Meta-Analysis of the Turnover of Intestinal Epithelia in Preclinical Animal Species and Humans

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ABSTRACT

Due to the rapid turnover of the small intestinal epithelia, the rate at which enterocyte renewal occurs plays an important role in determining the level of drug-metabolizing enzymes in the gut wall. Current physiologically based pharmacokinetic (PBPK) models consider enzyme and enterocyte recovery as a lumped first-order rate. An assessment of enterocyte turnover would enable enzyme and enterocyte renewal to be modeled more mechanistically. A literature review together with statistical analysis was employed to establish enterocyte turnover in human and preclinical species. A total of 85 studies was identified reporting enterocyte turnover in 1602 subjects in six species. In mice, the geometric weighted mean (WX) enterocyte turnover was 2.81 ± 1.14 days (n = 169). In rats, the weighted arithmetic mean enterocyte turnover was determined to be 2.37 days (n = 501). Humans exhibited a geometric WX enterocyte turnover of 3.48 ± 1.55 days for the gastrointestinal epithelium (n = 265), displaying comparable turnover to that of cytochrome P450 enzymes in vitro (0.96–4.33 days).

Statistical analysis indicated humans to display longer enterocyte turnover as compared with preclinical species. Extracted data were too sparse to support regional differences in small intestinal enterocyte turnover in humans despite being indicated in mice. The utilization of enterocyte turnover data, together with in vitro enzyme turnover in PBPK modeling, may improve the predictions of metabolic drug-drug interactions dependent on enzyme turnover (e.g., mechanism-based inhibition and enzyme induction) as well as absorption of nanoparticle delivery systems and intestinal metabolism in special populations exhibiting altered enterocyte turnover.

Introduction

Physiologically based pharmacokinetic (PBPK) models of oral drug absorption and gut-wall metabolism may be implemented at varying degrees of complexity from a simple first-order rate of absorption into the enterocytes located in the gut wall to more sophisticated segmented models of the intestinal tract, such as the advanced compartmental absorption and transit model and the advanced dissolution absorption and metabolism, allowing the incorporation of regional variations in permeability, mucosal volumes, metabolic capacity, and more (Agoram et al., 2001; Darwich et al., 2010). PBPK modeling of drug bioavailability allows the estimation of the gut-wall extraction and further implementation to accommodate mechanistic prediction of drug-drug interactions (DDIs) in the small intestine, including reversible inhibition, mechanism-based inhibition (MBI), and enzyme induction (Fahmi et al., 2009; Rowland Yeo et al., 2010).

Dynamic modeling of intestinal metabolism requires knowledge of the level of enzyme in the gut wall, in which the amount at steady state is a product of the rate of synthesis and degradation [enzyme turnover rate (kdeg)]. Enzyme turnover plays an important role in model-based predictions of MBI effects and enzyme induction in the gut, where the reliance upon kdeg becomes especially apparent for drugs exhibiting high small intestinal metabolism and for substrates of cytochrome P450 (P450) 3A (Yang et al., 2008). Current PBPK models use a lumped first-order enzyme turnover rate to describe the combined turnover of enzyme and enterocytes. Lumping kdeg of the enzymes and the enterocytes does not consider the difference in dynamics between these two in which steady state levels of the enzyme will be disrupted following MBI, whereas the enterocyte steady state levels may not. Widely used values of kdeg in the area of PBPK tend to be informed via indirect measures, such as clinical MBI studies or grapefruit juice studies, in which estimates of kdeg can vary up to fourfold (0.02–0.08 hour⁻¹) (Takanaga et al., 2000a, 2000b; Yang et al., 2008).

To give an indication of the impact of the lumped kdeg on predictions of the level of MBI, simulations were carried out using the Simcyp Simulator v13 (Simcyp, Sheffield, UK) minimal PBPK model. Predictions of level of MBI were performed in the gut wall following five 60 mg doses of oral diltiazem (dose interval of 8 hours), as per Rowland Yeo et al. (2010), varying kdeg between 0.01 and 0.05 h⁻¹ for CYP3A4 in the gut wall, resulting in about 20–30% variation in the activity of CYP3A4 at 1 and 24-hour postdiltiazem dosing (Fig. 1), thus illustrating the importance of a well-characterized kdeg parameter.

Although earlier research has characterized the indirect and in vitro enzyme turnover utilizing meta-analysis, no similar efforts have been made to determine enterocyte turnover (Takanaga et al., 2000a, 2000b; Yang et al., 2008). Physiologically, the enterocytes are produced through cell division of progenitor stem cells in the crypt at the base of the villi in the intestinal tissue. Matured enterocytes will migrate up the crypt-villous axis, where the turnover will be governed by apoptosis or

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ABBREVIATIONS: DDI, drug-drug interaction; GL, gastrointestinal; kdeg, enzyme turnover rate; MBI, mechanism-based inhibition; P450, cytochrome P450; PBPK, physiologically based pharmacokinetics; WX, weighted mean.

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shedding into the gut lumen at the tip of the villi. The time from enterocyte generation to shedding has been reported to occur within days after proliferation (Wilson and Potten, 2004; Malato et al., 2011).

Having reliable estimates of enterocyte turnover may not only impact predictions in the area of DDIs, but may also further aid the predictions of oral absorption of nanoparticle drug delivery systems subject to trapping in the enterocytes, where intestinal shedding may act to limit their absorption (Ensigh et al., 2012).

The aim of the current study was to conduct a comprehensive literature review and meta-analysis of published studies to assess the enterocyte turnover in preclinical species and humans and to identify the most commonly used methods for determining enterocyte turnover. Further simulation-based sensitivity analysis was to be employed to test the sensitivity of the level of enzyme turnover on the level of MBI.

**Materials and Methods**

A comprehensive literature search was performed to identify published data on direct determination of gastrointestinal (GI) enterocyte turnover, life span, cell cycle, or migration rate in healthy adults in preclinical species (including rats, mice, pigs, guinea pigs, rabbits, and hamsters), as well as methods used to determine the turnover using PubMed (1950 to November 2013). The search for human data was extended to include enterocyte turnover in patient populations due to the sparsity of data. Additional sources were identified through publication citations for all species.

Data were analyzed using descriptive statistics, calculating arithmetic weighted means (WX). Where data allowed, the geometric mean (GM) and S.D. of the enterocyte turnover were calculated. Statistical analysis was carried out using Welch’s t test (P < 0.05) with post hoc Dunn-Sidak correction where applicable in Matlab R2010a (Mathworks, Natick, MA) (Supplemental Material).

**Results**

Overall, a total of 85 studies was identified reporting enterocyte turnover in 1602 subjects in six different species. In 84% of the studies, determination of enterocyte turnover was carried out using isotope labeling, including [³H]thymidine and bromodeoxyuridine, and the remaining 16% were carried out using mitotic arrest methods and biopsy analysis. The outcome of the data analysis of enterocyte turnover follows below.

**Methods for Determining the Turnover of Enterocytes**

Various in vivo and in vitro methods were identified for the determination of enterocyte turnover. The GI epithelia differ from other physiologic cell lines as it is a highly organized tissue, allowing the study of cell migration within the tissue (Wilson and Potten, 2004).

**Isotope-Labeling Methods.** The most commonly used method for measuring enterocyte turnover was the pulse-chase method, in which DNA of a population of cells is labeled with an isotopic nucleoside, such as [³H]thymidine, or a synthetic analog, such as bromodeoxyuridine. Following administration of the isotopic nucleotide, the label will incorporate into the DNA during the synthetic phase (S-phase). The cell line can be monitored postlabeling using autoradiography, allowing quantitative determination of the different phases (Quastler and Sherman, 1959; Creamer, 1967; Scragg and Johnson, 1980).

With the knowledge of the duration of the S-phase ($T_S$) and the labeling index of the cell population ($LI$), the turnover of a cell line can be determined in vitro or in vivo, where turnover time ($T_{ot}$) is equal to the ratio of $T_S$ and $LI$ (eq. 1) (Scragg and Johnson, 1980).

$$T_{ot} = \frac{T_S}{LI}$$

The enterocyte turnover can be determined by estimating migration time of cells from the villous-crypt junction to the tip of the villi if their height has been determined. The migration time is therefore equal to the enterocyte life span or turnover (Leblond and Stevens, 1948).

**Mitotic Arrest Methods.** The mitotic arrest method involves stathmokinetic agents (such as colchine, colcemid, vinblastine, or vincristine) arresting cells in the metaphase entering mitosis. This will result in an accumulation of mitotic figures, allowing the determination of number of cells in metaphase by histologic examination. Mitotic arrest can be used in vitro or in vivo by administering the blocking agent via intravenous or intraperitoneal injection (Scragg and...
Johnson, 1980). Cell production rate can be determined from the relation between the number of cells in metaphase against time. The relation can be used to estimate the cell cycle times defined as the inverse relation of the rate of cells entering mitosis ($MI/\tau$) (eq. 2).

$$T_C = \frac{1}{MI/\tau}$$

(2)

Additionally, the villous transit time can be determined as a ratio between the villous population and the cell influx per villous (eq. 3) (Al-Nafussi and Wright, 1982).

$$Transit\ time = \frac{Villous\ population}{Cell\ influx/villous}$$

(3)

Cytophotometric Methods. Cytophotometric methods involve staining of the cell line DNA using dyes, such as ethidium bromide, binding specifically to DNA, and fluorescing with an intensity proportional to the amount of bound DNA. This allows the different cell cycle states to be quantitatively determined along with the proliferative index of a population (Scragg and Johnson, 1980).

Enterocyte Turnover in Preclinical Species

The literature search of enterocyte turnover in healthy adult subjects of preclinical species identified a total of 68 studies ($n$ subjects = 1337). In the rat, 24 studies consisting of 501 subjects were identified (Supplemental Table 1), in which the arithmetic WX GI enterocyte turnover was determined to be 2.37 days. Segmental turnover of the small intestinal epithelia was within close proximity of each other, with arithmetic WX of 1.89, 2.22, and 1.40 days, in the duodenum, jejunum, and ileum, respectively. The colon and stomach appeared to display longer turnover as compared with the small intestine with WX of 2.76 and 2.84 days, respectively. Reported S.D.s were limited to two radiographic studies of the duodenum and jejunum. The geometric WX and combined S.D. of these reports produced an enterocyte turnover of 2.76 ($\pm 1.68$) days ($n$ rats = 14). The scarcity in reported variance statistical verification of any regional differences was limited, albeit reported turnover in the duodenum (1.20 ± 0.20 days, $n$ = 4) and jejunum (3.95 ± 0.54 days, $n$ = 10) displayed a statistically significant difference ($P < 0.05$) (Fig. 2) (Leblond and Stevens, 1948; Stevens and Leblond, 1953; Bertalanffy, 1960; Loran and Althausen, 1960; Messier, 1960; Bertalanffy and Lau, 1962; Koldovsky et al., 1966; Altmann and Enesco, 1967; Shambaugh et al., 1967; Tutton and Barkla, 1976; Sunter et al., 1978, 1979; Menge et al., 1982; Holt et al., 1983; King et al., 1983; Cheeseman, 1986; Finney et al., 1989; Holle, 1991; Alam et al., 1994; Nsi-Emvo et al., 1994; Thomson et al., 1994; Gomes and Alvares, 1998; Macallan et al., 1998; Qi et al., 2009).

In the mouse, a total of 31 studies consisting of 651 healthy adult mice was identified reporting the enterocyte turnover in the small intestinal epithelia (Supplemental Table 2). The geometric WX enterocyte turnover for the GI tract was 2.81 ($\pm 1.14$, $n$ = 169 mice) days. The duodenum, jejunum, ileum, and colon displayed enterocyte turnovers of 2.83 ($\pm 1.06$, $n$ = 40), 2.97 ($\pm 1.05$, $n$ = 35), 2.56 ($\pm 1.05$, $n$ = 35), and 2.67 ($\pm 2.04$, $n$ = 38) days, respectively. All small intestinal segments differed at a statistically significant level from each other ($P < 0.05$), whereas colonic turnover was not significantly different compared with that of the small intestine due to high combined variability (CV = 76%; $P > 0.05$). Gastric epithelial turnover displayed an arithmetic WX of 3.05 ($n$ = 19) days, lacking reported variance (Fig. 3) (Leblond and Messier, 1958; Walker and Leblond, 1958; Quastler and Sherman, 1959; Creamer et al., 1961; Fry et al., 1961, 1962; Lesher et al., 1961; Lipkin and Quastler, 1962; Thrasher, 1967; Grey, 1968; Merzel and Leblond, 1969; Aluwihare, 1971; Chang and Leblond, 1971, 1974; Bottomley and Cooper, 1973; Kovacs and Potten, 1973; Potten et al., 1974; Chang and Nadler, 1975;

Enteroocyte Turnover in Humans

The literature search for human enterocyte turnover data identified 17 studies, with a total sample size of 265 individuals (Supplemental Table 3), reporting the turnover of human GI epithelial cells in the form of mean and S.D. ($n = 86$), mean only ($n = 153$), and ranges ($n = 26$). The majority of turnover values were from colonic, rectal ($n = 60$), and gastric epithelial cells ($n = 36$), whereas jejunum and ileum were limited to sample sizes of 3 and 9, respectively. A geometric WX turnover of 3.48 ($\pm 1.55$) days was obtained for the whole GI epithelia ($n = 86$). Analysis of independent GI segments identified a geometric WX of 1.50 ($\pm 2.90$, $n = 3$) days for the duodenum, 2.83 ($\pm 1.60$, $n = 30$) days for the stomach, and 4.12 ($\pm 1.32$, $n = 53$) days for the colorectal region. The stomach displayed a statistically faster turnover as compared with the colorectal region utilizing post hoc test ($P < 0.05$) (Fig. 4) (Bertalanffy and Nagy, 1961; Cole and McKalen, 1961; Deschner et al., 1963; Lipkin et al., 1963a, 1963b; MacDonald et al., 1964; Shorter et al., 1964, 1966; Bell et al., 1967; Lipkin, 1969; Bleiberg et al., 1970; Weinstein, 1974; Bleiberg and Galand, 1976; Wright et al., 1977; Potten et al., 1992; Patel et al., 1993; Bullen et al., 2006).

Summary Results on Enterocyte Turnover

Data on GI enterocyte turnover were further identified for healthy adult rabbits, guinea pigs, and hamsters, albeit being sparse. The arithmetic WX enterocyte turnover in rabbits, guinea pigs, and hamsters corresponded to 3.40 ($n = 20$ rabbits), 2.08 ($n = 68$), and 1.37 ($n = 29$) days, respectively. Statistical analysis identified mouse enterocyte turnover to be significantly shorter as compared with humans ($P < 0.05$) (Fig. 5) (Sawicki et al., 1968; Sawicki and Rowinski, 1970; Sawicki and Sawicki, 1972; Hattori and Fujita, 1976; Cremaschi et al., 1982, 1984, 1986; Smith et al., 1984; Grant and Specian, 2001).

Discussion

Turnover of the GI epithelia was established based on a large sample size in rats, mice, and humans, and based on sparse data in rabbits, guinea pigs, and hamsters. Statistical analysis indicated a shorter enterocyte turnover in preclinical species as compared with humans, in which the mice displayed a significantly shorter turnover time. The limitation of data did, however, not allow a conclusive analysis for the remaining species in which human turnover was mainly derived from disease populations.

Statistical analysis of regional differences in enterocyte turnover indicated the ileum to display the fastest turnover, followed by the duodenum and jejunum in mice. A similar trend was indicated in rats, although a majority of the data lacked variance and could therefore not be determined statistically. Human data on GI epithelial turnover identified the colorectal cell renewal to be slower as compared with the stomach, whereas data from the small intestinal regions were too sparse to observe any regional differences. Additional indications of regional differences in enterocyte turnover include observations from neonatal pig, in which the distal small intestine displayed a slower turnover (10.2 $\pm 1.5$ days, $n = 16$) as compared with the proximal region (4.7 $\pm 0.4$ days, $n = 16$) (Fan et al., 2001). In summary, evidence for regional differences in enterocyte turnover in humans is inconclusive, which would favor the utilization of a single parameter value of 3.48 ($\pm 1.55$) days for the intestine. Generation of further human enterocyte turnover data may allow the utilization of regional differences in enterocyte turnover, giving a more true depiction of the enzyme renewal along the small intestine. This may be of importance in the prediction of MBIs, in which the perpetrator or victim drug displays a short absorption window and an overall average enterocyte turnover is not representative of the intestinal segment in question.
Several factors have been identified to alter the turnover of the enterocytes, and may therefore account for variability seen in the data set; these factors include the age of the subjects, in which several publications have reported slower enterocyte turnover in neonatal or infant pigs, guinea pigs, rats, mice, and hamsters as compared with adults (Creamer et al., 1961; Koldovsky et al., 1966; Grey, 1968; Rundell and Lecce, 1972; Al-Nafussi and Wright, 1982; Holt et al., 1983; Cremaschi et al., 1986; Fan et al., 2001; Leaphart et al., 2008). In addition, altered enterocyte turnover has been reported in rats subject to numerous environmental changes and disease states, including small intestinal resection, dietary changes, diabetes, and irradiation (Menge et al., 1982, 1983; Cheeseman, 1986; Thomson et al., 1994). It can therefore be concluded that the enterocyte turnover is highly sensitive to numerous environmental factors.

In addition, the method used for determining enzyme turnover may significantly impact the produced value. Turnover based on mitotic figures may greatly underestimate the true turnover of the cell line as in particular the enterocytes are thought to be mainly eliminated via shedding into the gut lumen; in such case, radiolabeling, such as [H]thymidine labeling, is a more reliable candidate for determining turnover. Radiolabeling does, however, impose some ethical issues as a radioactive compound is used; thus, there are little data from healthy volunteers in humans. In situ or ex vivo determination of enterocyte turnover may not be representative of the turnover in vivo as the conditions seen in vivo are difficult to meet in vitro (Messier and Leblond, 1960; Bertalanffy and Nagy, 1961).

The quality of data posed the perhaps most significant limitation in this study, with a large number of publications only reporting mean data or ranges of enterocyte turnover; this was especially true for the human data in which labeling studies tended to be performed in colorectal cancer patients, which may influence the turnover. The enterocyte turnover in mice, rats, and humans could, however, be established in a reasonable large data set.

The human enterocyte turnover, as determined in this analysis (approximately 3.48 days), suggests the GI epithelia to display a comparable rate of renewal to that of the P450 enzymes as determined in vitro, in which Yang et al. (2008) found reported P450 turnover to vary between 0.96 and 4.33 days. The turnover of the enzyme and the enterocytes exhibits hierarchal dependencies in which the individual impact of these processes on enzyme recovery following DDIs is not completely straightforward to compare. The rate of turnover of the enzymes will follow a first-order rate following mechanism-based inhibition, whereas the enterocytes can be assumed to be renewed at a zero-order rate as steady-state levels of the enterocyte population will remain undisrupted following enzyme inhibition. Exploring the impact of nesting enzyme and enterocyte turnover for the prediction of DDIs and MBI requires a more sophisticated model and may be considered particularly well suited for


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