



Lipid-based formulations enhance the apparent intestinal permeability of BGP-15: physicochemical characterization and Caco-2 transport studies

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ABSTRACT

BGP-15 is a pleiotropic small molecule with cytoprotective, anti-inflammatory, and insulin-sensitizing effects, but its biopharmaceutical properties remain insufficiently characterized. This study investigated its pH-dependent distribution, Caco-2 permeability, and the effect of lipid-based formulations on apparent absorptive transport. The octanol–buffer distribution coefficient was determined over pH 1.5–10.5. BGP-15-loaded liposomes (LS) and solid lipid particles (SLP) were characterized for particle size, polydispersity, zeta potential, drug loading, and encapsulation efficiency, and selected formulations were evaluated in bidirectional Caco-2 transport studies. BGP-15 remained predominantly hydrophilic under acidic and near-neutral conditions and showed low apical-to-basolateral permeability as a free compound (1.40×10^{-6} cm/s), with marked directional transport asymmetry. LS3 and SLP3 significantly increased apical-to-basolateral permeability to 2.06×10^{-5} and 1.32×10^{-5} cm/s, respectively, while reducing the calculated efflux ratios to 1.18 and 1.30. ATPase assays showed no consistent concentration-dependent interaction with MDR1, ABCG2, or MRP2. These findings demonstrate a formulation-associated increase in the apparent absorptive transport of BGP-15 and support further investigation of lipid-based delivery approaches.

1. Introduction

BGP-15 is a nicotinamide amidoxime derivative, also known as (O-[3-piperidino-2-hydroxy-1-propyl]-nicotinicamidoxime) and it was developed by a Hungarian N-Gene Research Laboratories to treat insulin resistance (Nagy et al., 2010). BGP-15 is a small molecule that attracted attention due to its anti-inflammatory, anti-diabetic, and antioxidant properties. Many research groups are working with the molecule and investigating the effects of it. BGP-15 is known for its anti-inflammatory properties, as it inhibits the acetylation of heat shock factor 1 (HSF1), which enhances the induction of heat shock proteins (HSPs). This process helps in reducing inflammation (Pető et al., 2020). HSPs are pivotal in cellular stress responses and are involved in numerous diseases. In disease model systems, BGP-15 has been shown to increase heat shock

protein expression (Gombos et al., 2011; Literáti-Nagy et al., 2012), and protected cells against cell death (Nagy et al., 2010; Racz et al., 2002). It has been also shown that BGP-15 has a notable effect on the c-Jun N-terminal kinase (JNK) pathway, namely BGP-15 inhibits the activation of the abovementioned pathway (Sarnyai et al., 2020; Sarszegi et al., 2012), thereby preventing JNK-mediated inhibition from blocking the phosphorylation of the insulin receptor, which leads in enhanced insulin sensitivity (Crul et al., 2013; Hooper et al., 2014). BGP-15 also inhibits the activation of p38 MAPK (Chung et al., 2008; Sapra et al., 2014; Sarszegi et al., 2012). In the last few years, numerous pharmacological effects of BGP-15 have been investigated (Pető et al., 2020). The insulin-sensitizing effect was also studied in the last decade and it seems a promising drug candidate in the treatment of type 2 diabetes, moreover, it has been evaluated in phase II clinical trials for the

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sensitization of insulin resistance and it can control the metabolic side effects of atypical antipsychotics (Literáti-Nagy et al., 2012).

Although the pharmacological effects of BGP-15 have been widely investigated, its biopharmaceutical properties, particularly its membrane permeability, remain insufficiently characterized. A better understanding of the relationship between aqueous solubility and intestinal permeability is important for evaluating its potential for oral administration and for identifying possible limitations to its absorption. The reported aqueous solubility of BGP-15 in deionized water is 28 mg/mL at 25 °C (Sigma Aldrich, 2026; U.S. National Library of Medicine, 2026). However, equilibrium solubility across the physiologically relevant pH range has not been established, and membrane permeability data have not previously been reported. Therefore, the present study did not aim to establish a regulatory BCS classification of BGP-15. Instead, we sought to characterize its pH-dependent distribution behavior and apparent permeability across Caco-2 cell monolayers and to investigate whether lipid-based formulation could modify its apparent absorptive transport.

For this purpose, BGP-15-loaded liposomes and solid lipid particles (SLP) were prepared and characterized in terms of particle size, polydispersity index, zeta potential, drug loading, and encapsulation efficiency. The present study focused on two structurally distinct lipid-based delivery platforms, liposomes and solid lipid particles (SLPs), to investigate whether lipid formulation could improve the apparent epithelial transport of the highly hydrophilic BGP-15. Liposomal compositions were varied to obtain systems with different membrane and surface-charge characteristics, including positively and negatively charged components. In contrast, the SLP carrier matrix (Precirol® ATO 5/Tween 60) was kept constant, while the initial BGP-15 content was progressively increased. This design allowed assessment of whether increasing the drug-to-lipid ratio could improve BGP-15 incorporation without introducing changes in carrier composition. Selected formulations with the most favorable physicochemical properties were subsequently evaluated in bidirectional Caco-2 transport studies, and their apparent permeability coefficients (P_{app}) were compared with those of free BGP-15.

2. Materials and methods

2.1. Materials

BGP-15 was kindly provided by Meditop Pharmaceutical Ltd. (Pilisborosjenő, Hungary). Precirol® ATO 5 was kindly provided from Gattefosse (Lyon, France). Soy lecithin (Epikuron 200), and hydrogenated soy lecithin (Epikuron 200 SH) were purchased from Cargill, while cholesterol (Ph. Eur. 9.0) was supplied by Molar Chemicals, DOTAP and DSPG are products of Avanti Polar Lipids. Tween 60, Octadecyl amine, octanol, chloroform and other reagents were purchased from Sigma Aldrich (St. Gallen, Switzerland).

2.2. Determination of distribution coefficient for BGP-15

1-Octanol and different (1.5–10.5) pH buffer (500 µL) were combined in a 2 mL Eppendorf tube and vortex-mixed vigorously for 1 min to pre-equilibrate the phases. Subsequently, 10 µL of a 10 mM BGP-15 stock solution (prepared in DMSO) was added. The tube was sealed and rotated on a Loopster digital rotator (IKA, Germany) at room temperature for 24 h to allow phase partitioning to reach equilibrium. After incubation, the phases were allowed to separate, and 20 µL aliquots were carefully withdrawn from both the aqueous and octanol phases for analysis.

Quantification was performed by high-performance liquid chromatography (HPLC) using a Merck-Hitachi ELITE LaChrom system. Separation was achieved on an Aquasil C18 column (3 µm particle size) under isocratic conditions with a mobile phase of acetonitrile/water (10:90, v/v) containing 0.1% (v/v) formic acid, at a flow rate of 1.0 mL/min.

Detection was carried out using a diode-array detector (DAD) at 252 nm. The injection volume was 20 µL.

The distribution coefficient (D) was calculated according to Eq. (1):

$$\log D = \log_{10}(C_{oct} / C_{aq}) \quad (1)$$

where C_{oct} and C_{aq} are the equilibrium concentration of BGP-15 in the octanol and aqueous phases, respectively.

2.3. Preparation of BGP-15-loaded liposomes

BGP-15-loaded liposomes were prepared using the thin-film hydration method. A 15% (w/w) BGP-15 solution was prepared in purified water, and the pH was adjusted to 4.0; this solution was used as the hydration medium. The compositions of the lipid solutions (LS) are provided in Table 1. Briefly, 120 µL aliquots of each lipid solution were transferred into 5 mL Eppendorf tubes, and the chloroform was evaporated under a gentle nitrogen stream to form a thin lipid film. Subsequently, 3.5 mL of the BGP-15 hydration solution was added to each tube, and the mixtures were sonicated for 30 min. The resulting liposome dispersions were then extruded using an Avanti Liposome Extruder equipped with 200 nm polycarbonate membranes. Each dispersion was passed through the membrane for 10 extrusion cycles to obtain a narrower size distribution.

2.4. Preparation of BGP-15-loaded solid lipid particles

The lipid phase was prepared by melting Precirol® (glyceryl palmitostearate) in a water bath. BGP-15 was dispersed in the molten lipid phase (Table 2). In parallel, an aqueous phase consisting of purified water and Tween® 60 (1%w/w) was heated to approximately 65 °C. Using a hot homogenization approach, the molten lipid dispersion containing BGP-15 was added to the preheated aqueous phase and homogenized at 20,000 rpm using an Ultra-Turrax T25 Easy Clean homogenizer (IKA Werke GmbH & Co. KG, Staufen, Germany) fitted with a dispersing tool (18 G). The resulting emulsion was then cooled gradually to room temperature under continuous stirring to allow lipid solidification, yielding the SLP dispersion. The dispersions were subsequently frozen at –80 °C in a freezer (Arctiko ULUF 450) and lyophilized overnight using a ScanVac CoolSafe freeze dryer (Labogene, Allerød, Denmark) to obtain a dry, solid product.

2.5. Particle characterization

Particle size and polydispersity index (PDI) were measured using dynamic light scattering, while zeta potential was determined by electrophoretic light scattering using a Malvern Zetasizer Nano ZSP, (Malvern Instruments Ltd., Worcestershire, UK). Measurements were performed in triplicate immediately after preparation and again following reconstitution of the freeze-dried solid lipid particles (SLP). In all cases, samples were diluted 100-fold prior to analysis. All measurements were performed in triplicate at 20 °C using PBS dispersion medium.

2.6. Encapsulation efficiency and drug loading

The encapsulation efficiency (EE) of liposomes was determined as follows: 1.5 mL of extruded liposome dispersion was placed into dialysis tubing (Spectra/Por® 3, MWCO 3.5 kDa) and dialyzed against 100–150 mL of pH 3.5 buffer for 24 h. The amount of BGP-15 diffused into the dialysis solution was measured by UV-Vis photometry as unbound BGP-15. The dialyzed liposome content was subsequently lyophilized, and the dry mass was recorded.

For determination of unbound BGP-15 part in SLPs, the procedure began with reconstitution of lyophilized BGP-15-loaded particles. Briefly, 100 mg of SLP powder was suspended in 4.9 mL of pH 3.5

Table 1

Composition of lipid solutions. Lipid component were dissolved in chloroform.

Component	LS1	LS2	LS3	LS4
Epikuron 200	77.75 mmol/L	72.73 mmol/L	140.00 mmol/L	160.00 mmol/L
Epikuron 200 SH	77.75 mmol/L	72.73 mmol/L	-	-
Cholesterol	44.5 mmol/L	36.36 mmol/L	20.00 mmol/L	20.00 mmol/L
DOTAP	-	18.18 mmol/L	-	-
Octadecylamine	-	-	40.00 mmol/L	-
18:0 DSPG	-	-	-	20.00 mmol/L

Table 2

Composition of BGP-15 loaded SLPs.

No	BGP-15	Precirol® ATO 5	Tween 60	Purified water
SLP1	0.5 g	10.0 g	1.0 g	99.0 g
SLP2	1.0 g	10.0 g	1.0 g	99.0 g
SLP3	2.0 g	10.0 g	1.0 g	99.0 g

phosphate buffer under continuous agitation for 1 min. The suspension was centrifuged at 4500 rpm for 15 min (Eppendorf Centrifuge 5804 R, Eppendorf Ltd., Stevenage, UK). The supernatant, containing unbound drug, was analyzed using a UV–Vis spectrophotometer (Shimadzu UV-1900, Shimadzu Scientific Instruments Inc., Maryland, USA). For analysis, 100 µL of supernatant was diluted in 4.90 mL of pH 3.5 phosphate buffer, and absorbance was recorded in triplicate over the range of 235–260 nm.

To determine the total BGP-15 content in lipid formulations, 100 mg of lyophilized powder was dispersed in 5.0 mL of chloroform and vortexed for 5–10 min to dissolve the lipid matrix. Subsequently, 5.0 mL of pH 3.5 phosphate buffer was added, and BGP-15 was extracted from the organic phase by agitation for 24 h at 55 rpm (IKA Loopster digital rotator). The extracted amount of BGP-15 was then determined by UV–Vis spectrophotometry. The recovery of BGP-15 was 94%.

Encapsulation efficiency and drug loading (DL) were calculated using the following equations:

$$EE(\%) = (m_{Total} - m_{Unbound}) / m_{Total} \times 100 \quad (2)$$

$$DL(\%) = (m_{Total} - m_{Unbound}) / m_{LP} \times 100 \quad (3)$$

where m_{Total} is the total amount of BGP-15 in the liposome or SLP powder, $m_{Unbound}$ is the amount of non-entrapped drug (surface-associated or interliposomal), and m_{LP} is the mass of the liposome or SLP powder.

2.7. Cell culture

Human adenocarcinoma Caco-2 intestinal epithelial cells [European Collection of Cell Cultures (ECACC, UK); catalogue no. 86010202] were grown routinely in Dulbecco's Modified Eagle's Medium (DMEM) with the supplementation of 10% fetal bovine serum (FBS), and 1% penicillin-streptomycin solution at 37 °C in 5% CO₂ atmosphere. The passage number of Caco-2 cells was 15–30 in the study.

2.8. Investigation of the cytotoxicity of BGP-15 by real-time cell analysis

To discover the cytotoxicity of different concentrations of BGP-15, an xCELLigence Real-Time Cell Analysis system (RTCA DP Instrument, ACEA Biosciences Inc., San Diego, CA, USA) with E-Plates was used. Cells (2×10^4 cells/well) were seeded on RTCA plates in each well. A culture medium (100 µL) was added to each well for background measurements. After cells were attached to the wells (6 h later), different increasing concentrations (1–1000 µM) of BGP-15 were added and cultured in the E-Plates. Caco-2 cells were kept in an incubator at 37 °C at 5% CO₂ atmosphere; 1% Triton-X was used as a positive control (Inácio et al., 2011). Data acquisition and analysis were performed using

RTCA Software version X.X.

2.9. Measurement of transepithelial electric resistance TEER

Transepithelial Electric Resistance (TEER) is a widespread quantitative technique to measure the integrity and confluency of Caco-2 cell monolayers which reflects the tight junction dynamics in cell culture models. These values are indicators of the integrity of the cellular barriers before evaluation for transport of BGP-15. Cells (2×10^5) were seeded on Corning Transwell® polycarbonate filters, and the culture medium was renewed with fresh medium every three days in the inserts. After 14–21 days, cells formed monolayers, which were used for transport experiments. To measure the TEER values, Millicell-ERS volttohmmeter electrodes (Merck KGaA, Darmstadt, Germany) were submerged into the apical and basal chambers of the culture medium. In this experiment, Caco-2 cells were cultured until the 900 Ω cm² TEER value, which is a correct indicator for the confluence of the Caco-2 monolayer. Caco-2 cells were cultured without stimulants until the 900 Ω cm² TEER value threshold was reached for each monolayer. TEER values were measured immediately before and after the permeability experiment. TEER data were analyzed using one-way ANOVA followed by Tukey's multiple-comparisons test. Post-treatment values were compared among the untreated control, free BGP-15, LS3, and SLP3 groups. The p value of <0.05 was considered statistically significant.

2.10. Measurement of the permeability

For permeability studies, 2×10^5 cells/insert were seeded on 1.12 cm² permeable Transwell® polycarbonate filters with a 0.4 µm pore size. When Caco-2 cells became confluent and the transepithelial electrical resistance reached 900 Ω cm², cells were treated with BGP-15 in 100 µM concentration. Bidirectional transport was performed. Caco-2 monolayers were washed and pre-incubated with Hanks' Balanced Salt Solution (HBSS) for 20 min at 37 °C. The first group of the cells was treated in the upper chamber with 100 µM BGP-15 and the drug transport was investigated from the upper compartment to the lower. At specified times, 100 µL samples were collected from the lower compartment (15 min, 30 min, 60 min, 90 min, 120 min) and the volume was supplemented with HBSS. The concentration of the BGP-15 was determined by LC-MS. The other group of cells was treated with BGP-15 in the lower chamber and investigated the transport from the lower compartment to the upper. At certain time points, samples were collected from the upper compartment, and the volume was supplemented with HBSS.

The apparent permeability coefficients (P_{app}) were calculated with the following equation:

$$P_{app} = dQ/dt \times 1 / (C_0 \times A),$$

Where P_{app} is the apparent permeability coefficient (cm/s); dQ/dt is the permeability rate of substances (mol/s); C_0 is the initial concentration of the substances in the upper/lower compartment (mol/mL), and A is the surface area of the membrane (cm²).

The efflux ratio for BGP-15, liposome and SLP formulations were calculated with the equation:

$$ER = P_{app}(B \rightarrow A) / P_{app}(A \rightarrow B)$$

Free BGP-15, LS3, and SLP3 were applied at an equivalent BGP-15 concentration of 100 μM in the donor compartment. The volume of each formulation was calculated on the basis of its experimentally determined BGP-15 content. Bidirectional permeability experiments were performed in three independent biological replicates, with three technical replicates per condition.

Permeability data were analyzed using two-way analysis of variance (two-way ANOVA), with formulation and transport direction as independent factors. Tukey's multiple-comparisons test was applied for post hoc pairwise comparisons. The p value of <0.05 was considered statistically significant.

2.11. Investigation of efflux transporters by ATPase assay

The interaction of BGP with the efflux transporters P-glycoprotein (P-gp/MDR1), breast cancer resistance protein (BCRP/ABCG2), and MRP2 was investigated using transporter-specific ATPase assays. ATPase activity was measured using the MDR1/ABCG2/MRP2-specific Predeasy ATPase Assay Kits (Solvo Biotechnology, Szeged, Hungary), which employ Sf9 membrane vesicles expressing the respective human transporter. To evaluate the effects of BGP on transporter function, both activation and inhibition assays were performed. In activation assays, membrane vesicles were incubated with increasing concentrations of BGP to determine its ability to stimulate basal ATPase activity of the respective transporters. In inhibition assays, the effect of BGP on ATPase activity induced by the corresponding reference activator was assessed.

All assays were performed in 96-well microplates according to the manufacturer's instructions. BGP was added to assay samples in 1 μL DMSO, and vehicle control samples received the same volume of DMSO. Verapamil was used as the reference activator for MDR1, whereas Sulfasalazine served as the reference activator for ABCG2 and MRP2. Transporter ATPase activity under the different experimental conditions was determined by quantifying the release of inorganic phosphate (Pi) using a colorimetric assay. Absorbance was measured at 600 nm with a microplate reader, and Pi concentrations were calculated from a calibration curve. ATPase activity was expressed as the difference in Pi release between paired samples incubated in absence and presence of sodium orthovanadate, a selective inhibitor of ABC transporter ATPase activity.

ATPase activity data were analyzed separately for each transporter and assay mode (activation or inhibition). Statistical comparisons were performed using one-way analysis of variance (ANOVA) followed by Dunnett's multiple-comparisons test, with each BGP-15 concentration compared against the corresponding vehicle control (activation assays) or stimulated control (inhibition assays).

For each transporter, three independent biological experiments were performed, each containing three technical replicates. Technical replicates were averaged prior to statistical analysis, and the mean value from each independent experiment was considered a single biological replicate. Data are presented as mean $\pm$ standard deviation (SD) of three independent biological experiments ($n = 3$). Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. LogD results of BGP-15

For BGP-15, negative logD values were obtained in the pH range 1.5–5.5, indicating that BGP-15 is predominantly present in the aqueous

phase in its ionic form (Table 3). With increasing pH, logD increased progressively, followed by a steep transition in the ~ 8.5 –9.5 range (Fig. 1). This region contains the pH at which logD equals 0 (i.e., $D = 1$), where BGP-15 partitions equally between octanol and water. Linear interpolation between the values measured at pH 8.5 and 9.5 indicated that logD reached zero at approximately pH 8.8. At higher pH values, the neutral form becomes increasingly dominant, exhibiting greater solubility in the octanol phase.

3.2. Characteristics of extruded liposomes and solid lipid particles

Among the liposomal formulations, LS3 showed the smallest mean particle size and the highest positive zeta potential, whereas LS4 exhibited the lowest PDI and a negative surface charge (Table 4Table 5). The SLP formulations were considerably larger, with mean diameters ranging from approximately 1.5 to 3.7 μm . SLP3 showed a mean diameter of 1492 nm, a PDI of 0.564, and a slightly negative zeta potential.

3.3. Drug loading and encapsulation efficiency

Drug loading and encapsulation efficiency values are summarized in Table 6. Among the liposomal formulations, drug loading ranged from 2.11% to 2.59%, while encapsulation efficiency varied between 11.7% and 17.9%. LS2 showed the highest drug loading ($2.59 \pm 0.31\%$), whereas LS3 exhibited the highest encapsulation efficiency ($17.9 \pm 0.15\%$). LS1 and LS4 showed comparable drug-loading values of $2.11 \pm 0.12\%$ and $2.21 \pm 0.13\%$, respectively, with corresponding encapsulation efficiencies of $11.7 \pm 0.13\%$ and $15.2 \pm 0.16\%$.

The solid lipid particle formulations showed higher drug loading and encapsulation efficiency than the liposomal formulations. Drug loading increased from $5.45 \pm 0.78\%$ for SLP1 to $9.11 \pm 0.83\%$ for SLP2 and $24.9 \pm 1.94\%$ for SLP3. A similar increasing trend was observed for encapsulation efficiency, which reached $13.2 \pm 0.16\%$, $22.1 \pm 0.15\%$, and $43.87 \pm 0.24\%$ for SLP1, SLP2, and SLP3, respectively.

The 24-h dialysis procedure used for liposomes may reflect not only initially unencapsulated drug but also formulation-dependent release occurring during dialysis. Therefore, the liposomal EE values are

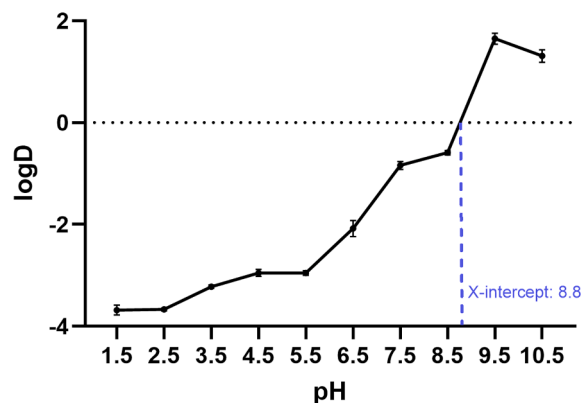


Fig. 1. pH-dependent distribution coefficient (logD) of BGP-15. The dashed line indicates the interpolated pH at which logD = 0, corresponding to $D = 1$ and equal distribution between the aqueous and octanol phases. Data are presented as mean $\pm$ SD ($n = 3$).

Table 3

LogD values of BGP-15 on different pH values. The number of repetitions is $n = 3$.

pH of aqueous phase	1.5	2.5	3.5	4.5	5.5	6.5	7.5	8.5	9.5	10.5
logD	-3.68	-3.67	-3.22	-2.93	-2.95	-2.08	-0.84	-0.59	1.65	1.31
$\pm$ SD	± 0.10	± 0.03	± 0.03	± 0.07	± 0.04	± 0.16	± 0.08	± 0.04	± 0.11	± 0.12

Table 4

Summary of liposome formulations regarding the size distribution and surface properties.

Code of formulation	Z average (nm) ±SD	PDI±SD	Zeta potential (mV) ±SD
LS1 + BGP-15 (pH 4.0)	274.1 ± 2.1	0.565 ± 0.005	+10.8 ± 0.9
LS2 + BGP-15 (pH 4.0)	400.9 ± 15.1	0.979 ± 0.017	+27.9 ± 0.4
LS3 + BGP-15 (pH 4.0)	180.9 ± 1.2	0.392 ± 0.005	+31.1 ± 2.2
LS4 + BGP-15 (pH 4.0)	248.3 ± 2.3	0.257 ± 0.008	−9.87 ± 1.4

Table 5

Summary of SLPs formulations regarding the size distribution and surface properties.

Code of formulation	Z average (nm) ±SD	PDI±SD	Zeta potential (mV) ±SD
SLP1	3744 ± 192.4	0.465 ± 0.055	−0.35 ± 0.13
SLP2	1485 ± 282.0	0.797 ± 0.161	−3.19 ± 0.35
SLP3	1492 ± 184.2	0.564 ± 0.118	−5.01 ± 0.09

Table 6

Drug loading and encapsulation efficiency of BGP-15 in liposomal (LS) and solid lipid particle (SLP) formulations.

Code of formulation	DL%±SD	EE%±SD
LS1 + BGP-15 (pH 4.0)	2.11 ± 0.12	11.7 ± 0.13
LS2 + BGP-15 (pH 4.0)	2.59 ± 0.31	13.5 ± 0.11
LS3 + BGP-15 (pH 4.0)	2.37 ± 0.26	17.9 ± 0.15
LS4 + BGP-15 (pH 4.0)	2.21 ± 0.13	15.2 ± 0.16
SLP1	5.45 ± 0.78	13.2 ± 0.16
SLP2	9.11 ± 0.83	22.1 ± 0.15
SLP3	24.9 ± 1.94	43.87 ± 0.24

interpreted as operationally determined values obtained under the standardized dialysis conditions. Because different separation procedures were required for the two carrier classes, absolute EE and DL values should be compared within, but not directly between, the LS and SLP series.

3.4. Concentration-dependent cellular tolerability of BGP-15 by real-time cell analysis

The cytotoxicity effects of different BGP-15 concentrations on Caco-2 cells were analyzed in real time by the xCELLigence system (RTCA method) when the cells reached the maximum cell index value (Fig. 2). We applied BGP-15 in 6 different concentrations (1 µM–1 mM). BGP-15

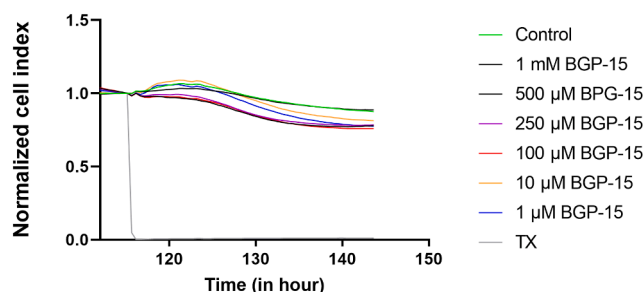


Fig. 2. Normalized cell index of Caco-2 cell layers treated with different concentrations of BGP-15: 1 µM, 10 µM, 100 µM, 250 µM, 500 µM, 1 mM and with 1% Triton-X-100 (TX). The cell index was normalized to the time of treatment. Data are presented as mean ± SD (n = 3).

did not show a significant decrease in the cell index in contrast with 1% Triton-X, which was used as a positive control.

3.5. Measurements of permeability

Free BGP-15 exhibited low apparent permeability in the apical-to-basolateral direction, with a P_{app} value of $1.40 \times 10^{-6} \pm 4.74 \times 10^{-7}$ cm/s. In the opposite direction, the P_{app} value was significantly higher, reaching $1.38 \times 10^{-5} \pm 3.05 \times 10^{-6}$ cm/s. The inclusion of BGP-15 in the liposome increased the value of P_{app} in the A→B direction approximately 14.7-fold ($2.06 \times 10^{-5} \pm 1.42 \times 10^{-6}$; $p < 0.01$ AB BGP-15 vs. AB LS3) and significant increase (approximately 9.4-fold) was observed in the case of the SLP formulation also ($1.32 \times 10^{-5} \pm 8.33 \times 10^{-7}$; $p < 0.05$ AB BGP-15 vs. AB SLP3). However, the P_{app} value of none of the formulations changed significantly in the case of transport in the B→A direction (Table 7).

TEER values were measured before and after the permeability experiments to assess the integrity of the Caco-2 cell monolayers (Fig. 3). Post-treatment TEER values remained close to the corresponding pre-treatment values in all groups. Neither free BGP-15 nor the LS3 and SLP3 formulations caused a statistically significant decrease in TEER compared with the untreated control group ($p > 0.05$). These results indicate that the treatments did not induce a marked loss of monolayer integrity under the applied experimental conditions.

3.6. Investigation of efflux transporters by ATPase assay

The potential interaction of BGP-15 with MDR1, ABCG2, and MRP2 was evaluated by measuring vanadate-sensitive ATPase activity in activation and inhibition assays over a concentration range of 1–1000 µM (Fig. 4).

3.6.1. MDR1 ATPase activity

In the MDR1 activation assay, BGP-15 caused a moderate increase in vanadate-sensitive ATPase activity compared with the vehicle control. ATPase activity was not significantly altered at 1, 10, or 100 µM BGP-15, whereas a statistically significant increase was observed at 1000 µM ($p < 0.05$). However, the response was not monotonic across the investigated concentration range and therefore did not demonstrate a clear concentration-dependent activation of MDR1 ATPase activity.

In the MDR1 inhibition assay, ATPase activity remained comparable to the stimulated control at 1, 10, and 1000 µM BGP-15. A significant increase, rather than inhibition, was observed at 100 µM ($p < 0.05$). Since BGP-15 did not reduce the reference activator-stimulated ATPase activity at any tested concentration, the results do not indicate inhibition of MDR1 ATPase activity. The isolated increase detected at 100 µM was not concentration dependent.

3.6.2. ABCG2 ATPase activity

In the ABCG2 activation assay, ATPase activity varied across the tested concentrations, with lower mean values at 1 and 10 µM and higher values at 100 and 1000 µM compared with the vehicle control. However, none of these differences reached statistical significance. Thus, BGP-15 did not induce statistically significant or concentration-dependent activation of ABCG2 ATPase activity.

In the ABCG2 inhibition assay, the mean ATPase activity was moderately lower in the presence of BGP-15 than in the stimulated control. Nevertheless, no statistically significant differences were observed at any investigated concentration, and the reduction did not follow a concentration-dependent pattern. Therefore, the results do not provide evidence for concentration-dependent inhibition of ABCG2 ATPase activity.

3.6.3. MRP2 ATPase activity

BGP-15 did not significantly alter MRP2 ATPase activity in the activation assay. The measured activities remained close to the vehicle-

Table 7

Apparent permeability coefficients (P_{app}) of free BGP-15 and BGP-15-loaded lipid formulations across Caco-2 cell monolayers. The “ns” indicates no significant difference between the two transport directions within the same formulation.

$P_{app} \pm SD$	BGP-15 (cm/s)	LS3 (cm/s)	SLP3 (cm/s)
A→B	$1.40 \times 10^{-6} \pm 4.74 \times 10^{-7}$	$2.06 \times 10^{-5} \pm 1.42 \times 10^{-6} p < 0.01$ (vs AB BGP-15)	$1.32 \times 10^{-5} \pm 8.33 \times 10^{-7} p < 0.05$ (vs. AB BGP-15)
B→A	$1.38 \times 10^{-5} \pm 3.05 \times 10^{-6} p < 0.05$ (AB vs.BA BGP-15)	$2.44 \times 10^{-5} \pm 9.55 \times 10^{-7} ns$ (AB vs. BA LS3)	$1.72 \times 10^{-5} \pm 5.14 \times 10^{-6} ns$ (AB vs. BA SLP3)
Efflux ratio	9.86	1.18	1.30

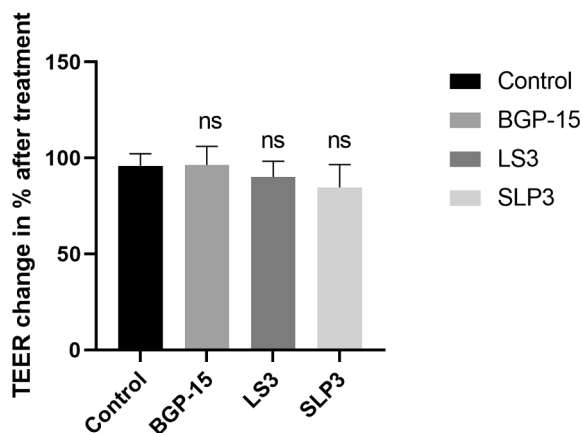


Fig. 3. Changes in transepithelial electrical resistance (TEER) following treatment during the permeability study. Post-treatment TEER values were expressed as a percentage of the corresponding pretreatment values. Data are presented as mean $\pm$ SD, $n = 3$. Statistical analysis was performed using one-way ANOVA followed by Tukey’s multiple-comparisons test. No significant differences were observed between the treated groups and the control group (ns, $p > 0.05$).

control value throughout the concentration range of 1–1000 μ M, with no apparent concentration–response relationship.

Similarly, no significant inhibition of reference activator-stimulated MRP2 ATPase activity was observed. ATPase activity remained comparable to the stimulated control at all tested concentrations. These findings indicate that BGP-15 did not produce detectable concentration-dependent activation or inhibition of MRP2 ATPase activity under the applied experimental conditions.

Overall, the ATPase assays did not reveal a consistent concentration-dependent interaction of BGP-15 with MDR1, ABCG2, or MRP2. Although isolated statistically significant increases in MDR1 ATPase activity were observed at 1000 μ M in the activation assay and at 100 μ M in the inhibition assay, these changes were not monotonic and did not demonstrate a consistent substrate-like stimulation of MDR1 ATPase activity. No significant effects were detected for ABCG2 or MRP2.

Vanadate-sensitive ATPase activity was measured in the presence of BGP-15 at concentrations of 1, 10, 100, and 1000 μ M. Panels A, C, and E show the activation assays for MDR1, ABCG2, and MRP2, respectively. Panels B, D, and F show the corresponding inhibition assays performed in the presence of the transporter-specific reference activator. ATPase activity is expressed as nmol inorganic phosphate released per mg protein per minute. Data are presented as mean $\pm$ SD from three independent biological experiments ($n = 3$), each performed with three technical replicates. Statistical analysis was performed using one-way ANOVA followed by Dunnett’s multiple-comparisons test against the corresponding control. ns, not significant $p > 0.05$; * $p < 0.05$.

4. Discussion

Although BGP-15 has a broad pharmacological profile, including insulin-sensitizing, cytoprotective, and anti-inflammatory effects, its biopharmaceutical properties have remained insufficiently

characterized (Pető et al., 2020). The present study therefore investigated the pH-dependent distribution behavior and bidirectional Caco-2 permeability of BGP-15 and evaluated whether lipid-based formulations could improve its apparent absorptive transport. Solid lipid-based systems have also been reported to enhance the apparent Caco-2 permeability of orally administered drugs, although formulation size and composition differ substantially among studies (Shah et al., 2021).

The logD results demonstrated strongly pH-dependent distribution behavior. BGP-15 remained predominantly in the aqueous phase throughout the acidic-to-neutral pH range and exhibited a negative logD value at pH 7.5. These findings indicate limited affinity for a lipid-rich environment under near-physiological conditions and are consistent with a predominantly ionized state. According to the pH-partition concept, ionized compounds generally exhibit reduced passive diffusion across lipid membranes (Camenisch et al., 1998; O’Hagan and Kell, 2015; Zheng et al., 2015). The pH-dependent increase in the distribution coefficient suggests that the fraction of less ionized, more lipophilic BGP-15 may increase as luminal pH rises, which could theoretically favor passive transcellular diffusion in more distal intestinal regions. However, the present logD data alone are insufficient to predict site-specific intestinal absorption, because regional absorption is also influenced by epithelial surface area, residence time, transporter expression, and other physiological factors. In agreement with this physicochemical profile, free BGP-15 showed low apparent permeability in the apical-to-basolateral direction (1.40×10^{-6} cm/s), whereas its basolateral-to-apical permeability was approximately one order of magnitude higher (1.38×10^{-5} cm/s). The experimentally determined low apical-to-basolateral permeability, together with the previously reported aqueous solubility of 28 mg/mL in deionized water, provides useful information on the biopharmaceutical properties of BGP-15. However, the reported solubility value was obtained under a single experimental condition and cannot be used to establish high solubility according to regulatory BCS criteria. Since equilibrium solubility across the physiologically relevant pH range was not determined in the present study, no BCS classification is assigned to BGP-15 (Council et al., 2019; WHO, 2019).

The investigated liposomal and solid lipid formulations displayed markedly different physicochemical characteristics. LS3 was a nano-sized, positively charged liposomal formulation with a mean diameter of 180.9 nm and a zeta potential of +31.1 mV, although its PDI of 0.392 indicated a moderately broad size distribution. In contrast, SLP3 had a mean diameter of 1492 nm, a PDI of 0.564, and a slightly negative zeta potential of −5.01 mV. Thus, SLP3 should be considered a micrometer-scale solid lipid particle system rather than a nanoparticle formulation. SLP3 showed substantially higher drug loading and encapsulation efficiency than LS3, reaching 24.9% and 43.87%, respectively, compared with 2.37% and 17.9% for LS3. Nevertheless, its relatively large particle size and broad size distribution represent limitations from a colloidal formulation perspective. These findings show that drug incorporation capacity and colloidal properties should be considered as separate formulation attributes.

Both selected lipid formulations significantly increased the apparent absorptive permeability of BGP-15. LS3 increased the apical-to-basolateral P_{app} approximately 14.7-fold, to 2.06×10^{-5} cm/s, whereas SLP3 produced an approximately 9.4-fold increase, to 1.32×10^{-5} cm/s. The greater permeability enhancement observed with

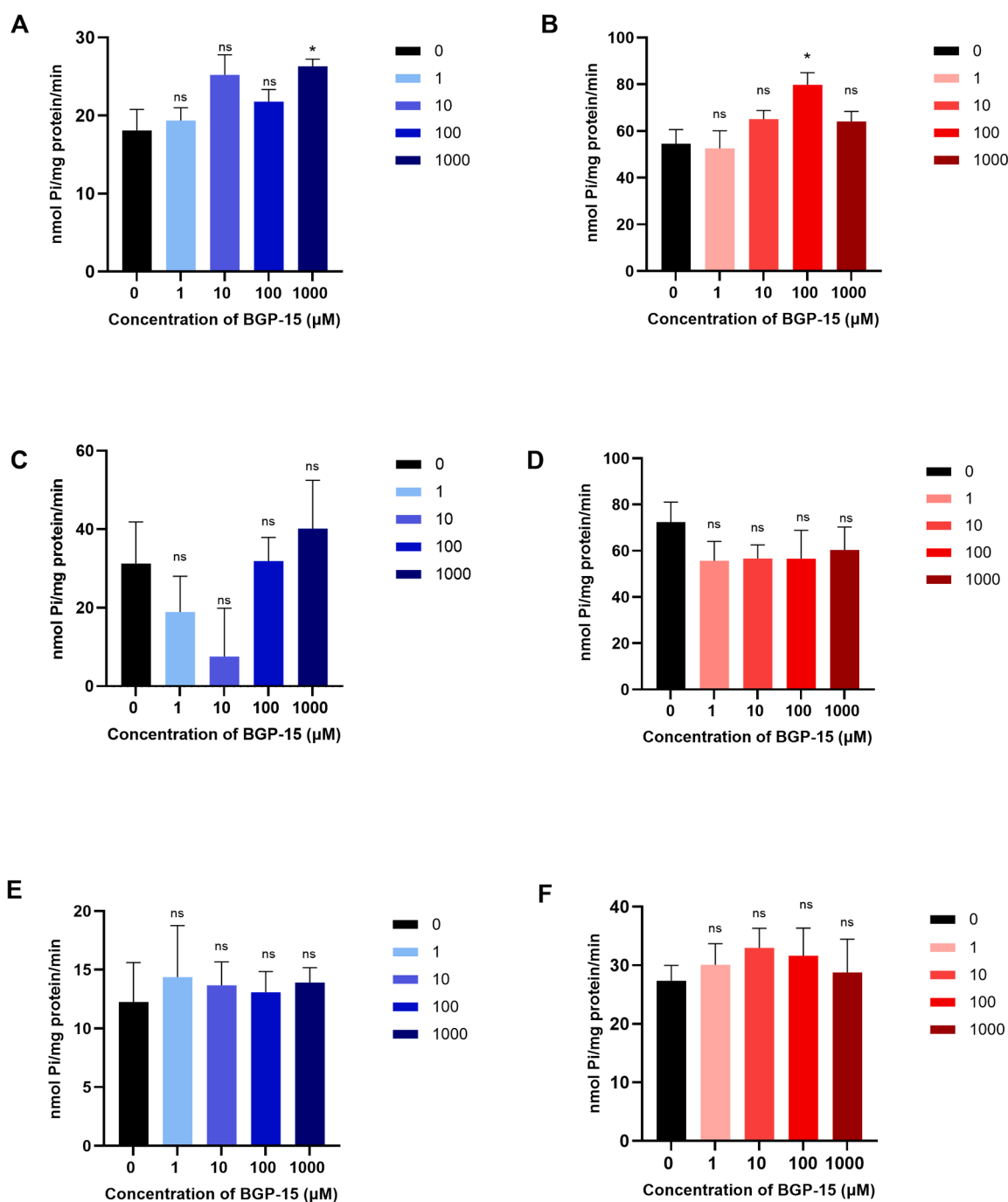


Fig. 4. Effect of BGP-15 on the ATPase activity of MDR1, ABCG2, and MRP2.

LS3, despite its lower drug loading and encapsulation efficiency, suggests that maximal drug incorporation alone did not determine transport performance. The smaller particle size and positive surface potential of LS3 may have promoted closer interaction with the negatively charged epithelial surface and thereby contributed to enhanced apparent transport. Previous studies using Caco-2 cells have shown that liposome association with intestinal epithelial cells is influenced by particle size and surface charge, with cationic liposomes generally exhibiting stronger cellular association than neutral or anionic systems (Kono et al., 2018; Paramshetti et al., 2024). However, cellular uptake, drug release, and formulation stability in the transport medium were not directly investigated. Consequently, the present data do not allow us to distinguish between transport of formulation-associated BGP-15 and transport of

BGP-15 released from the carrier before or during exposure to the Caco-2 monolayer. In addition, intracellular accumulation of BGP-15 was not quantified; therefore, transmonolayer transport cannot be distinguished from cellular retention or uptake. Therefore, no conclusions can be drawn regarding controlled release or intact carrier-mediated transport.

Importantly, post-treatment TEER values did not differ significantly between the untreated control, free BGP-15, LS3, and SLP3 groups. This finding indicates that the increased apparent permeability was not associated with a marked or persistent loss of Caco-2 monolayer integrity under the applied experimental conditions. Nevertheless, cellular uptake, drug release, formulation stability in the transport medium, and paracellular marker permeability were not directly investigated.

Therefore, the relative contributions of particle-associated transport, released BGP-15, epithelial membrane interaction, and subtle or transient modulation of tight-junction permeability cannot be fully distinguished.

The efflux ratio of free BGP-15 was 9.86, reflecting marked directional asymmetry. In contrast, the efflux ratios of LS3 and SLP3 were reduced to 1.18 and 1.30, respectively. The reduction in efflux ratio resulted primarily from increased apical-to-basolateral permeability rather than from a decrease in basolateral-to-apical transport. Similar observations have been reported for phospholipid-based formulations, which may alter the balance between passive entry and carrier-mediated efflux without necessarily acting as direct transporter inhibitors (Kapitza et al., 2007). Thus, the findings do not demonstrate direct inhibition of efflux transporters by the formulations. Instead, they suggest that the carriers improved the apparent entry or passage of BGP-15 from the apical side of the Caco-2 monolayer. The absence of a significant post-treatment decrease in TEER further supports the interpretation that the reduction in transport asymmetry was not simply caused by marked disruption of the epithelial monolayer.

The high efflux ratio of free BGP-15 could initially suggest the involvement of apically localized ABC transporters, including MDR1/P-gp, ABCG2, and MRP2. In the ATPase assays, isolated increases in MDR1 ATPase activity were detected at 1000 μ M in the activation assay and at 100 μ M in the inhibition-assay configuration. However, these changes were not monotonic and did not form a consistent concentration–response relationship. Moreover, BGP-15 did not reduce reference activator-stimulated MDR1 ATPase activity, arguing against direct MDR1 inhibition. No significant effects were detected in the ABCG2 or MRP2 assays. Taken together, the ATPase results do not indicate a consistent substrate-like or inhibitory interaction of BGP-15 with these transporters under the applied experimental conditions. Nevertheless, ATPase assays assess transporter-associated ATP hydrolysis and cannot, on their own, determine whether a compound undergoes directional transport across an intact epithelial monolayer. Moreover, weak or absent ATPase modulation does not exclude transporter-mediated flux (Brück et al., 2017; da Silva Junior et al., 2015). Consequently, the contribution of MDR1, ABCG2, MRP2, or other uptake and efflux transporters to the directional transport of BGP-15 remains uncertain. Because BGP-15 is a polar, potentially cationic compound under near-physiological conditions, transporters involved in organic cation disposition may also warrant consideration; however, the intestinal role of transporters such as OCT1 remains controversial and may include elimination rather than classical absorptive uptake (Wenzel et al., 2021). A high efflux ratio alone does not identify the transporter responsible for directional asymmetry. Accordingly, the high efflux ratio observed for free BGP-15 should not be attributed to a specific efflux transporter based on the present data. Confirmation of transporter-mediated flux generally requires bidirectional Caco-2 studies in the presence of selective probe inhibitors, as demonstrated for established P-gp substrates such as digoxin and quinidine (Patil et al., 2010; Smalley et al., 2007).

The RTCA measurements indicated that free BGP-15 did not cause a marked reduction in the Caco-2 cell index over the investigated concentration range, whereas Triton X-100 produced the expected cytotoxic response. This finding supports the cellular tolerability of free BGP-15 under the applied conditions. Although the lipid formulations themselves were not separately evaluated in the RTCA assay, post-treatment TEER measurements provided additional information on epithelial barrier integrity during the permeability experiments. Neither free BGP-15 nor LS3 or SLP3 caused a statistically significant reduction in TEER compared with the untreated control. Together, these findings suggest that the enhanced apical-to-basolateral transport was not primarily caused by overt cytotoxicity or marked disruption of the epithelial barrier. However, because TEER mainly reflects ionic conductance across the monolayer, it cannot exclude subtle, transient, or solute-selective changes in paracellular permeability (Bunchongprasert and

Shao, 2020). Additional studies using paracellular markers and time-resolved TEER measurements would be required to characterize such effects more comprehensively.

Overall, LS3 and SLP3 demonstrated complementary advantages. SLP3 provided the highest drug loading and encapsulation efficiency, whereas LS3 produced the greatest enhancement in apparent absorptive permeability. These findings indicate that the selection of a suitable BGP-15 carrier should not be based solely on encapsulation parameters. Particle size, surface properties, drug release, colloidal stability, and interactions with the epithelial barrier are also likely to influence transport performance. The absence of a significant post-treatment decrease in TEER supports preservation of overall monolayer integrity under the experimental conditions. Further studies should nevertheless include time-resolved TEER monitoring, paracellular marker permeability, formulation stability and release testing in biorelevant media, mass-balance analysis, direct cellular uptake studies, and transporter-specific inhibition experiments. Such investigations are required before the observed increase in apparent Caco-2 permeability can be translated into conclusions regarding improved oral absorption or bioavailability.

5. Conclusions

BGP-15 exhibited strongly pH-dependent distribution behavior and remained predominantly hydrophilic under acidic and near-neutral conditions. In the Caco-2 model, free BGP-15 showed low apparent absorptive permeability and marked directional transport asymmetry. Both selected lipid-based formulations significantly increased the apparent permeability of BGP-15 in the apical-to-basolateral direction. The LS3 liposomal formulation produced the greatest permeability enhancement, whereas SLP3 showed the highest drug loading and encapsulation efficiency. The ATPase assays did not demonstrate concentration-dependent activation or inhibition of MDR1, ABCG2, or MRP2, although isolated increases in MDR1 ATPase activity were detected at individual BGP-15 concentrations. These findings support further investigation of lipid-based systems as a strategy to improve the apparent absorptive transport of BGP-15. However, additional studies addressing drug release, formulation stability, barrier integrity, mass balance, and transporter-specific mechanisms are required before conclusions regarding oral bioavailability can be drawn.

CRedit authorship contribution statement

Ágnes Klusóczyki: Writing – original draft, Methodology, Investigation, Data curation. Gábor Vasvári: Writing – original draft, Methodology, Investigation, Data curation. Attila Kiss-Szikszai: Validation, Methodology, Investigation. Katalin Goda: Validation, Methodology, Investigation, Conceptualization. Ferenc Fenyvesi: Writing – review & editing, Supervision, Formal analysis. Katalin Réti-Nagy: Validation, Supervision, Formal analysis. Ildikó Bácskay: Resources, Funding acquisition. Judit Váradi: Writing – review & editing, Validation, Supervision, Methodology, Conceptualization.

Declaration of competing interest

We wish to confirm that there is no conflict of interest associated with this publication.

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Data availability

Data will be made available on request.

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