QPG-1030, Releasable Pegylation of Teduglutide Using Uni-Qleaver® Results in Improved PK/PD Properties in Rats Aiming for Once-Weekly Administration

Supaporn Sawadjoon, Marcus Bosson, Achim Orzechowski, Christian Sund, and Vidar Wendel-Hansen
QuiaPEG Pharmaceuticals AB, Virdings Allé 32 B, 754 50 Uppsala, Sweden

Introduction

Short bowel syndrome (SBS) is a rare malabsorption disorder resulting from a lack of a functional small intestine. The Glucagon-like peptide 2 (GLP-2) analogue, teduglutide (Gattex®), indicated for the treatment of patients with SBS has an increased half-life (2-3 hrs) and stability as compared to the native GLP-2 (7 min), but still requires daily dosing and a multistep process of reconstitution. In order to extend the half-life of drugs and drug candidates, thereby enabling less frequent dosing, QuiaPEG Pharmaceuticals AB has developed Uni-Qleaver®, a half-life extension platform technology, in which a drug is covalently attached to polyethylene glycol (PEG) by the releasable linker that slowly releases the original drug over time under physiological conditions (Fig. 1). Due to the ability of the PEG moiety to improve the solubility and stability of drugs in blood circulation and extend their half-life by reducing their renal excretion, PEG has been used in a wide range of FDA approved drugs. However, a permanent PEGylation can lead to loss of the biological activity by sterically blocking the active site of an enzyme.

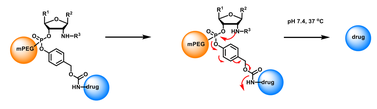

Figure 1. Schematic representation of release mechanism of linker cleavage.

Methods

Preparation of QPG-1030

PEGylation of teduglutide (QPG-1030) was prepared by treating teduglutide in 0.3 M HEPES pH 7.4 at room temperature for 2 hrs with Uni-Qleaver®, followed by treatment with citric acid, purification using anion exchange column and desalting using size exclusion chromatography.

In vitro release studies

QPG-1030 was incubated in phosphate buffer pH 7.4 at 37 °C. At selected time points, the hydrolysis samples were analyzed by analytical RP-HPLC.

GLP-2 receptor binding assay

The biological activity of QPG-1030 was assessed in a PathHunter GLP-2 Bioassay.

PK studies

The PK profile of teduglutide was studied following single subcutaneous (SC) administration of 108, 217, or 541 nmol/kg of QPG-1030to rats (Groups 1-3) or single intravenous (IV) of 108 nmol/kg QPG-1030 (Group 4). In addition, 400 nmol/kg of teduglutide, were directly administered subcutaneously to Group 5. Blood samples were taken from each animal (n=3/dose group) at different time points up to 72 hrs post-dose and analyzed by LC-MS/MS.

PD studies

Rats were treated daily by SC injections with vehicle control (n=5), teduglutide (100 nmol/kg, n=4), apraglutide (75nmol/kg, n=4) or QPG-1030(75 nmol/kg, n=4) for 6 days.

His-Gly-Asp-Gly-Ser-Phe-Ser-Asp-Glu-Met-Asn-Thr-Ile-Leu-Asp-Asn-Leu-Ala-Ala-Arg-Asp-Phe-Ile-Asn-Trp-Leu-Ile-Gln-Thr-Lys-Ile-Thr-Asp

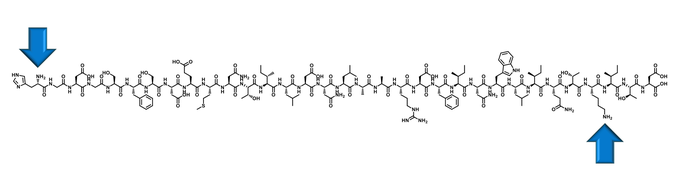

Figure 2. Molecular structure of teduglutide.

Results

The conjugate formation was verified by Sodium Dodecylsulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) analysis (Fig. 4). The native teduglutide was released from QPG-1030 with an in-vitro half life of 16 hrs under physiological conditions (Fig. 5). Results from in vitro drug hydrolysis of QPG-1030 in rat plasma at 37 °C showed a sustained teduglutide release with Cmax reached after 24 hrs (Fig. 6). The identity of the released teduglutide was confirmed by LC-MS/MS (Fig. 6 and 8). QPG-1030 displayed a 1000-fold reduction in receptor binding affinity compared to teduglutide (Fig. 7) Following a single SC dose, QPG-1030 was absorbed slowly from the subcutaneous compartment and Cmax for released teduglutide was generally observed at 30-40 hrs post dose. After reaching the peak concentration, teduglutide was eliminated from the systemic circulation in rats with a mean elimination half-life ($t_{1/2}$) in the range of 14.5 -19.1 hrs following dosing of QPG-1030. This is considerably longer than the elimination half-life of teduglutide when dosed directly, which was estimated to around 0.8 - 1 hrs, demonstrating that PEG-conjugation of teduglutide significantly increases the elimination half-life (Fig. 8). Following 6 days of repeated daily subcutaneous dosing of QPG-1030 to rats, a significant and dose-dependent increase in small intestinal weight was observed (Fig. 9), indicative of potent GLP-2 agonist activity. The effect was significantly enhanced compared to that of teduglutide at comparable dose levels and comparable to equimolar daily doses of apraglutide.

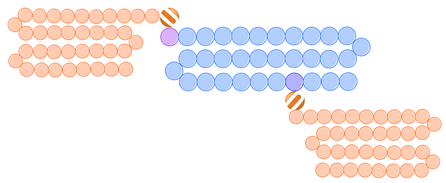

Figure 3. Structure of QPG-1030.

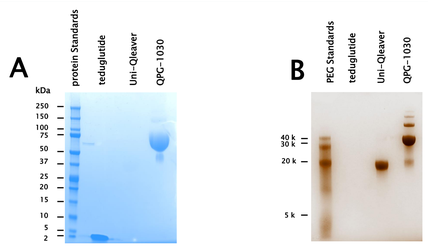

Figure 4. Electrophoresis of native teduglutide, Uni-Qleaver® and QPG-1030 on SDS-PAGE gels. (A) Specifically stained for protein with Coomassie blue. (B) Specifically stained for PEG with iodine.

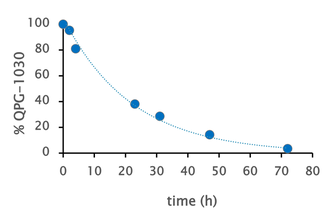

Figure 5. Hydrolysis of QPG-1030 in phosphate buffer (pH 7.4) at 37°C.

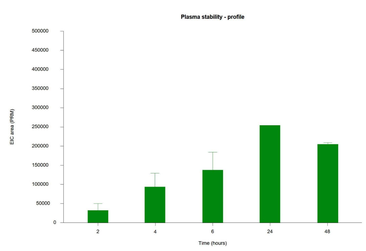

Figure 6. Teduglutide release from QPG-1030 in rat plasma at 37°C measured by LC-MS/MS.

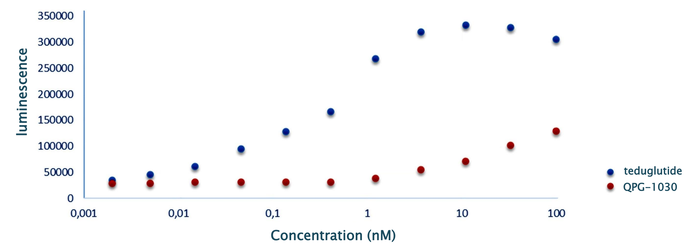

Figure 7. GLP-2 receptor binding assessed by a cAMP bioassay comparing teduglutide and QPG-1030.

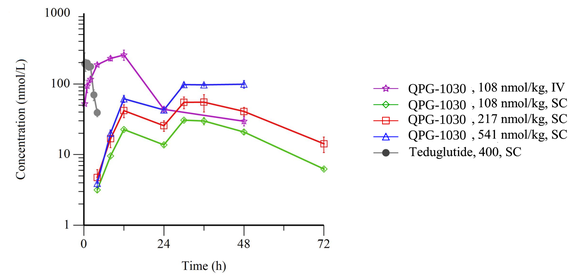

Figure 8. PK profiles of QPG-1030 and teduglutide.

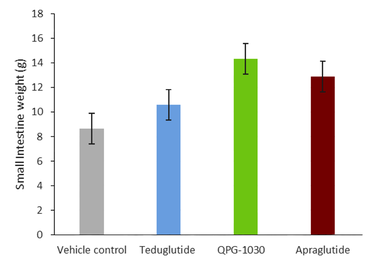

Figure 9. Intestinotrophic effects of QPG-1030 in comparison to apraglutide, teduglutide and vehicle.

Conclusion

QGP-1030 was found to have the following properties:

- In vitro half-life was 16 hrs (at 37°C, pH 7.4).
- Does not activate GLP-2 receptor, as determined by the cAMP Hunter bioassay, thus functioning as a prodrug.
- After single-dose subcutaneous administration in rats, peak plasma concentrations were reached at 30-40 hrs.
- In vivo half-life was 14.5 -19.1 hrs, significantly increased compared to teduglutide and similar to apraglutide.
- In a rat model evaluating intestinal growth induction, QGP-1030 displayed a significant increase in intestinal growth compared to teduglutide, similar to apraglutide.

These results indicate that QPG-1030 has the potential to be developed as a once-weekly subcutaneously administered therapeutic.

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Contact information

QuiaPEG Pharmaceuticals AB
Email: info@quiapeg.com