Available online at www.sciencedirect.com

SCIENCE DIRECT

Journal of Pharmacological and Toxicological Methods 51 (2005) 65–72

Journal of Pharmacological and Toxicological Methods

www.elsevier.com/locate/jpharmtox

Original article

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

Ruben de Kanter[a,*], Annemarie Tuin[b], Esther van de Kerkhof[b], Marcella Martignoni[a], Annelies L. Draaisma[b], Marina H. de Jager[b], Inge A.M. de Graaf[b], Dirk K.F. Meijer[b], Geny M.M. Groothuis[b]

[a]*Nerviano Medical Science Srl., Viale Pasteur 10, 20014 Nerviano (MI), Italy*
[b]*Groningen University Institute for Drug Exploration (GUIDE), Dept. Pharmacokinetics and Drug Delivery, Groningen, The Netherlands*

Received 24 December 2001; accepted 26 July 2004

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.

* Corresponding author. Tel.: +39 0331 581484; fax: +39 0331 581105.

E-mail address: ruben.dekanter@nervianoms.com (R. de Kanter).

1056-8719/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.vascn.2004.07.007

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,