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GLUCURONIDATION IN THE RAT INTESTINAL WALL

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Since the intestine is an important entry point for xenobiotics it is not surprising that all major biotransformational enzyme activities can be found in the intestinal wall [1-4]. In comparison with phase I drug metabolism, phase II metabolism is relatively important [1, 5]. Systemic availability of orally administered phenolic xenobiotics like phenol [6], 1-naphthol [7] and morphine [8] can be very effectively reduced by intestinal first-pass metabolism. Indirect pharmacokinetic evidence suggests that also β-sympathomimetics are metabolized in the intestinal wall during absorption [5]. The glucuronidation of more than 40 different compounds in various preparations of the intestinal wall has been established ([9] for review). This communication summarizes our recent studies of the intestinal glucuronidation of 1-naphthol, morphine and six β_2-sympathomimetics in the rat.

METHODS

Intestinal epithelial cells were isolated from male Wistar rats using procedures that have been described in detail elsewhere [10-13]. A mixture of villus tip cells and crypt cells is obtained with this method [13]. Intestinal microsomes are prepared from isolated cells by Ultra-Turrax homogenization and differential centrifugation [10, 11]. Isolated intestinal segments are perfused on both the mucosal and serosal surface [14].

COMPARISON OF IN VITRO SYSTEMS (1-NAPHTHOL)

The glucuronidation of 1-naphthol was studied on the subcellular (microsomes), cellular (isolated mucosal cells) and organ level (perfused segments) in order to elucidate the qualitative and quantitative relationship between these model systems.

The microsomal UDP-glucuronosyltransferase (UDPGT) appears to follow an ordered Bi-Bi reaction sequence in which 1-naphthol and UDP-glucuronic acid (UDPGA) are the first and second binding substrates and UDP and 1-naphthol glucuronide the first and second products, respectively [15]. Latency of UDPGT in intestinal microsomes is comparable to latency in liver microsomes [11] and is possibly explained by endproduct inhibition by UDP [15].

The glucuronidation rate in intestinal microsomes (whether activated or not by $MgCl_2$ and Triton X-100) is substantially higher than in isolated cells [11]. Glucuronidation in mucosal cells is directly dependent on supply of UDPGA from extracellular carbohydrates (glucose or fructose) and is decreased by adding D-galactosamine [16]. No intracellular carbohydrate

reserves, able to support glucuronidation, are present in intestinal cells [16].

In isolated perfused intestinal segments the situation is more complicated. Glucuronidation after mucosal administration of 1-naphthol appears to be limited by entry of the substrate into the metabolizing compartment. When 1-naphthol is added to the serosal side a 3- to 4-fold higher glucuronidation rate is observed than after mucosal administration [14].

These results suggest that both cellular uptake of substrate and availability of endogenous UDPGA can be limiting for intestinal glucuronidation in the rat. Intestinal cells or perfused segments are to be preferred over microsomes to investigate intestinal metabolism of xenobiotics. Microsomes can be used to clarify biochemical details such as enzyme kinetics and activation phenomena.

GLUCURONIDATION OF MORPHINE AND β_2-SYMPATHOMIMETICS

The glucuronidation of morphine and six β_2-sympathomimetics (orciprenaline, terbutaline, fenoterol, salbutamol, ritodrine, bamethan) in isolated mucosal cells was investigated. Only conjugates with glucuronic acid could be detected. Intrinsic clearances (Cl_{int}), calculated from cellular V_{max} and K_m^{app} values varied from 0.15 (terbutaline) to 0.80 (bamethan) ml/min·kg rat [17]. Predicted intestinal extraction ratios of 0.1 (terbutaline) to 0.9 (bamethan) can be calculated from a model in which both Cl_{int} and mucosal blood flow are taken into account [18]. The estimated intestinal extraction ratio of morphine (0.3) reasonably well predicts the *in vivo* observed extraction ratio (0.5, Ref. 8).

These results suggest that isolated mucosal cells are a suitable model system that presents a reasonably accurate prediction of intestinal glucuronidation. Additional *in vitro* and *in vivo* experiments are presently being performed to definitely establish the main factors governing the extent of intestinal first-pass metabolism in the intact animal.

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