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Review

# *In situ* perfusion in rodents to explore intestinal drug absorption: Challenges and opportunities

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ABSTRACT

The *in situ* intestinal perfusion technique in rodents is a very important absorption model, not only because of its predictive value, but it is also very suitable to unravel the mechanisms underlying intestinal drug absorption. This literature overview covers a number of specific applications for which the *in situ* intestinal perfusion set-up can be applied in favor of established *in vitro* absorption tools, such as the Caco-2 cell model. Qualities including the expression of drug transporters and metabolizing enzymes relevant for human intestinal absorption and compatibility with complex solvent systems render the *in situ* technique the most designated absorption model to perform transporter-metabolism studies or to evaluate the intestinal absorption from biorelevant media.

Over the years, the *in situ* intestinal perfusion model has exhibited an exceptional ability to adapt to the latest challenges in drug absorption profiling. For instance, the introduction of the mesenteric vein cannulation allows determining the appearance of compounds in the blood and is of great use, especially when evaluating the absorption of compounds undergoing intestinal metabolism. Moreover, the use of the closed loop intestinal perfusion set-up is interesting when compounds or perfusion media are scarce. Compatibility with emerging trends in pharmaceutical profiling, such as the use of knockout or transgenic animals, generates unparalleled possibilities to gain mechanistic insight into specific absorption processes.

Notwithstanding the fact that the *in situ* experiments are technically challenging and relatively time-consuming, the model offers great opportunities to gain insight into the processes determining intestinal drug absorption.



**Contents**



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## 1. Introduction

Since oral intake remains the preferred route of drug administration, the need to develop and validate suitable models to evaluate intestinal absorption is self-evident. In the pharmaceutical industry, there is a strong tendency towards the use of *in vitro* tools to study intestinal permeability because of their suitability to be implemented in high-throughput programs (Bohets et al., 2001). The Caco-2 model is nowadays considered the gold standard in intestinal permeability screening. This cell line expresses most of the transporters that are relevant for drug absorption in humans, rendering it useful to study absorption mechanisms. Moreover, for compounds that are passively absorbed and exhibit low intestinal metabolism, permeability values observed in the Caco-2 model allow good predictions of the fraction of the administered dose of a drug that will be absorbed in humans (Artursson et al., 2001). Nevertheless, despite its wide applicability in permeability profiling, this *in vitro* model sometimes fails to address the complexity of intestinal processes which eventually determine *in vivo* intestinal absorption. Two major downsides of using Caco-2 cells include (i) the very low expression levels of P450 enzymes, important for compounds undergoing significant intestinal metabolic extraction and (ii) the absence of a protective mucus layer, causing the cells to be vulnerable upon direct contact with more complex media, including human and simulated intestinal fluids of the fed state. Moreover, the lack of a mucus layer renders the Caco-2 cells more sensitive to pH changes of the apical media, as compared to mammalian intestinal tissue (Lee et al., 2005). Additionally, the Caco-2 model cannot be used for regional absorption studies, for obvious reasons.

Therefore, the use of more robust, biorelevant and versatile models is crucial to understand and predict key mechanisms defining drug transport across the small intestinal barrier. The *in situ* intestinal perfusion technique in rodents has been around for decades and since its introduction by Schanker in 1958, this model has exhibited the ability to adapt to contemporary challenges (Schanker et al., 1958). This versatility has rendered the *in situ* intestinal perfusion model indispensible in the field of intestinal absorption research.

This review aims to provide a critical overview of the use and applications of the *in situ* intestinal perfusion technique in rodents. More specifically, some unique assets of this model will be discussed, such as its applicability in evaluating the transporter–metabolism interplay, regional absorption processes and its compatibility with complex media, which is of utmost importance in the study of food effects and absorption enhancing strategies.

## 2. Permeability assessment–disappearance ($P_{eff}$) *versus* appearance ($P_{app}$)

### 2.1. Measuring disappearance from the perfusion solution–effective permeability

In the original set-up of the *in situ* intestinal perfusion, a segment of the small intestine of an anesthetized animal is cannulated and perfused with a solution containing a predefined concentration of a drug of interest. During the experiment, the animal is kept unconscious and its body temperature is maintained by the use of a heating pad or an overhead lamp. Upon perfusion of the intestinal segment, drug will be absorbed to some extent, depending on its physicochemical and biopharmaceutical properties, and the drug concentration in the perfusion solution will decrease. Through comparison of the donor concentration and the concentration of the solution that exits the isolated segment, the amount of drug that has permeated the apical membrane of the small intestinal barrier (transcellular transport) or has passed through the intercellular space (paracellular transport) can be calculated. By correcting the amount of drug that disappeared from the perfusion solution over time for the donor concentration and the absorptive area of the intestinal segment, the effective permeability value can be calculated using Eq. (1):

$$P_{\text{eff}} = F \times \frac{(1 - C_{\text{out}}/C_{\text{in}})}{2\pi RL} \quad (1)$$

with $F$ the flow rate of the perfusion solution, $C_{\text{out}}$ and $C_{\text{in}}$ the outlet and inlet concentration, respectively, and $R$ the radius and $L$ length of the perfused intestinal segment. Due to the fact that water absorption or secretion upon intestinal perfusion may influence the measured concentrations, correction methods for this water flux have been introduced, including the use of non-absorbable markers in the perfusion solution or gravimetric methods (Sutton et al., 2001).

Cao et al. demonstrated a good correlation between the effective permeability of rat intestine and human intestine for a series of 17 compounds, exhibiting both passive and transporter-mediated absorption Cao et al. (2006). Human intestinal permeability values used in this study were obtained from jejunal perfusion studies using the Loc-I-gut® technique (Lennernäs et al., 1992).

### 2.2. Measuring appearance in the blood–apparent permeability

It is essential, however, to be aware of the fact that the effective permeability does not necessarily give a reliable prediction of the amount of drug that will appear in the blood. Non-specific binding to perfusion tubing or the isolated intestinal segment can result in