Enterocytes in the upper region of the small intestine play a significant role in the absorption of nutrients as well as the first-pass metabolism of many orally ingested xenobiotics. For this reason, functionally active and stable intestinal subcellular fractions are required to assess the first-pass metabolism of drugs by cytochrome P450, UDP-glucuronosyltransferases and other drug-metabolizing enzymes. The present study summarizes enzymatic activity data from individual and pooled human and animal intestinal microsomes that were prepared from fresh duodenum/jejumum based on an enterocyte elution method with EDTA and various protease inhibitors. All samples were analyzed for their ability to catalyze testosterone 6β-hydroxylation, 4-methylumbelliferyl glucuronidation, and NADPH-cytochrome c reduction. Microsomes prepared from chemically eluted enterocytes had substantially greater CYP3A activity than those prepared from small intestinal samples subjected to mechanical scraping. Freezing/thawing small intestinal microsomes for up to 5 cycles did not cause significant loss of CYP3A, NADPH-cytochrome c reductase or UDP-glucuronosyltransferase (UGT) activity. These results suggest that our elution method for processing small intestines is well suited to preserving microsomal enzymatic activities.

ABSTRACT

Enterocytes from fresh small intestinal (duodenum and jejunum) tissue from various species were used to determine a variety of cytochrome P450 (CYP) and UDP-glucuronosyltransferase (UGT) activities. These results suggest that our elution method, which was performed with or without protease inhibitors, is well suited to preserving small intestinal CYP activities and other drug-metabolizing enzymes. The present study summarizes enzymatic activity data from individual and pooled human and animal intestinal microsomes that were prepared from fresh duodenum/jejumum based on an enterocyte elution method with EDTA and various protease inhibitors. All samples were analyzed for their ability to catalyze testosterone 6β-hydroxylation, 4-methylumbelliferyl glucuronidation, and NADPH-cytochrome c reduction. Microsomes prepared from chemically eluted enterocytes had substantially greater CYP3A activity than those prepared from small intestinal samples subjected to mechanical scraping. Freezing/thawing small intestinal microsomes for up to 5 cycles did not cause significant loss of CYP3A, NADPH-cytochrome c reductase or UDP-glucuronosyltransferase (UGT) activity. These results suggest that our elution method for processing small intestines is well suited to preserving microsomal enzymatic activities.

INTRODUCTION

Metabolic enzymes (Phase I and II) within the mature enterocytes of the villus tips. Enterocytes are rapidly renewed and the lifespan of the enterocyte is a few days, hence the enterocytes are less metabolically active than cells found in the lower parts of the villus. In particular, the majority of drug-metabolizing enzymes are located in the duodenum and jejunum and that CYP content dramatically decreases distal to the jejunum. It is therefore important to ensure that the majority of enterocytes that are processed to microsomes and other metabolically suitable subcellular fractions are derived from the upper portions of the duodenum and proximal jejunum.

Traditionally, intestinal microsomes are prepared from enterocytes that have been collected by one of two general methods, namely mechanical scraping of the intestinal lumen or elution of enterocytes.

Scraping Method

- Intestine is cut longitudinally and the lumen is exposed
- A gel knife or glass slide is used to scrape the mucosa and discard the entercytes
- This leads to contamination with unwanted cell types or immature enterocytes
- Quality can vary greatly between preparations
- Many of the enterocytes are lysed and exposed to digestive enzymes found in the lumen
- Harvesting is quick and total protein yield is high, but the specific activity of CYP enzymes and other drug-metabolizing enzymes is low

Elution Method

- Intestine is cut into manageable sections (8-12 inches)
- Enterocytes from the intestinal lumen are isolated by an elution buffer (typically chemically-based with protease inhibitors)
- Harvesting is time-consuming and protein yield is low, but the specific activity of CYP enzymes and other drug-metabolizing enzymes is high

MATERIALS AND METHODS

In this study, microsomes were prepared with an elution procedure with EDTA and various protease inhibitors. For comparative purposes, intestinal microsomes were also prepared by a scraping method with or without protease inhibitors.

Enterocyte harvest

Enterocytes were harvested from fresh small intestinal (duodenum and jejunum) tissue from various species. An ethylenediaminetetraacetic acid (EDTA)-based elution method with various protease inhibitors, including phenylmethylsulfonyl fluoride, was used to separate mature enterocytes from the underlying lamina propria.

Preparation of microsomes

The eluates were subjected to low-speed centrifugation to concentrate and wash the enterocytes. The concentrated enterocyte pellets were suspended in homogenization buffer containing protease inhibitors and mechanically lysed with a motor-driven Teflon pestle.

Teflon pestle.

Centrifugation of the homogenates resulted in preparation of S9 fractions, which were subjected to ultracentrifugation to isolate the microsomal fraction. Microsomes were resuspended in 250 μM sucrose and stored at -70°C or below.

Note: These microsomes were prepared with buffers containing the protease inhibitor phenylmethylsulfonyl fluoride (PMF), which irreversibly inhibits carboxylesterases.

Enzymatic assays

Small intestinal microsomes from various species were incubated with testosterone, 4-methylumbelliferyl glucuronidation, or cytochrome c to measure the activities of CYP3A, UDP-glucuronosyltransferase (UGT), and NADPH-cytochrome c reductase, respectively, as essentially described in Pearce et al.

Figures 1-2. In all species, CYP3A and UGT activity was considerably greater in enterocytes than in crypt cells. Microsomes prepared by XenoTech’s elution procedure can be stored at -70°C or below for at least nine months without loss of CYP3A4 activity. The same results were obtained for UGT activity.

RESULTS

Individual samples of human intestinal microsomes were assayed for testosterone 6β-hydroxylase activity to assess the inter-individual variation in CYP3A4 expression. Testosterone 6β-hydroxylase activity varied about 10-fold from one sample to the next (from 283 to 2620 pmol/mg protein/min), as shown in Fig. 5.

The results of this study demonstrate that microsomes prepared from various species using an EDTA-based elution method with various protease inhibitors possess systemically greater CYP3A and UGT activity compared with microsomes prepared by mechanical scraping of the intestine. Intestinal microsomes prepared by XenoTech’s elution method are stable over multiple freeze/thaw cycles and can be stored at -70°C or below for at least nine months when stored at -70°C or below for at least nine months.

REFERENCES


#181