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Original article

A new technique for preparing precision-cut slices from small intestine and colon for drug biotransformation studies

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Abstract

Introduction: A new technique was developed to prepare precision-cut slices from small intestine and colon with the object of studying the biotransformation of drugs in these organs. **Methods:** Rat intestinal slices were prepared in two different ways. In the first method, slices were punched out of the small intestine. In the second method, precision-cut slices were made from agarose-filled and -embedded intestines, using the Krumdieck tissue slicer. This method was also applied to colon tissue. Viability of the slices was determined by analysis of intracellular ATP and RNA levels and morphology. Drug metabolizing activity was studied using lidocaine, testosterone, and 7-ethoxycoumarin (7-EC) as phase I substrates, and 7-hydroxycoumarin (7-HC) as a phase II substrate. **Results:** Precision-cut slices made from agarose-filled and -embedded intestine better preserved ATP levels than tissue that was punched out of the intestinal wall. After 24 h of incubation, morphology in precision cut-slices showed was quite well preserved while punched out tissue was almost completely autolytic after incubation. In addition, total RNA amount and quality was much better maintained in precision-cut slices, when compared to punched out tissue. Both intestinal slices and punched-out tissue showed high, and comparable, phase I and phase II biotransformation activities. **Discussion:** It is concluded that preparing precision-cut 0.25 mm slices out of agarose-filled and -embedded intestine provides an improvement, compared with punched-out tissue, and that both intestinal and colon slices are useful preparations for in vitro biotransformation studies.

Keywords: Biotransformation; Rat; Small intestine; Colon; Morphology; Methods

1. Introduction

The intestines can contribute significantly to the biotransformation of xenobiotics because of their high content of drug-metabolizing enzymes (Krishna & Klotz, 1994).

Drug safety issues such as potential drug–drug interactions, species differences, and prediction of the in vivo clearance and toxicity can, in principle, be studied using in vitro preparations, as has been proven with several liver-derived in vitro models in the past. One particular model, the model of precision-cut liver slices has been shown to be very valuable for all these aspects while at the same time, simple and convenient to use (Lerche-Langrand & Toutain, 2000). The slice technique has also been successfully applied to kidney and lung tissue in studies on drug metabolism and toxicity, as reviewed by us before (de Kanter, Monshouwer, Meijer, & Groothuis, 2002).

Abbreviations: 7-EC, 7-ethoxycoumarin; 7-HC, 7-hydroxycoumarin; 7-HC GLUC, 7-hydroxycoumarin glucuronide; 7-HC SULF, 7-hydroxycoumarin sulphate; MEGX, monoethylglycinexylidide.

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Analogous to the model of precision-cut liver, lung, and kidney slices, our present aim was to develop a convenient and simple in vitro slice-model of the intestine for biotransformation studies. Until now, slices from intestinal tissue have only been used rarely. Examples are studies on the metabolism of drugs in human intestinal slices (Vickers et al., 1992, 1995, 2001) and the metabolic activation in rat colon slices (Malfatti, Connors, Mauthe, & Felton, 1996).

In a previous report, we showed that both rat and human small intestinal slices showed high biotransformation activities towards four model compounds, when compared to liver, lung or kidney slices (de Kanter et al., 2002). In contrast to the observed high capacity of intestinal slices for biotransformation processes, ATP levels were relatively low in these slices that were punched out of the intestinal wall (de Kanter et al., 2002). Therefore, we tried to improve the particular preparation technique in order to retain a better viability of the intestinal slices. We considered the possibility that the thickness of the punched out intestinal tissue was too large to allow efficient supply of oxygen and substrates to all the cells. Therefore, we prepared slices of 0.25 mm thickness, perpendicular to the intestinal wall, by filling and embedding the intestines in agarose and by using the Krumdieck tissue slicer. Viability was assessed by measuring intracellular ATP and RNA levels, and by examining histomorphology. Drug-metabolizing activity was studied through the quantification of the formed metabolites from incubations with lidocaine, testosterone, 7-ethoxycoumarin (7-EC), and 7-hydroxycoumarin (7-HC) as model substrates. Furthermore, we applied the technique of filling and embedding intestines in agarose on large intestine (colon), and compared the metabolic capacity of slices from small intestine and colon.

2. Methods

2.1. Materials

The following compounds were obtained from the sources indicated: lidocaine from Centrachemie (Etten-Leur, the Netherlands); 16β-hydroxytestosterone from Steraloids (Newport, RI, USA); 7-EC from Fluka (Buchs, Germany); *N*-benzylimidazole, 2β-, 6β-, and 11β-hydroxytestosterone, testosterone, androstenedione, 7-HC, 7-hydroxycoumarin glucuronide (7-HC GLUC), and low melting agarose (type VII-A) from Sigma-Aldrich Chemie (Zwijndrecht, the Netherlands); amphotericin B (Fungizone) and Williams' medium E (with Glutamax) from Invitrogen (Breda, the Netherlands). Monoethylglycinexylidide (MEGX) was a kind gift of AstraZeneca (Södertälje, Sweden), and 7-hydroxycoumarin sulphate (7-HC SULF) was a kind gift from GlaxoWellcome (Herts, UK). All other chemicals were of analytical grade and were obtained from commercial sources.

2.2. Rat tissue

Male Wistar (HsdCpb:WU) rats (Harlan, Horst, the Netherlands) were housed in standard cages and had free access to food (standard 'RMH' chow, Hope Farms, Woerden, the Netherlands) and tap water. All experiments were performed with approval of the animal experimental regulatory authorities concerned. Rats (mean weight, 390 g) were anaesthetised by isofurane and N_2O/O_2, and the intestines were excised and placed in Krebs–Henseleit buffer (KHB) containing 10 mM HEPES and 25 mM glucose, pH 7.4, on ice. The intestines were flushed thoroughly with ice-cold Krebs–Henseleit buffer to remove the contents. Small pieces of each organ were cut off before the organs were excised and snap-frozen to determine in vivo ATP levels as described in 'viability of slices and punches'.

2.3. Preparation of slices and punches

To discern these agar embedded slices (see below) from punched-out tissue slices, the latter will be further called 'punches' in this paper. Slices and punches were prepared from the same intestine, and care was taken that they were prepared from the same region of the intestine. Punches were made using skin biopsy cores (diameter, 3 mm) from Stiefel (Sligo, Ireland), which were pushed through the intestinal wall after the intestine was cut open. For some experiments, the muscle layer was carefully removed (stripped).

To prepare agarose-filled slices, the intact intestines were first cut in 5–10 cm parts that were subsequently ligated on one side. These parts were then filled with 3% (w/v) low melting agarose solution in 0.9% (w/v) NaCl at 37 °C and were allowed to gel in ice-cold Krebs–Henseleit buffer. The agarose-filled intestines were cut in 1 cm parts and were embedded in the agarose solution at 37 °C using the Tissue Embedding Unit from Alabama R&D (Munford, AL, USA) so that agarose gel cylinders with a diameter of 16 mm were formed (Fig. 1). These cylinders were used to prepare precision-cut slices, with a diameter of 16 mm and a thickness of 0.25 mm, using a Krumdieck tissue slicer (Alabama R&D), precooled and filled with oxygenated, ice-cold (0–4 °C) Krebs–Henseleit buffer (KHB). Slices and punches were stored in ice-cold KHB until use for a maximum of 2 h between excision from the rat and start of the incubation. When the slices were transferred to the incubation plates (see below), the agarose surrounding the slices was separated from the slice, so that only the ring of intestinal tissue (diameter about 3–5 mm) was used, as illustrated in Fig. 1.

2.4. Incubation of slices and punches

Slices and punches were incubated in 3.2 ml Williams medium E, prewarmed and gassed with 95% O_2/5% CO_2,

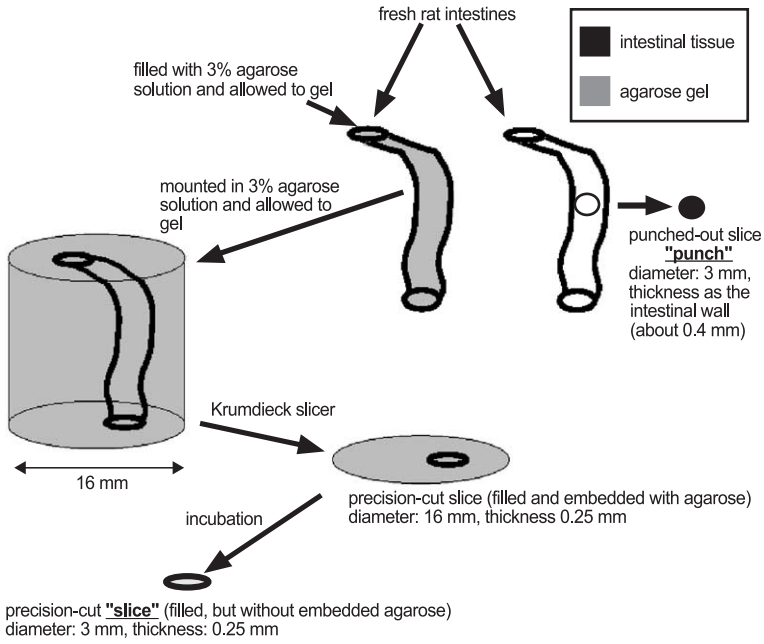

Fig. 1. Schematic of the preparation of precision-cut intestinal slices using filled and embedded intestinal tissue and the Krumdieck tissue slicer.

and supplemented with glucose (final concentration, 25 mM). To inhibit bacterial and fungus growth, gentamicin (50 μg/ml) and amphotericin B (Fungizone; 2.5 μg/ml) were added. These agents are poorly metabolized themselves and therefore are assumed not to interfere with biotransformation enzymes. Slices were individually incubated in 6-well culture plates, which were placed in a plastic container and gassed with humidified 95% O_2/5% CO_2, and shaken back and forth (90 times/min) in a cabinet at 37 °C.

2.5. ATP measurements

ATP content was determined by rapidly putting tissue in 1 ml 70% ethanol (v/v) containing 2 mM EDTA (pH 10.9) and snap-freezing in liquid nitrogen. After storage at −80 °C and homogenizing by sonication, ATP extracts were diluted 10 times with 0.1 M Tris–HCl/2 mM EDTA solution (pH 7.8) buffer to lower the ethanol concentration. The ATP content was measured using ATP Bioluminescence Assay Kit CLS II from Roche (Mannheim, Germany) and a 96-wells Lucy1 luminometer (Anthos, Durham, NC, USA).

2.6. Total RNA analysis

Total RNA was extracted by putting the intestinal tissue in RNAlater (Ambion, Austin, TX, USA) at 4 °C. RNA was then extracted within a month of storage at 4 °C using Rneasy® mini kit (Qiagen, Crawley, UK). The quality of the isolated RNA was assessed using the RNA 6000 Nano Assay and the Agilent 2100 bioanalyzer (Palo Alto, CA, USA). The bioanalyzer uses gel electrophoresis in the confines of a microfabricated chip and highly sensitive laser-induced fluorescence detection using an intercalating dye, which is added to the polymer.

2.7. Morphology

To study histomorphology, slices and punches were fixed in formaline and were stored at 4 °C. After embedding in paraffin, cross-sections were made and stained by haematoxylin and eosin staining using standard procedures.

2.8. Metabolic activity of slices and punches

Metabolism of lidocaine (5 mM), testosterone (0.25 mM), 7-EC (0.5 mM), and 7-HC (0.5 mM) was studied in slices and punches by the addition of 100× concentrated stock solutions in water (lidocaine), or methanol (testosterone, 7-EC, and 7-HC). Metabolism was studied during 3 h of incubation, and was shown to be linear during 3 h of incubation. Medium samples (1 ml) of 7-EC incubations were acidified by adding 10 μl 2 M HCl, after sampling, in order to prevent spontaneous formation of metabolites that occurs when the pH of the incubation exceeds 7.0 (de Kanter et al., 2002). Lidocaine, 7-EC, and 7-HC medium samples were stored at −20 °C until analysis. Preliminary experiments showed that no significant amounts of metabolites were retained in the slices (results not shown). Samples were analyzed using HPLC as described earlier for the MEGX metabolite of lidocaine (Bargetzi, Aoyama, Gonzalez, & Meyer, 1989) and for the metabolites of 7-EC and 7-HC (Walsh, Patanella, Halm, & Facchine, 1995). For all other metabolites, we used authentic standards. For the metabolic biotransformation of testosterone, slices/punches

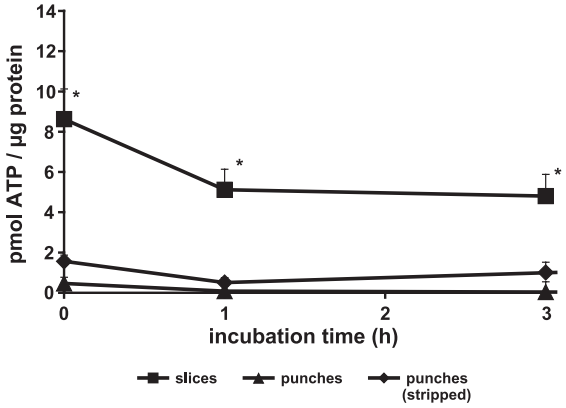

Fig. 2. ATP levels in small intestine slices and punches during 3 h of incubation. Data are expressed as mean±S.E.M. from 4–7 independent experiments, in which the mean of 3 slices (-■-) and punches (-▲-) was determined, at each time point. Stripped punches (-◆-) refer to intestinal preparations from where the muscle layer was stripped of (see text). *p<0.05 vs. punches.

and their incubation medium were harvested together and were homogenized using sonication to extract the metabolites from the slice. The homogenates were stored at −20 °C. After thawing, 5 µl 11β-hydroxytestosterone, dissolved in methanol, was added as internal standard to 1 ml of the homogenate and 6 ml dichloromethane was then added. After removal of the water phase and protein aqueous interphase, the organic phase was evaporated, and testosterone and its metabolites were dissolved in 130 µl 50% (v/v) methanol which was analyzed using HPLC as described earlier (van 't Klooster, Blaauboer, Noordhoek, & van Miert, 1993).

2.9. Protein content of slices and punches

After incubation, five slices or punches from each organ were taken and homogenized in their own incubation medium by sonication and then diluted with 0.1 M NaOH. The protein content of the diluted homogenate was determined using Bio-Rad protein assay dye reagent (Bio-Rad, Munich, Germany) against a BSA standard curve. All metabolic activities and ATP levels are expressed per microgram (µg) protein.

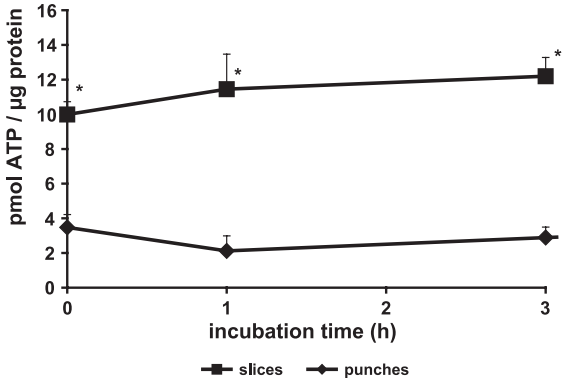

Fig. 3. ATP levels in colon slices and punches during 3 h of incubation. Data are expressed as mean±S.E.M. from four independent experiments, in which the mean of 3 slices (-■-) and punches (-◆-) was determined, at each time point. *p<0.05 vs. punches.

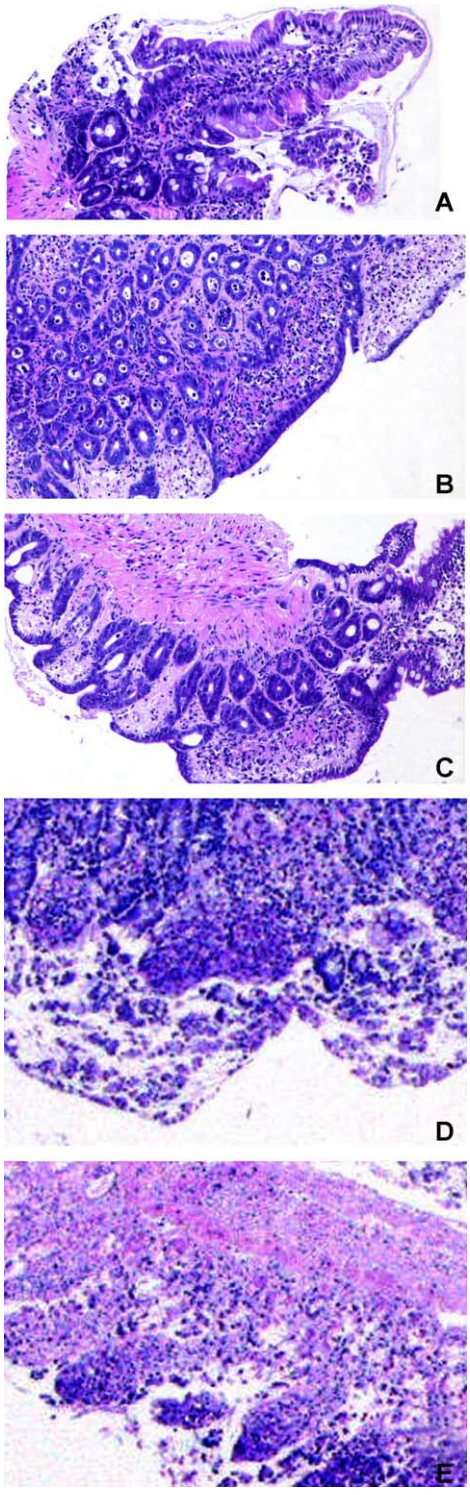

Fig. 4. Morphology of small intestine (jejunum), after filling and embedding in agarose and slicing, but not incubated (A), after incubation for three hours (B), and 24 h (C). Morphology of punched-out intestinal tissue after incubating for 8 (D) and 24 h (E).

3. Results

3.1. Viability as judged by ATP content

To determine the relative viability in culture of intestine slices and punches, ATP levels were assessed in vivo by cutting a small piece of tissue from the intestine or colon from the anaesthetised rats, before, and directly after preparation of the slices and punches, and after 1 and 3 h of incubation.

In small intestinal punches with the muscle layer stripped off, ATP levels were retained at slightly higher levels than in punches with the muscle layer still attached (Fig. 2). ATP levels in precision-cut slices that were made from agarose-filled and -embedded small intestine and colon were significantly higher than ATP levels in punches from these organs, at all time points ($p<0.05$) as shown Figs. 2 and 3.

ATP levels in precision-cut slices, but less so for punches, were up to fourfold higher than in vivo values, which were 1.8±0.9 and 2.4±0.7 pmol ATP/μg protein for small intestine and colon, respectively (values±S.E.M., $n=7$). The ATP level of the tissue remained constant during washing (1.4±0.4 pmol ATP/μg protein) and embedding (1.6±0.7 pmol ATP/μg protein), but increased during slicing to 8.6±1.5 pmol ATP/μg protein. We also observed this phenomenon in earlier studies for the in vivo values of liver, lung, and kidney, compared with precision-cut slices from these organs (de Kanter et al., 2002) which could be ascribed to ATP synthesis at 4 °C in the oxygenated KHB.

In all intestinal slice preparations, ATP levels were decreased after 24 h of incubation to 40–60% of the values after 3 h of incubation (not shown).

3.2. Viability as judged by morphology

To get more insight in the viability of small intestine and colon slices and punches, microscopic examination was undertaken. All sections were stained with haematoxylin and eosin and were viewed at a magnification of ×100.

In Fig. 4a, a section of an agarose-filled and -embedded intestinal slice is shown that is not incubated, showing preserved morphology. After 3 h of incubation (Fig. 4b), flattening of the villi is observed in small intestine slices, and multifocal crypts are mildly dilated by sloughed epithelial cells and the lamina propria is expanded by necrotic debris. Also after 24 h of incubation (Fig. 4c), the lamina propria is expanded by necrotic debris and edema, and multifocal crypts are mildly dilated by sloughed epithelial cells. However, the overall morphology and architecture of the slice are quite well preserved, with intact epithelial cells and minimal autolytic changes after 24 h of incubation.

In contrast to the slices, punches from the intestine show damaged morphology after incubation for 3 h with diffuse autolytic changes: moderately expanded lamina propria by edema and necrotic debris and diffuse sloughing of epithelial lining from the surface of the villi (Fig. 4d). After 8 h, the punches are almost completely autolytic (Fig. 4e).

3.3. Viability as judged by RNA

To get more insight in the viability, total RNA was extracted from the preparations and analysed for integrity by gel separation of the 18S and 28S bands using an Agilent 2100 Bioanalyser. As shown in Fig. 5, total RNA in slices incubated for 6 and 24 h showed a typical, intact RNA pattern, comparable to intestinal tissue that was not

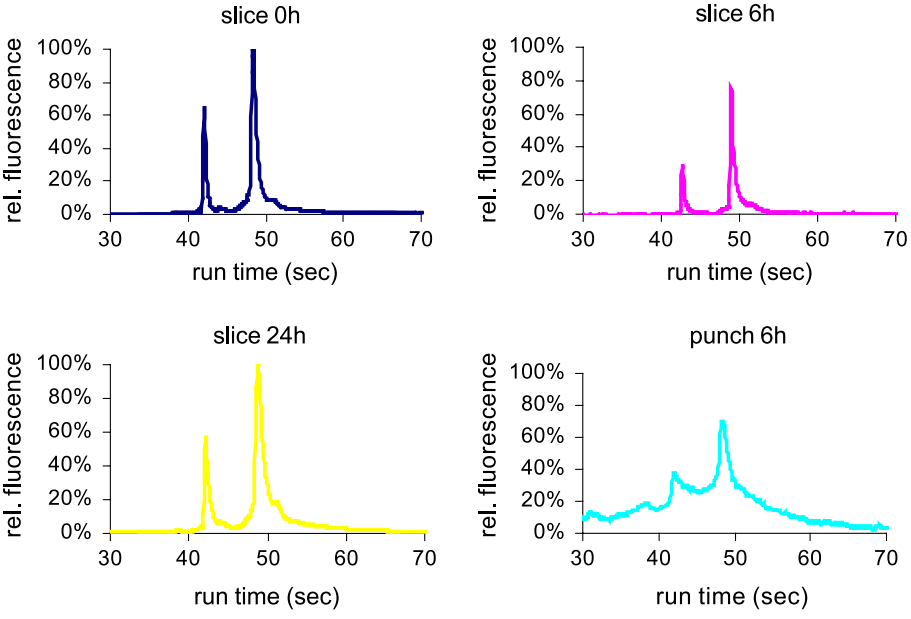

Fig. 5. Total RNA integrity assessment from slices incubated for 0, 6 and 24 h and from punches incubated for 6 h. The fluorescence peaks at 42 and 50 s gel electrophoresis run time represent the 18S and 28S bands, respectively.

incubated. Punched-out tissue showed significant reduction of the 18S and 28S RNA peaks and a shift in the RNA electropherogram to other fragment sizes, indicating significant degradation of RNA. This RNA degradation strongly suggests decreased viability of the punches, while slices show the same rRNA profile as not-incubated tissue (Fig. 5). RNA isolated from intestine slices incubated for 24 h was successfully used to study cytochrome *P*450 gene induction in vitro using quantitative RT-PCR (to be published elsewhere), an application that requires cellular RNA of high quality.

3.4. Metabolism

To reveal the metabolic capacity of intestinal slices and punches, incubations were performed with lidocaine, testosterone, 7-EC, and 7-HC for the first 3 h of incubation.

In this study, the *N*-deethylated metabolite, MEGX, of lidocaine was quantified. Punches had a similar capacity to form MEGX as precision-cut intestinal slices. Colon slices showed about half the capacity to form MEGX, when compared to intestinal slices (Fig. 6).

It is shown in Fig. 7 that androstenedione was the main metabolite detected in testosterone incubations for both small intestine and colon. Punches had a lower capacity to form androstenedione compared to precision-cut intestinal slices. Colon slices showed the same capacity to form androstenedione if compared to intestinal slices. Colon slices did not form detectable amounts of hydroxylated testosterone metabolites and only minor amounts of 6β-hydroxytestosterone were formed in some of the small intestinal preparations. Very minor amounts of 2β- and 16β-hydroxytestosterone were produced by punches of the small intestine, while these metabolites were not detected from incubations with precision-cut slices. Other hydroxylated metabolites could not be detected. The presence of less tissue in the precision-cut slice incubations is most likely the cause of undetectable amounts of other metabolites than androstenedione, compared to punches that contain more intestinal tissue. Slices contained 0.16 mg of protein, while

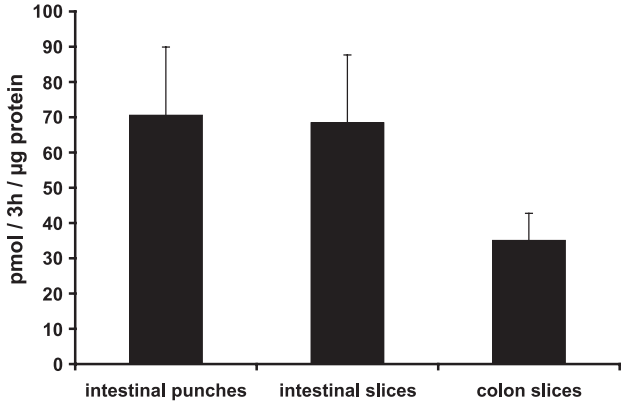

Fig. 6. Lidocaine (5 mM) metabolism during 3 h incubation (data are means of 4 organs±S.E.M. and 3 slices/punches per organ).

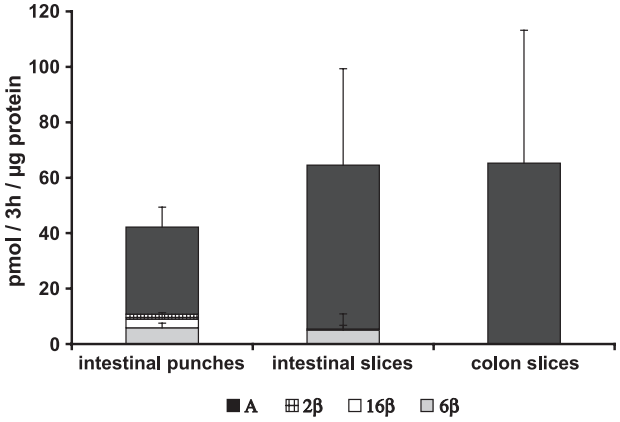

Fig. 7. Testosterone (0.25 mM) metabolism towards androstenedione (A) and 2β-, 16β- and 6β-hydroxytestosterones during 3-h incubation (data are means of 4 organs±S.E.M. and 3 slices/punches per organ).

punches had a protein content of 0.35 mg. Note that all metabolic data are normalized for protein content.

The metabolism of 7-EC is a commonly used parameter to investigate both phase I and integrated phase II metabolism in vitro. When small intestinal punches and slices were compared, it appeared that slices show more 7-HC from 7-EC. However, less 7-HC is glucuronidated towards 7-HC GLUC by slices compared to punches from the small intestine. In colon slices, no metabolism was detectable from incubations with 7-EC. No 7-HC SULF could be detected after intestinal slice or punch incubation with 7-EC.

In Fig. 9, the conjugation (phase II) capacity of intestinal slices and punches is shown, using 7-HC as a model substrate. The main conjugated metabolite formed is the glucuronide conjugate; 7-HC SULF is also formed by all intestinal preparations (see Fig. 9). Colon slices have a twofold higher capacity to form 7-HC GLUC compared to intestinal slices. No differences between the different preparations of small intestine were observed.

4. Discussion

The aim of the present study was to develop a method for the preparation of slices from the intestines that retain adequate viability. Previous reports (Vickers et al., 1992, 1995; Malfatti et al., 1996) in which intestinal slices have been used, gave almost no details about the viability. As far as we were aware, morphology data of intestinal slices have not been shown before.

We showed that ATP levels were somewhat higher in punches from the small intestine after stripping off the muscle layer than in punches with intact muscle layer (Fig. 2). A possible reason could be that removing the muscle layer facilitates diffusion of oxygen and nutrients. However, the thickness of the intestinal wall itself, which is about 0.4 mm in the rat (own observation), may be a limiting factor for diffusion into the inner cell layers,

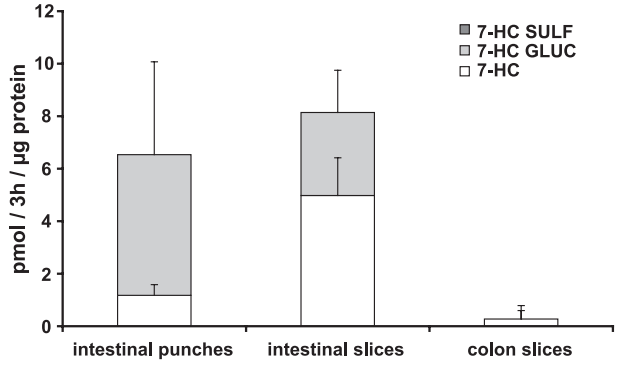

Fig. 8. 7-EC (0.5 mM) metabolism during three hours incubation towards 7-HC, 7-HC GLUC and 7-HC SULF (data are means of 4 organs±S.E.M. and 3 slices/punches per organ).

explaining the low ATP levels. In addition, the procedure of stripping is difficult and may be damaging to the intestinal tissue.

To overcome this problem, we set-up a method to prepare slices from agarose-filled and -agarose embedded small intestine, resulting in slices of only 0.25 mm thickness. As shown for liver slices, this may be thin enough to allow sufficient diffusion of oxygen and substrates to the inner cell layers. Indeed, we showed here that ATP levels, RNA integrity, and morphology in precision-cut slices from agarose-filled and -embedded small intestine were better preserved than in punches. Therefore, we conclude that agarose-filled and -embedded intestine slices are an improved preparation compared to punched out preparations.

Intestinal slices show considerable biotransformation activity with regard to the four model substrates, exhibiting several metabolic routes as deethylation, oxidation, hydrolysis, as well as glucuronide and sulphate conjugation. These reactions are considered to take place in the epithelial mucosal cell layer (Kaminsky & Fasco, 1991). Because the villi that are partly stripped off from the slices did not contribute to the biotransformation activity (results not shown), the epithelial cells that are present in the slice account for the metabolic activity. Therefore, we conclude that these slices are useful for biotransformation studies because they show a relatively high metabolizing activity (for discussion, see below), and a better morphological appearance and ATP/RNA content. Because we did not directly measure metabolic performance of intestinal tissues in vivo, it remains to be studied whether the observed activity is a good representation of the in vivo situation. It cannot be excluded that the damage of the intestines, as was observed by histomorphology, may affect the biotransformation activity in the slices.

Lidocaine deethylation towards MEGX is considered to be a result of cytochrome *P*450 activity in both man and rat (Imaoka et al., 1990). We observed that MEGX formation took place in both small intestine and colon slices (Fig. 6). This is in contrast with the earlier observation that colon punches lacked cytochrome *P*450 activity (Malfatti et al., 1996). This might be either due to the improved preparation technique of colon slices used in the present study or to the possibility that MEGX formation in the colon is catalyzed by other enzymes than cytochrome *P*450 alone.

Earlier, testosterone metabolism in enterocytes was described to result in androstenedione formation (which is an oxidation reaction) in vivo, but in vitro reduction towards dihydroxytestosterone was favored (Farthing, Vinson, Edwards, & Dawson, 1982), possibly due to the relative hypoxic circumstances in vitro. Here, we show that oxidative metabolism of testosterone takes place, producing androstenedione (Fig. 7). This is in agreement with in vivo results in the rat (Farthing et al., 1982). In rat liver, androstenedione formation is known to be catalyzed both by cytochrome *P*450 (Yamazaki & Shimada, 1997) and by 17β−hydroxysteroid oxidoreductase (Farthing et al., 1982). Because of the low cytochrome *P*450 content in the colon (Malfatti et al., 1996), it is more likely that 17β−hydroxysteroid oxidoreductase is involved in androstenedione formation.

After incubation with 7-EC, we detected mainly 7-HC and 7-HC GLUC in small intestinal punches and slices. Hardly any metabolism was detected in colon slices. The formation of 4-ethoxy-2-hydroxyphenyl propionic acid (EPPA) as reported in earlier studies appeared due to spontaneous formation at the pH used during incubations. In isolated intestinal cells of the rat, the V_{max} of total 7-EC metabolism was reported to be about 2.4 nmol/min/g intestine (Borm, Koster, Frankhuijzen-Sierevogel, & Noordhoek, 1983), which is lower than the total formation of 7-ethoxycoumarin metabolites in slices (Fig. 8), being 5.5 nmol/min/g intestine (using a protein content of 12.5%). A reason for this difference might lay in the possible selection of a subset of cells during the isolation procedure of intestinal cells together with the relatively low yield of viable isolated intestinal cells.

All intestinal preparations showed extensive phase II metabolism of 7-HC (Fig. 9). Conjugation of 7-HC was observed to be higher in colon slices than in slices and

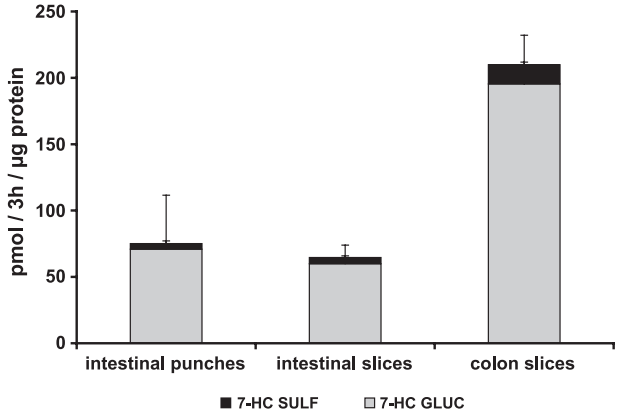

Fig. 9. 7-HC (0.5 mM) metabolism during three hours incubation towards 7-HC GLUC and 7-HC SULF (data are means of 4 organs±S.E.M. and 3 slices/punches per organ).

punches from the small intestine, which is in agreement with the higher reported content of conjugation enzymes in the colon (Malfatti et al., 1996). When normalized for protein content, colon slices appear to have even a higher capacity to conjugate 7-HC than liver slices (de Kanter et al., 2002a). The current rates of 7-HC GLUC and 7-HC SULF formation, being 40 and 3.2 nmol/min/g small intestine, respectively (using a protein content of 12.5%) in rat intestinal slices, are also higher than the reported V_{max} values of about 14.4 and 1.8 nmol/min/g intestine for 7-HC glucuronidation and sulphation, respectively, as determined in isolated intestinal epithelial cells from the rat (Koster & Noordhoek, 1983). Like the difference in 7-EC metabolism, the improved viability of the slices together with the relatively low yield of viable isolated intestinal cells can be an explanation of this quantitative difference in rates of metabolism of 7-HC.

Recently, this method of preparing intestine slices by filling and embedding in agarose has also been applied to study biotransformation of three candidate drugs in the rat, using in vivo, and in vitro methods (de Graaf, Van Meijeren, Pektas, & Koster, 2002). It appeared that the small intestine slice preparation was a proper model because they formed large amounts of a particular metabolite that was also the main metabolite observed in vivo. In contrast, this metabolite was only slightly formed by liver slices and isolated hepatocytes, and was not formed at all in lung or kidney slices, S9-mix, or microsomes from the liver (de Graaf et al., 2002).

In conclusion, precision-cut intestine slices represent an improved in vitro technique compared to intestinal punches. The use of intestinal slices is attractive in biotransformation studies because these slices show a high drug transformation capacity. Further studies are necessary to further improve the morphology of incubated slices and to control whether these slices quantitatively correctly reflect the in vivo activity of the intestines in various species.

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