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Abstract

Oral delivery of therapeutic peptides reduces patient burden and increases adherence. Nevertheless, existing technologies have limited performance and versatility. AdOral® is an oral delivery platform based on non-specific MoA (Mechanism of Action) via intestine paracellular passage leading to improved absorption.

The MoA of AdOral® and its permeation effect on several peptides were evaluated in vitro, using gold-standard Caco-2 monolayers. Then, AdOral® platform was evaluated in vivo, in rat and dog. In vitro, apparent permeability coefficient of insulin lispro and semaglutide were improved by 16 and 25-fold vs. control, mostly due to the concentration-dependent and reversible tight junctions opening, leading to paracellular passage of peptides. AdOral® has a higher permeation enhancing properties compared to SNAC (effective concentration of 10 mM vs. 100 mM for SNAC).

In rats, AdOral® had a good safety profile after daily oral administration for 2 weeks. In dogs, AdOral® capsules allowed oral absorption of semaglutide and insulin lispro, demonstrating the versatility of the platform.

AdOral® semaglutide showed 2.5-fold improvement in median semaglutide AUC0-5h and bioavailability vs. Rybelsus®. Thus, AdOral® is a versatile and efficient platform that could lead to cost-effective oral formulations for a wide range of therapeutic peptides.

Introduction & Background

- Peptides and proteins are widely used as drugs. However, almost all these drugs only exist in injectable form. Oral forms improve patient adherence and compliance, provide comfort of use, simplify drug administration in children and overall, help patients to achieve their therapeutic goals.
- Most peptides and proteins have very low oral bioavailability due to their very low permeability through the gastrointestinal (GI) epithelium and their susceptibility to proteases present in the GI tract.
- AdOral® is an oral delivery platform based on non-specific MoA (Mechanism of Action) via intestine paracellular passage leading to improved absorption.

Methods

- Caco-2 permeability assay: Formulations were added into the apical compartment of a differentiated Caco-2 cells cultured in a Transwell system. One hour after the addition of formulations, cell viability was determined through ATP quantification. Only conditions for which more than 45% of cells were viable were considered for permeability assessment. Lispro and semaglutide were quantified by ELISA in basal compartments and apparent permeability coefficient (P_{app}) were calculated. For reversibility demonstration, TEER (Trans Epithelial Electric Resistance) were measured before and after treatments. In parallel the permeability to semaglutide simultaneously (direct protocol) or 16h after (reverse protocol) AdOral® addition was quantified.

AdOral®—A Versatile Platform for Improved Oral Peptide Absorption

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- Tight junctions of differentiated Caco-2 cells (cultured on glass) were visualized by specific labelling of ZO-1.
- Safety study in rats: AdOral® was evaluated in a maximum tolerated dose study in Male Wistar rats (5 rats per group), by daily oral gavage for 14 days.
- Oral administration: Beagle dogs (N=10) were fasted overnight before administration of AdOral® capsules. Lispro concentrations were measured in plasma samples by ELISA, semaglutide concentration by LC-MS/MS.

AdOral® has a concentration-dependent permeation effect mediated by tight junction opening

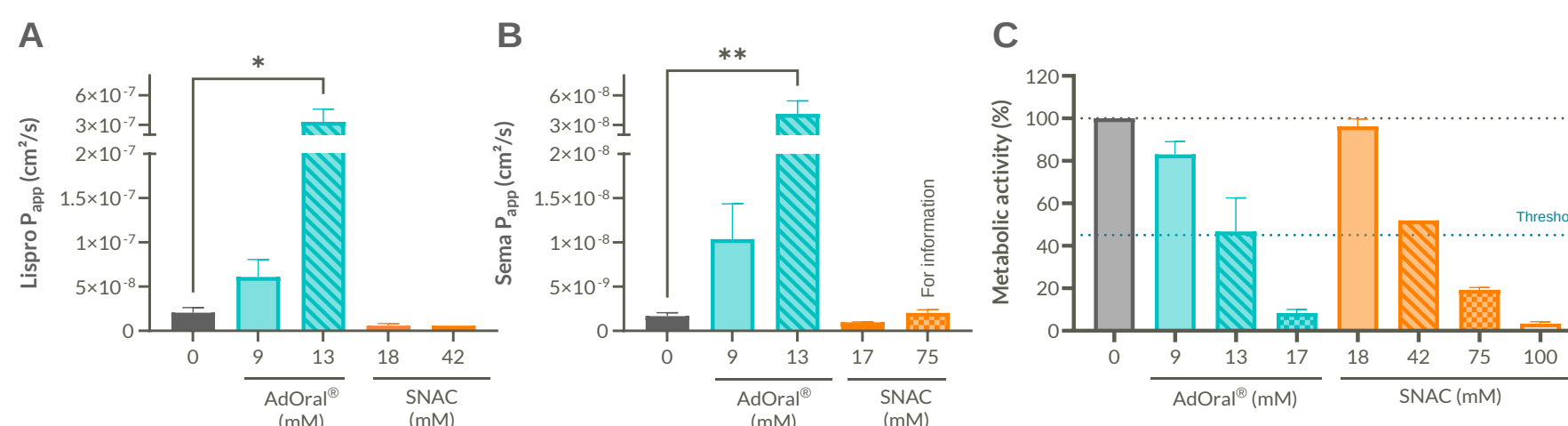


Figure 1: Impact of AdOral® and SNAC on lispro and semaglutide in vitro absorption. Lispro (A) and semaglutide (B) P_{app} measured in the basal compartment in N=3 and 5 independent experiments, respectively. C. Metabolic activity of Caco-2 cells after 1h exposure with formulations (A, N=2 independent experiments). One-Way ANOVA, *: $p<0.05$, **: $p<0.01$.

- AdOral® is a dose dependent permeation enhancer for semaglutide and lispro: P_{app} of lispro and semaglutide increase up to 16- and 25-fold in the presence of AdOral®, respectively.
- AdOral® is an efficient permeation enhancer at lower concentrations compared to SNAC.

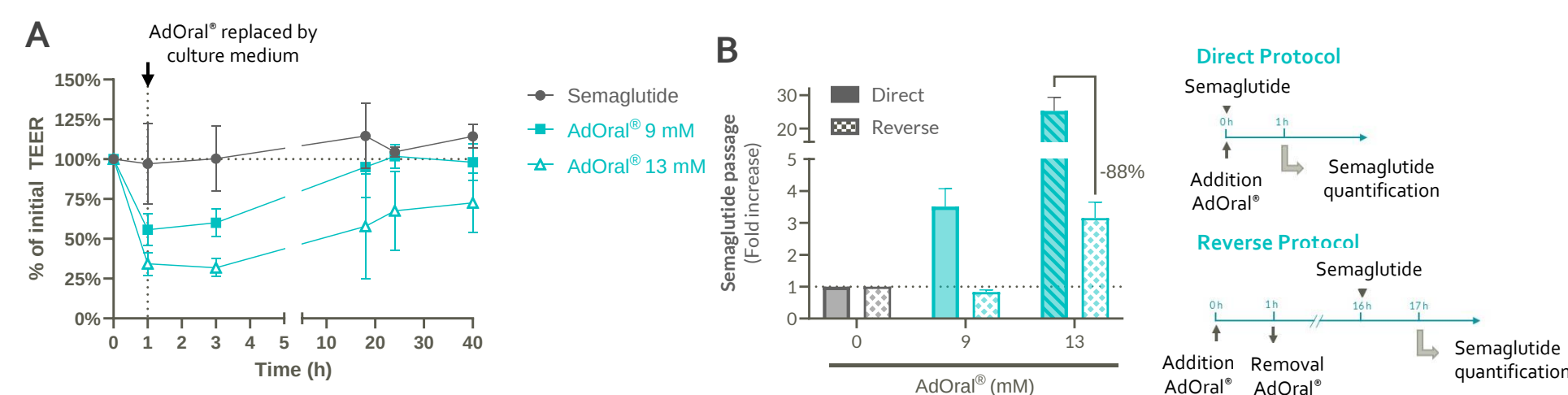


Figure 2: AdOral® enhances the permeability of Caco-2 monolayer in a reversible manner. A. Evolution of the TEER in response to AdOral® exposition for 1 hour and recovery for up to 40 hours. N=4 independent experiments. B. Differentiated Caco-2 cells (transwell) were incubated for 1h with AdOral®. Semaglutide 100 μ M was added either at the same time as AdOral® (direct protocol) or after a washing period of 16h (Reverse protocol). Semaglutide was quantified 1 hour after treatment. N=2 independent experiments.

- AdOral® induces a reversible and dose dependent decrease of TEER
- In agreement, the permeability of semaglutide induced by AdOral® is reversible.

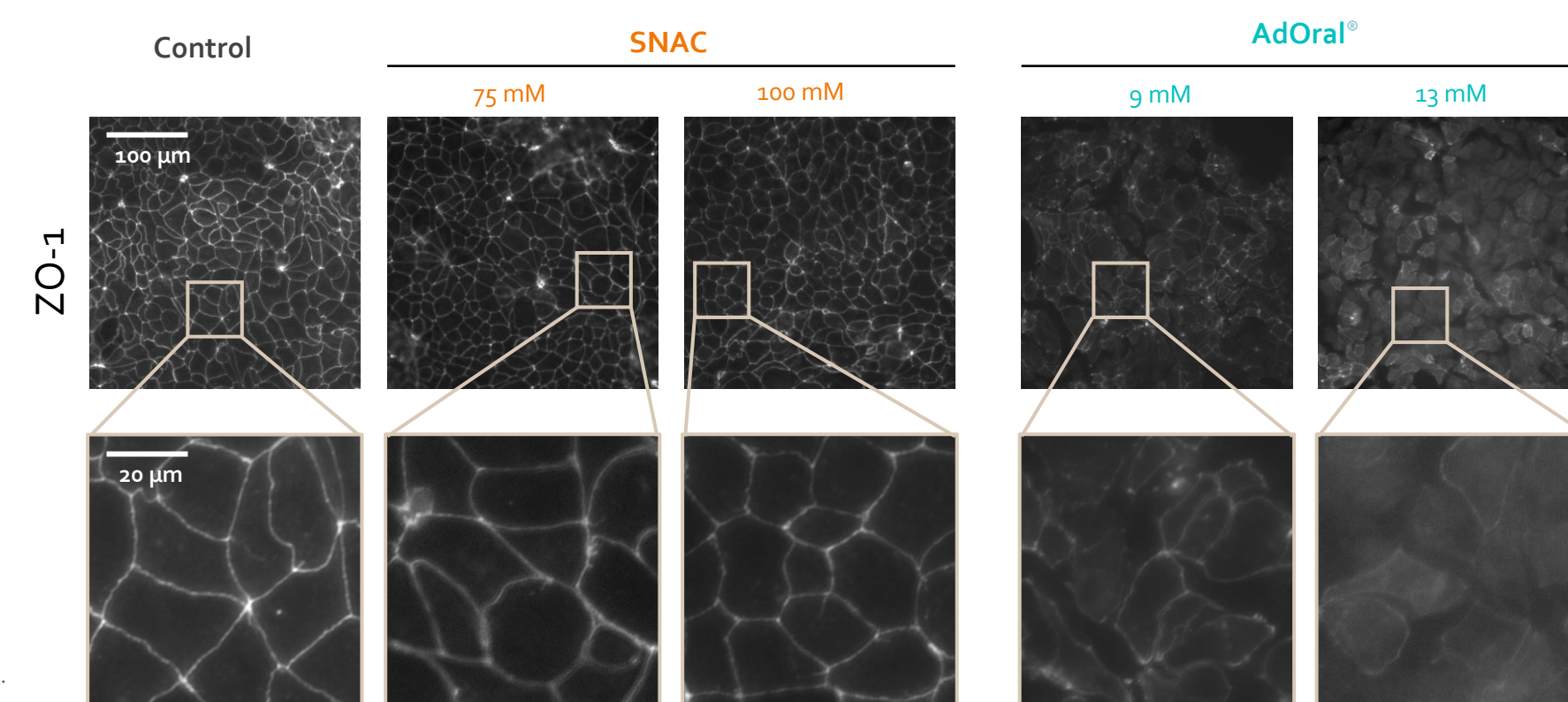


Figure 3: AdOral induces a concentration dependent opening of tight junctions. Localization of ZO-1 tight junction protein on differentiated Caco-2 cells either (A) untreated or exposed to (B) 75 or 100 mM SNAC or (C) 9 or 13 mM AdOral® for 1 hour. N=3 independent experiments.

- Contrary to SNAC, AdOral® induces a dose-dependent opening of tight junctions, leading to paracellular passage of peptide.

AdOral® is well tolerated after two weeks of daily administrations in rats:

AdOral® was well tolerated after oral administration for 14 days up to 500 mg/kg/day :

- No mortality or unexpected event,
- No effect on body weight, organ weight, hematology or coagulation,
- No treatment related adverse effect on clinical pathology,
- No adverse macroscopic observations,
- NOAEL established at 500 mg/kg/day in rats.**

AdOral® is easily formulated into solid oral dosage forms

AdOral® can be easily incorporated into solid oral dosage forms: for pre-clinical administration to Beagle dogs, the peptide and AdOral® were manually blended then filled into size 00 Vcaps Enteric capsules (Lonza/Capsugel). Capsules were sealed before administration.

- Enteric capsules were used as a prototype dosage form to deliver the formulation past the stomach:
- AdOral® is compatible with other oral dosage forms



AdOral® semaglutide outperforms Rybelsus® in Beagle dog

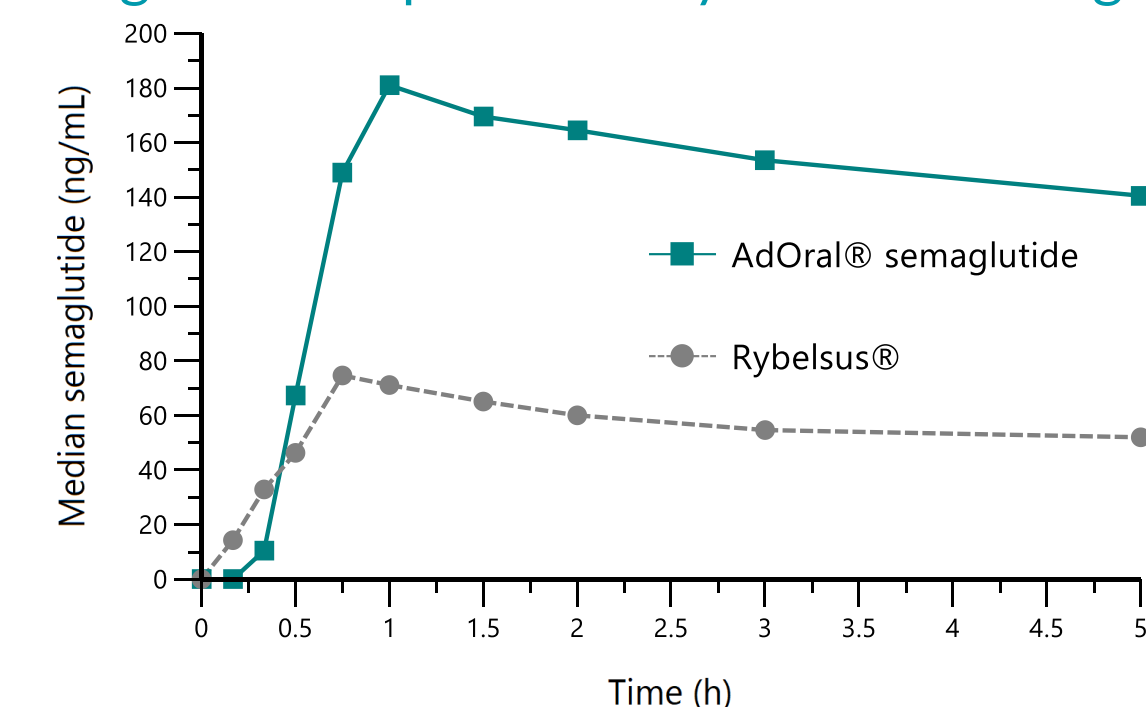


Figure 4: Median semaglutide concentrations after administration of AdOral® semaglutide 14mg (blue) or commercial Rybelsus® 14mg (grey) to 10 Beagle dogs

- AdOral® semaglutide 14mg leads to a median bioavailability ~2,5 times higher than the commercial comparator Rybelsus® 14mg.

AdOral® allows the oral delivery of bioactive insulin lispro in Beagle dog

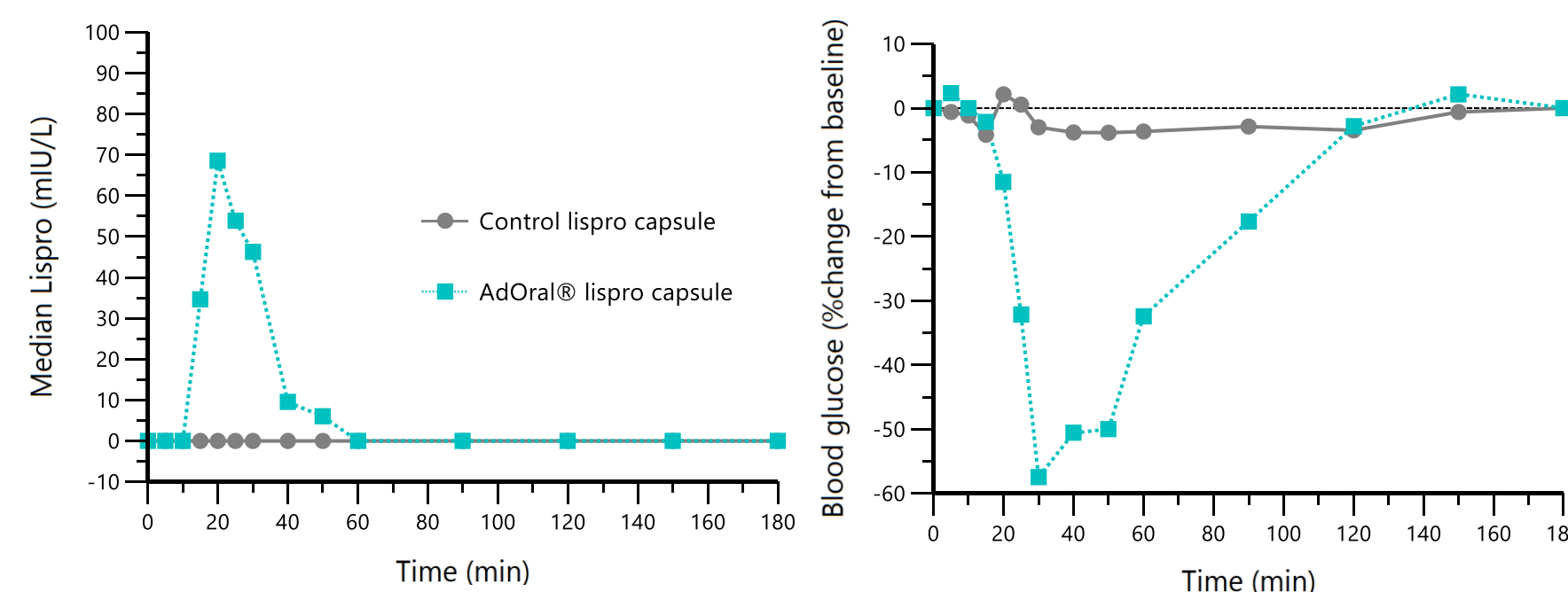


Figure 5: Median PK (left) and PD (right) profiles after administration of AdOral® Lispro (blue) or a control insulin lispro capsule (grey) to 10 Beagle dogs

- AdOral® Lispro leads to systemic exposure and hypoglycemic effect while orally administered insulin lispro control does not measurably reach the bloodstream

Conclusions

- AdOral® has a reversible tight junction opening effect which enhances peptide permeability through a paracellular pathway
- AdOral® is well tolerated in a rat model (NOAEL at 500 mg/ kg/day)
- AdOral® has demonstrated its ability to orally deliver two peptides with different properties in Beagle dogs (N=10)
- AdOral® semaglutide 14mg has a median bioavailability ~2,5 times higher than the commercial comparator Rybelsus® 14mg in beagle dogs