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OXIDATIVE DRUG METABOLISM IN THE RAT INTESTINAL WALL. *IN VITRO* - *IN VIVO* CORRELATIONS

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Intestinal drug metabolism may have important pharmacokinetic and/or toxicological consequences due to its rapid and significant modification by various dietary substances. We studied cytochrome P-450 mediated drug metabolism in different *in vitro* preparations of rat small intestine (everted segments, isolated mucosal cells and subcellular preparations). The aim of our studies is to make an adequate extrapolation of the data obtained *in vitro* to their *in vivo* significance.

7-Ethoxycoumarin (7-EC) and 1-naphthol were model compounds used to evaluate a proper cell isolation procedure and to assess the capability and biochemical characteristics of the *in vitro* systems to metabolize xenobiotics [1-3]. The intestinal metabolism of two pairs of structurally related drugs was studied *in vivo* and *in vitro*. Phenacetin (Ph) and ethoxybenzamide (EB) are analgesic drugs metabolized predominantly by oxidative O-deethylation and subsequent conjugation with sulphate or glucuronic acid. Hexamethylmelamine (HMM) and pentamethylmelamine (PMM) are antitumor agents that are metabolized both in man and rat by successive N-demethylations.

RESULTS AND DISCUSSION

A summary of the *in vitro* - *in vivo* correlations is presented in Table 1 with respect to the substrates studied and the *in vitro* system employed. A resonable correspondence between the metabolism in microsomes and cells is generally observed in control rats when corrections are made for microsomal recovery and cell viability. When studying induction, however, differences can arise due to toxicity of the inducing compound [4].

From the four drugs studied *in vivo* only HMM was subject to considerable oxidative intestinal first-pass metabolism [5, 6] in control rats. For phenacetin a 53% first-pass effect occurred only after previous treatment of the rats with 3-methylcholanthrene [7]. In the latter case the maximal intrinsic clearance (V_{max}/K_m) adequately predicted such behaviour [8]. The most remarkable observation was that PMM, being nothing else than HMM missing one of its six CH_3-moieties, did not show detectable intestinal metabolism. This in contrast with HMM, which is 73% extracted during its first passage in the gut wall. Such a difference was not predicted from *in vitro* data in isolated cells, but could be reproduced using perfused intestinal segments as an *in vitro* tool [9].

The final conclusion from our studies is that isolated intestinal cells are an easy and reliable tool to detect metabolism in the small intestine and to study its biochemical characteristics. However, to avoid false-positive predictions on the *in vivo* extraction ratio (EB, PMM) one has to be aware that absorption and metabolism are competitive processes *in vivo*. It is therefore necessary to study concomitantly the compound's absorption (and metabolism) in a different *in vitro* system.

Table 1. Presystemic metabolism in the rat small intestine from enzyme kinetic data in rat intestinal *in vitro* preparations and from *in vivo* experiments

Substrate[1]	In vitro system	V_{max}^{app}[2]	Cl_{int}[3]	$E_{g,calc}$[4]	$E_{g,vivo}$[5]	Ref.
7-EC	Microsomes	0.49	.33	.06 - .25	-	[1, 10]
	Cells	0.24	.13	.02 - .11		
PH	Microsomes	0.24	.10	.02 - .09	ND[1,5]	[7, 10]
	Cells	0.16	.09	.02 - .08		
EB	Microsomes	3.5	.39	.07 - .28	ND[1,5]	
	Cells	16.0	4.34	.54 - .84		
HMM	Microsomes	5.6	3.26	.40 - .76		
	Cells	7.0	3.45	.41 - .77	0.7	[5, 6]
	Segments	39.0	6.68	.83[6]		
PMM	Microsomes	-				
	Cells	6.7	4.05	.45 - .80	ND[1,5]	[5, 6, 9, 11]
	Segments	*		.27[6]		

[1]Abbreviations are 7-EC: 7-ethoxycoumarin, PH: phenacetin, EB: ethoxybenzamide, HMM: hexamethylmelamine and PMM: pentamethylmelamine. ND means not detectable.

[2]V_{max} is expressed in nmol·min^{-1}·g intestine^{-1} measured as total metabolites produced and is corrected for microsomal recovery (45%) and cell viability (85-95%).

[3]$Cl_{int} = V_{max}/K_m$ in ml·min^{-1}·kg rat^{-1}, assuming 8.0 g small intestine/250 g rat.

[4]E_g is the intestinal extraction ratio, calculated from $E_g = Cl_{int}/(Q_{muc} + Cl_{int})$. Mucosal blood flow ($Q_{muc}$) is varied between 1 and 5 ml·min^{-1}·kg^{-1}.

[5]$E_{g,vivo}$ calculated from $E = 1 - (AUC_{id}/AUC_{pv})$. If AUC_{id} and AUC_{pv} are not significantly different, no E_g can be calculated. Therefore ND means E_g < 10-20%.

[6]Extraction-ratio in segments was calculated dividing metabolic clearance by total clearance. See also 9 .

*No Michaelis-Menten kinetics. No K_m or V_{max} can be estimated.

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