confirmed by NMR and MS spectrum. The stabilities of the prodrug in PBS (pH 2.50, 5.00, 7.20, and 7.89) were studied. Cytotoxicity and uptake assay of the prodrug were perfomed on HK-2 and MDCK cell lines. The results showed that compared with prednisolone, the PSG not only did not increase the cytotoxicity but also improved the uptake to 2.2 times of prednisolone by the cells. Thus, it indicated that glucosamine might be a potential carrier for kidney-targeting delivery of prednisolone.

Keywords: Glucosamine; Prednisolone; Prodrug; Cytotoxicity; Uptake

Glucocorticoid such as prednisolone has been generally used for the therapy of nephrotic syndrome. However, the long-term administration of this kind of drug is always accompanied by systemic toxicities and side effects, including osteoporosis, amyotrophy, and central obesity, *etc.* Therefore, it is necessary to develop a novel kidney-targeted drug delivery system to increase the therapeutic effect and reduce the systemic toxicities. Several renal drug delivery systems have been previously developed, such as poly (vinylpyrrolidone-co-dimethyl maleic acid) (PVD) [1], low molecular weight proteins [2], sugar-modified low-molecular-weight peptides prodrugs, and randomly 50% *N*-acetylated low molecular weight chitosan (LMWC) [3]. However, their complex structures hinder their further development for clinical applications. Thus, our aim is to synthesize a kidney-targeted prodrug with a simple chemical structure. As the constitutional unit of chitosan, glucosamine was chosen to synthesize the prodrug.

In the current research, the following studies have been investigated: (1) synthesizing the prednisolone succinate–glucosamine conjugate (PSG); (2) the *in vitro* stabilities of the PSG; (3) cytotoxicity assay and uptake assay of the PSG on kidney cell lines.

The synthetic routes of the intermediate **10** and PSG were presented as Scheme 1 [4,5] and Scheme 2. The structures of the intermediate **10** and PSG were characterized by ^{1}H NMR, ^{13}C NMR and ESI-MS [6].

The stability of PSG was investigated in PBS (pH 2.50, 5.00, 7.20 and 7.89) at 37 ± 1 °C. The percentages of the residual PSG were determined by HPLC. In Fig. 1, it could be known that the prodrug was relatively stable in acidic environment (pH 2.50, 5.00), while in slightly basic environment (pH 7.20, 7.89), most of the PSG were hydrolyzed within 9 and 24 h, respectively.

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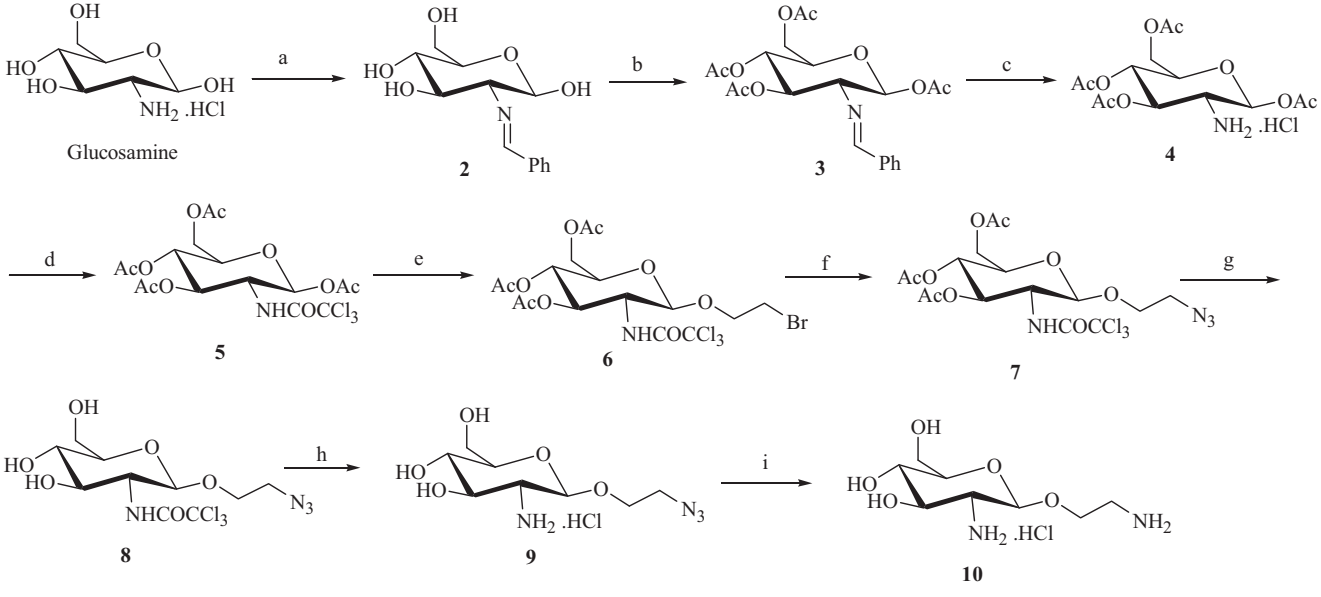
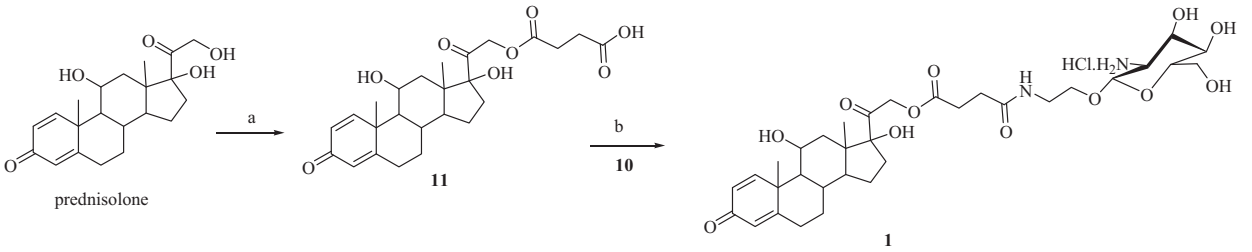


Scheme 1. Synthesis of 2-amino-1-(2-aminoethoxy)-glycose. *Reagents and conditions*: (a) benzaldehyde in NaOH aqueous solution; (b) Ac_2O, Py; (c) HCl; (d) Cl_3CCOCl, Et_3N; (e) 2-bromoethanol, $BF_3{\cdot}Et_2O$; (f) NaN_3, DMF; (g) CH_3ONa; (h) 0.3 mol/L NaOH; and (i) Pd/C (10%), H_2 in MeOH.



Scheme 2. Synthesis of prednisolone succinate–glucosamine conjugate (PSG). *Reagents and conditions*: (a) succinic anhydride; and (b) DCC/DMAP.

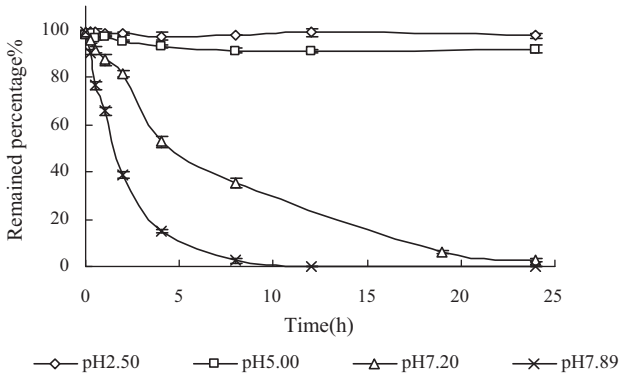


Fig. 1. Stability of PSG in PBS buffers at 37 ± 1 °C. Error bars represent standard deviation of the mean ($n = 3$).

The cytotoxicity of PSG and prednisolone were both determined on Madin-Darby Canine kidney (MDCK) cell lines and human proximal renal tubular epithelial (HK-2) cells after 24 h incubation at 37 °C using the MTT test. The results, reported in Fig. 2, showed the cell viability of both prednisolone and PSG higher than 90% when the concentration was lower than 0.28 μmol/mL. Compared with prednisolone, PSG showed no differences in cytotoxicity ($P > 0.05$).

Uptake experiments were also performed at 37 °C on MDCK and HK-2 cell lines. Prednisolone and PSG were respectively dissolved in the serum-free medium to a final concentration of 0.28 μmol/mL. Afterwards, they were incubated with the two cell lines for 2 h. Then, MDCK and HK-2 cells were collected for the determination of the drug contents. Compared with the prednisolone group, 2.2 times of the intracellular content of prednisolone in the PSG group was certified by the HPLC method ($P < 0.01$) (Fig. 3).

In conclusion, Prednisolone succinate–glucosamine conjugate was synthesized successfully. The cytotoxicity and uptake assay of PSG on MDCK and HK-2 cell lines showed that PSG did not increase the cytotoxicity while it could

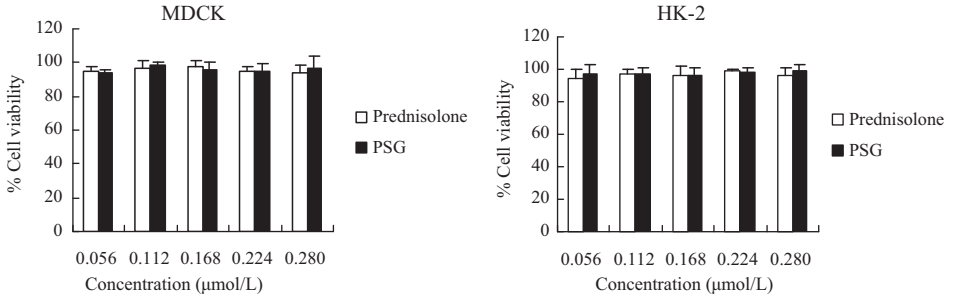


Fig. 2. Effect of prednisolone (white columns) and PSG (black columns) concentration on MDCK and HK-2 cell viability as determined by the MTT assay after 24 h incubation. Mean ± SD ($n = 3$).

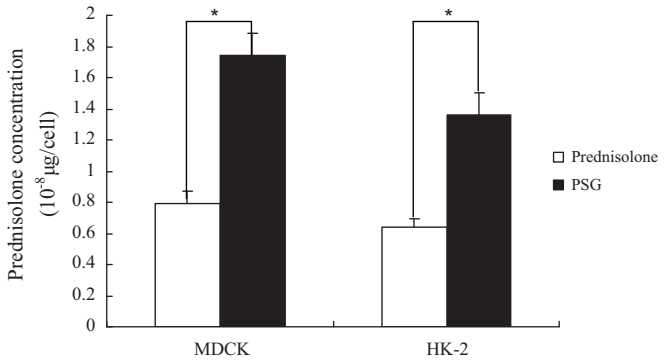


Fig. 3. *In vitro* uptake assay of prednisolone (white) and PSG (black) on MDCK and HK-2 cells. Mean ± SD ($n = 3$), (*$P < 0.01$).

improve the uptake 2.2 times of prednisolone by the cells. Therefore, the conjugate could be used as a potential kidney-targeted delivery system for prednisolone. Further studies of this conjugate are ongoing in our laboratory.

Acknowledgment

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[6] Analytical data for the intermediate **10**. yeild: 87%; ^{1}H NMR (400 MHz, D_2O): δ 4.71 (d, 1H, J = 18.8 Hz), 4.20 (m, 1H), 4.01 (m, 2H), 3.81 (d, 1H, J = 5.6 Hz), 3.79 (d, 1H, J = 5.6 Hz), 3.53 (m, 3H), 3.32 (m, 2H); ^{13}C NMR (200 MHz, D_2O): δ 36.8, 53.5, 57.9, 63.3, 67.0, 71.3, 73.5, 98.2; ESI-MS (*m*/*z*): 245.0 $[M+Na]^+$. Compound **1**. Yield: 57%; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.33 (d, 1H, J = 10.4 Hz), 6.16 (d, 1H, J = 10.4 Hz), 5.92 (s, 1H), 5.06 (d, 1H, J = 17.2 Hz), 4.76 (d, 1H, J = 17.6 Hz), 4.29 (d, 1H, J = 3.2 Hz), 4.07 (m, 2H), 3.48 (m, 4H), 3.15 (m, 2H), 3.04 (m, 4H), 2.60 (m, 3H), 2.42 (m, 3H), 2.31 (m, 2H), 2.02 (m, 2H), 1.64 (m, 3H), 1.38 (s, 3H), 1.25 (m, 2H), 1.02 (dd, 1H, J = 13.2 and 4 Hz), 0.89 (dd, 1H, J = 10.8 and 3.2 Hz), 0.77 (s, 3H); ^{13}C NMR (200 MHz, DMSO-d_6): δ 16.8, 21.1, 22.8, 23.7, 28.4, 28.9, 29.9, 31.1, 31.8, 34.2, 44.1, 47.3, 51.3, 55.6, 57.2, 61.2, 66.0, 68.1, 68.3, 70.2, 76.2, 77.4, 88. 8, 103.9, 115.9, 121.8, 127.3, 157.1, 172.2, 173.4, 174.3, 185.5, 205.6; ESI-MS (*m*/*z*): 665.4 $[M+H]^+$.