Release and Availability for Absorption of Prednisone Delayed-Release Formulations During Transit Through TNO Dynamic Gastrointestinal Models (TIM Systems)

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Introduction

- The aim of this study was to determine the release and bioaccessibility (BA) of prednisone from 4 delayed-release colon-targeted formulations during transit through the in vitro dynamic, computer-controlled model of the stomach plus small intestine (tiny-TIM) and the large intestine (TIM-2) – TNO systems
- Triskelion's in vitro computer-controlled, multicompartmental gastrointestinal (GI) models (TIM) give insight into the release, solubility, stability, and availability for absorption of pharmaceuticals and nutrients during passage through the upper gastrointestinal tract (Tiny-TIM system; **Figures 1** and **2**) and the lower gastrointestinal tract (TIM-2 system; **Figures 3** and **4**)
- The tiny-TIM system consists of a gastric compartment and small-intestinal compartment connected by peristaltic valves (**Figure 1**). This model mimics the intraluminal pH, enzyme activity, bile salt concentrations, peristaltic movements, and gastrointestinal transit of the contents. The set-points for gastrointestinal simulation are controlled and monitored by specific computer programs. Released and dissolved drug molecules are removed from the intestinal lumen by semipermeable membrane units connected to the small-intestinal compartment. This allows the assessment of the so-called bioaccessible fraction, ie, the fraction of the drug that is potentially available for small-intestinal absorption
- The TIM-2 system represents the large-intestinal compartment, wherein the following standardized conditions are simulated: body temperature, pH in the lumen, composition and rate of secretion, delivery of a predigested substrate from the ileum, mixing and transport of the intestinal contents, and absorption of water. Complex, high-density, metabolically active, anaerobic microbiota of human origin were used for the pilot experiments but were eliminated from the main study, owing to significant colonic metabolism of prednisone
- The different multiparticulate-based delayed-release formulation strategies that were considered were coated pellets prepared by fluid bed (Wurster) coating method and hot-melt extrusion (HME)-based formulation. The coated pellet formulations were formulated to result in delayed-pulse and sustained-release (SR) profiles in the colon, while the HME formulation was developed to generate delayed sustained-release profiles in the colon based on a pH and time-release concept. The bioaccessibility of the prednisone formulations was compared to the reference Rayos® delayed-release tablets

Figure 1. Two tiny-TIM units in one cabinet, comprising stomach compartment and small-intestinal compartment

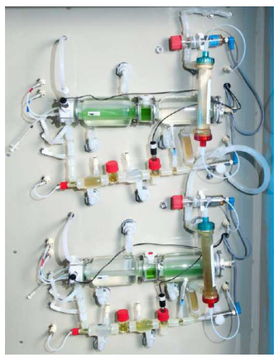


Figure 2. Schematic representation of tiny-TIM. a: gastric compartment; b: small-intestinal compartment; c: gastric secretion; d: pH electrodes; e: duodenal secretion; f: pyloric sphincter; g: filtrate fluid; h: hollow-fiber device with semipermeable membrane

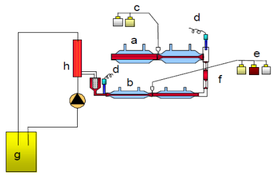


Figure 3. Close-up of the TIM-2 unit mimicking the large intestine

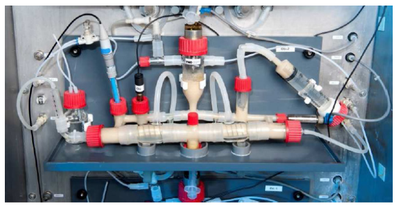
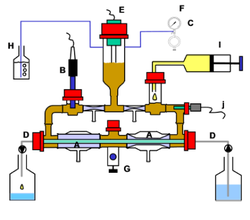


Figure 4. Schematic representation of TIM-2 system



A: peristaltic compartments; B: pH electrode; C: alkali pump; D: filtrate liquid circuit with hollow fibers; E: level sensor; F: N2 gas inlet; G: sampling port; H: gas outlet; I: « ileum effluent » container; J: temperature sensor.

Materials and Methods

- Coated pellets were prepared by drug layering of prednisone onto sugar cores (Suglets – ~1,000 µm dia.) using GPCG-2 Glatt fluid bed coater, followed by functional coating with either Eudragit® FS30D/L30D-55 or Eudragit RL/RS followed by coating with Eudragit FS30D/L30D-55 to generate delayed pulsatile- or sustained-release profiles, respectively
- For delayed SR HME extrudates, Eudragit S100 was granulated by addition of triethyl citrate in the Bohle granulator at 200 rpm impeller speed/500 rpm chopper speed, followed by blending with prednisone and extrusion using a 7.5 mm twin-screw extruder at 50 rpm screw speed and 170°C. The resulting granules were milled and sieved to particle size range of <2.0- 4 µm
- All multiparticulate formulations were encapsulated to have a final dose strength of ~20 gm
- USP apparatus II was used to determine drug release from the encapsulated formulations at 20 mg dose strength using a 3-stage dissolution setup, starting with SGF (pH 1.8), followed by FaSSIF (pH 6.5), and finally simulated colonic fluid -sCoF (pH 7.2)
- Two in vitro computer-controlled, multicompartmental gastrointestinal models were used to study the gastrointestinal behavior of 20 mg prednisone formulations during transit through the upper GI tract (stomach and small intestine – tiny-TIM)[1] and lower GI tract (colon – TIM-2)[2]. Four delayed-release formulations, including Rayos marketed tablet (delayed pulse release) and 3 capsule formulations containing pulse sugar pellets, sustained-release sugar pellets, and SR hot-melt extrusion pellets, were dosed into the stomach compartment of the tiny-TIM system; experiments lasted 4 + 21 hours (tiny-TIM + TIM-2), with GI conditions of young fasted healthy adults simulated during these runs. TIM samples were analyzed with HPLC-UV, and the bioaccessibility (fraction of API available for absorption) was calculated

Table 1. Average gastrointestinal parameters simulated in the TIM systems under simulation of average gastrointestinal physiological conditions of healthy young adults, as used for performance of the main study

Model	Parameter	Condition
Tiny-TIM	Stomach	
	Intake (total)	250 g
	Water and artifical saliva	240 g
	Gastric start residue	10 g
	Gastric emptying T1/2	20 min
	Housekeeper wave	Yes
	Gastric pH	3.0 to 1.7 in 30 min
	Small intestine	7.0
	Concentration bile + pancreatin	20%
	Residence time in tiny-TIM	4 hr
TIM-2	Substrate	Simulated ileal effluent medium for fasted conditions (SIEM without carbohydrates)
	pH gradient	5.8 (t = 0-2 hr) 6.4 (t = 2-10 hr) 6.8 (t = 10-18 hr) 7.2 (t = 18-21 hr)
	Microbiota/buffer	None/citrate buffer
	Residence time	21 hr

Calculation of Results

- Calculation of absolute amounts of prednisone
  The concentrations of prednisone analyzed in the TIM-1 samples were multiplied by the measured volume of the total samples, resulting in absolute amounts (µg or mg)
- Calculation of small-intestinal bioaccessibility:

$$\left(\frac{\Sigma\_tiny-TIM\_filtrate[mg]}{Intake[mg]-gastric\_residue[mg]-small\_intestinal\_residue[mg]}\right)\times 100$$

- The small-intestinal bioaccessibility, measured in tiny-TIM, expressed as percentage of the prednisone intake (20 mg) minus the gastric and small-intestinal residue, is shown in the above equation
- Calculation of large-intestinal bioaccessibility:

$$\left(\frac{\Sigma\_TIM-2\_filtrate[mg]}{Intake[mg]-Prednisone_{tiny-TIM}[mg]}\right)\times 100$$

- The large-intestinal bioaccessibility, measured in TIM-2, is expressed as percentage of the prednisone intake (20 mg) minus the bioaccessible amount in tiny-TIM minus the gastric and small-intestinal residue (tiny-TIM), as shown in the above equation
- Total bioaccessibility
  The total bioaccessibility is the sum of equation (1) and equation (2)
- Calculation of recovery
  The recovery (mass balance) is the sum of all analyzed sample fractions, expressed as percentage of the intake

Results

Drug-Release Studies

Figure 5. Delayed pulse-release profiles from coated pellet (LHS) and reference Rayos tablets (RHS)

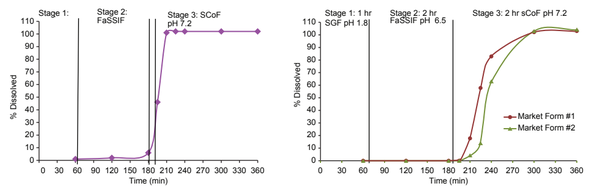
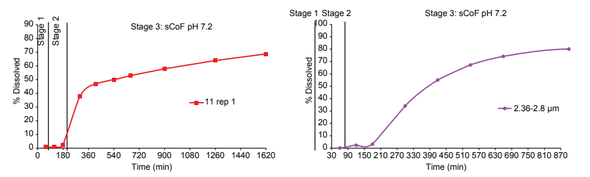


Figure 6. Delayed extended-release profiles from coated pellet of prednisone (LHS) and HME formulation of prednisone (RHS)



Bioaccessibility of Delayed-Release Formulations

Figure 7. Amounts of prednisone as released in tiny-TIM (time points 0-4 hr) and TIM-2 (time points 4-25 hr) from the tablet formulation (#1, blue bars), the capsule with pellets (#3, red bars), the HME capsule (#4, green bars), and the SR capsule (#5, purple bars), mean ± range

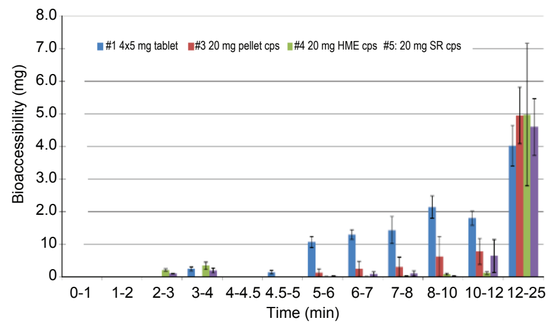


Table 2. Bioaccessible fractions of prednisone [in mg and % of intake] from formulations #1, #3, #4, and #5 after passage through tiny-TIM and TIM-2

Fraction	Formulation #1 4x5 mg tablet	Formulaton #3 20 mg pellet cps	Formulation #4 20 mg HME cps	Formulation #5 20 mg SR pellets
Σ Tiny-TIM filtrate (mg)	0.2±0.1	3.6+3.4	0.5+0.2	0.1+0.1
Tiny-TIM residue (mg)	6.3±0.8	2.3±2.2	0.6±0.3	0.5±0.4
Small intestinal BA (%)	**1.8±0.5**	**18.4±16.8**	**3.8±0.4**	**3.2±1.5**
Σ Tiny-2 filtrate	11.9±0.7	7.9±2.5	5.2±2.3	5.4±1.5
TIM-2 residue	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Large intestinal BA (%)	88.6±10.9	48.5±13.9	27.9±12.6	27.8±7.2
Total bioaccessible fraction (%) (tiny-TIM+TIM-2)	**90.3±11.5**	**66.9±2.9**	**31.7±13.0**	**29.5±5.7**

- Formulations #1 and #3 showed the highest bioaccessibility as released over the experimental period of 25 hr (4 hr transit through the upper GI tract and 21 hr through the lower GI tract). The majority of released prednisone occurred in the lower GI tract within 1 hr after administration to the TIM-2 system, confirming delayed-release performance. Bioaccessibility was lower for both slow-releasing formulations

TIM-2 Luminal Fraction

- Besides the fraction that is absorbed through the tiny-TIM and TIM-2 filtration units, the TIM-2 lumen fraction was measured. Luminal samples reflect a snapshot of the amount of released prednisone from its formulation at the time point of measurement

Figure 8. Amounts of prednisone measured in the lumen of TIM-2 (time points 4-25) from the tablet formulation (#1, blue line), the capsule with pellets (#3, red line), the HME capsule (#4, green line), and the SR capsule (#5, purple line), reflecting the released amount of prednisone

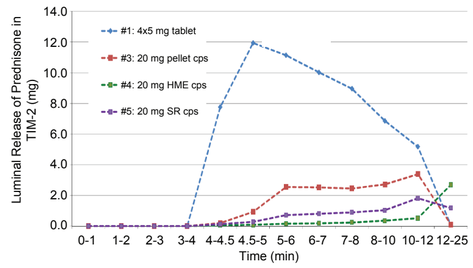


Table 3. Amount of prednisone (mg), as measured in the lumen of TIM-2 per time point

Fraction points (hours after tiny-TIM)	Formulation #1 4x5 mg tablet	Formulaton #3 20 mg pellet cps	Formulation #4 20 mg HME cps	Formulation #5 20 mg SR pellets
4.5	7.8±0.7	0.2+0.1	0.1+0.0	0.1+0.1
5	11.9±1.1	0.9±0.8	0.1±0.0	0.3±0.3
6	11.1±0.7	2.6±2.4	0.2±0.0	0.7±0.6
7	10.0±0.6	2.5±2.3	0.2±0.0	0.8±0.7
8	9.0±1.0	2.5±2.2	0.2±0.0	0.9±0.8
10	6.9±1.2	2.7±1.8	0.4±0.1	1.0±0.7
12	5.2±1.3	3.4±1.2	0.5±0.2	1.8±0.8
25	0.1±0.0	0.1±0.0	2.7±2.1	1.2±1.1

- The release profile, as measured in the TIM-2 lumen samples over time, suggests that formulations #1 and #3 completely released their API during passage through TIM-2 (**Figure 8**)
- In contrast, formulations #4 and #5 did not release their entire API content during the 25-hr experimental period. Extrapolation of the luminal curves of formulations #4 and #5 suggest that release continued after the 25-hr experimental period

Residue Fractions and Recovery

At the end of each experiment in tiny-TIM and TIM-2, the residues of the formulations were collected and analyzed. This gave information on the amount of prednisone remaining in the TIM systems at the end of the experiments as well as the amounts not yet released from the formulations.

The sum of all sample fractions sums up to the recovery of API and indicates whether or not an appropriate amount of API could (i) be recovered from the TIM systems and (ii) be analyzed appropriately.

Table 4. Overview of residual amounts of prednisone (mg) as measured from the tiny-TIM residue, the TIM-2 residue, the recovered amount of formulation after 25 hr, and the overall experimental recovery

Sample fraction	Formulation #1 4x5 mg tablet	Formulaton #3 20 mg pellet cps	Formulation #4 20 mg HME cps	Formulation #5 20 mg SR pellets
Tiny-TIM residue	6.3±0.8	2.3+2.2	0.6+0.3	0.5+0.4
TIM-2 residue	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Formulation residue	0.0±0.0	0.0±0.0	5.5±2.7	13.0±0.6
Recovery	18.5±1.6	13.1±1.4	14.5±2.0	20.2±1.6
Recovery (%)	92%±8%	65%±7%	73%±10%	101%±8%

- No remaining API was detected during analysis of the formulation remainders, confirming complete release from formulations 1 and 2
- High % of prednisone remains unreleased from formulations 4 and 5, as confirmed by high residual amount of formulation residues for 3 and 4 after 24-plus hr in TIM-2

References

1. Verwei M, Minekus M, Zeijdner E, Schilderink R, Havenaar R. *Int J Pharm.* 2016;498(1-2):178-186.
2. Tenjarla S, Romasanta V, Zeijdner E, Villa R, Moro L. *Adv Ther.* 2007;24(4):826-840.
3. Barker R, Abrahamsson B, Kruusmägi M. *J Pharm Sci.* 2014;103(11):3704-3712.
4. Gupta P, et al. CRS, 2016 (Poster 71).

Conclusion

- TIM systems have been used extensively to study drug product performance with a high predictive quality for the in vivo situation. These have also been used as a physiologically relevant alternative to animal PK and dissolution studies (Barker et al, 2014)[3] and can be used as a predictive tool to determine drug release behavior from MR formulations in vivo under dynamic GI conditions
- The pulse-release pellet and the Rayos reference tablets showed the highest bioaccessibility, with the majority of prednisone release occurring in the lower GI tract within 1 hr after administration to the TIM-2 system. Extended-release formulations seemed to show a much slower release of prednisone, which seemed to continue after the experimental period of 24 hr, resulting in lower overall bioaccessibility during the test period and substantiated by a high residual amount of API determined in the formulation remainders
- The lower bioaccessability from the SR sugar pellets and the HME pellets is believed to be in part due to (i) less-than-desirable release rate from the formulations (~70%-80% release) for the optimized SR pellets and (ii) partial ionization of the enteric polymers, leading to incomplete drug release from the slower-release SR formulations. The enteric polymers used in these formulations typically dissolve at a higher threshold pH (pH≥7.0) than the pH setting (pH≤6.8) used in the current TIM-2 setup for up to 18 hr of residence time in this experimental setup
- The results were generally in sync with the dog PK study results, wherein the relative bioavailability rank order was delayed pulse pellets (130%) > Rayos (100%)/SR pellets (100%) > SR extrudates (~63%)
- Specifically, for the above SR formulations, change in the experimental setup parameters for TIM-2 and/or polymeric coatings can help understand mechanistically the loss in the observed bioaccessible fraction and increase in the fraction of API available for drug absorption in the lower GI