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Drug metabolism in human and rat intestine
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Chapter 5

Induction of drug metabolism along the rat intestinal tract

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In preparation
Abstract
Induction of drug metabolizing enzymes in the intestine can result in a marked variation in the bioavailability of drugs and cause an imbalance between local toxification and detoxification. In vivo gradients of drug metabolizing activity are demonstrated along the intestinal tract, but knowledge on the regional differences in induction is scarce. Previously, we showed that induction can be studied in vitro using intestinal tissue slices. This method allows investigation of induction processes in the different regions of the intestine under identical conditions. With use of this method, we compared the extent of induction of drug metabolizing activity in duodenum, jejunum, ileum and colon in vitro with that in vivo. After in vivo (oral dosing) or in vitro (slice incubation) exposure to β-naphthoflavone (AhR ligand), dexamethasone (PXR/GR ligand) or phenobarbital (CAR modulator), the metabolic conversion rates of 7-ethoxycoumarin, testosterone and 7-hydroxycoumarin were determined in slices prepared from duodenum, jejunum, ileum and colon. The in vitro responses to β-naphthoflavone and dexamethasone were largely comparable with the responses in vivo with one exception. 7-ethoxycoumarin O-deethylation was highly induced in colon by exposure to β-naphthoflavone in vitro, but not in vivo. Furthermore, all regions of the intestine were highly sensitive for induction by β-naphthoflavone. Dexamethasone induction was prominent in duodenum and ileum, but the effect was virtually absent in jejunum and colon. These differences appeared to parallel the differences in the expression levels of the responsible nuclear receptors. Although significant PB responses could not be detected in vitro, androstenedione, 16α-hydroxytestosterone and 16β-hydroxytestosterone formation were induced in duodenum and jejunum after exposure in vivo. 16α- and 16β-hydroxytestosterone formations were also elevated in ileum, but not in colon despite the observation that CAR was equally expressed in all regions of the intestinal tract. In conclusion, induction responses detected with the intestinal precision-cut slice system satisfactorily represents the induction responses obtained after in vivo exposure, except for PB mediated induction. Moreover, comparison of in vivo with in vitro induction demonstrates that regional differences in induction in vivo can at least partly be explained by the different exposure to inducers.

The abbreviations used are:
Introduction

The sensitivity of drug metabolizing enzymes (DMEs) to inducers is a major concern in the development of new drugs. This can result in a marked variation in the bioavailability of drugs and can also cause an imbalance between toxification and detoxification [1]. The rat intestine possesses a broad spectrum of DMEs [2] and their sensitivity to inducers has been inferred from in vivo studies by administering prototypical inducers, such as β-naphthoflavone (BNF) or dexamethasone (DEX) orally [3,4] or intraperitonially [5,6].

The rat intestine is a heterogeneous organ in many aspects, particularly with respect to expression of DMEs [7,8] and nuclear receptors involved in regulation of DMEs. PXR is one of the important nuclear receptors (NR) involved in the regulation of the expression of DMEs, such as CYP3A [9] and its expression level is higher in rat colon than in small intestine [10]. The pattern of CAR (constitutively activated receptor) expression along the intestinal tract has not been described to date. The aryl hydrocarbon receptor (AhR) has been described to be constant [11] or decrease [6] in distal direction, whereas glucocorticoid receptor (GR) is higher in colon and ileum than in duodenum and jejunum [12].

Although intestinal DMEs have been proven to be sensitive to inducers, it remains unclear to what extent this induction takes place in the different regions of the intestinal tract. Knowledge about the extent to which induction occurs in intestinal regions can be very valuable for the interpretation of absorption data along the intestinal tract. Zhang et al. reported a decreasing induction of CYP1A1 in distal direction of rat small intestine after in vivo administration of BNF [6]. It has not been studied, however, whether this pattern was the result of a decrease in inducer concentration along the gut, or that the intestine in distal direction actually is less sensitive to induction. Therefore, an in vitro method is needed that enables determination of the potential of new drugs to induce DMEs in the different regions of the intestine under identical circumstances.

In addition, both animal welfare as well as the budget for drug safety research would benefit from such studies, since the slice technique represents an alternative to in vivo studies. Moreover, in vitro test methods offer the possibility to study induction in human organs. To adequately predict induction in vitro, however, a model should be selected in which the functionality of all the above-mentioned pathways has been proven. Induction of DMEs in intestine via the AhR pathway has been studied with Caco-2 cells, but these cells lack PXR expression and are therefore less suitable to study PXR responses [13]. Our laboratory recently presented rat intestinal precision-cut slices as an in vitro model for studying enzyme induction and showed that the results of these studies were in line with published in vivo data [14].

In the current study, we investigated the extent of induction of DME activity at various regions of the intestinal tract both in vivo and in vitro. For this purpose, animals were orally dosed with DEX, BNF or PB after which the induction responses at enzyme activity level were studied in tissue slices prepared from the various regions of the intestine. In addition, slices from the same regions of the intestine of untreated animals were exposed to DEX, BNF or PB in vitro, after which metabolic conversion of several substrates was measured. To facilitate the interpretation of the findings, the mRNA expression of villin (a specific
marker for enterocytes), GAPDH (a house keeping gene for all cell types) and the nuclear receptors AhR, CAR and PXR was measured relative to villin in samples from non-treated animals.

**Materials and methods**

**Chemicals**

6β-, 14α-, 15α-, 11β-hydroxytestosterone (TOH), testosterone, androstenedione, 7HC, 7HC-glucuronide (7HC-GLUC), low gelling temperature agarose (type VII-A), DMSO, and DEX were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Gentamicin, Williams medium E with glutamax-I and amphotericin B (Fungizone)-solution were obtained from Gibco (Paisley, UK). 7EC and BNF were obtained from Fluka Chemie (Buchs, Switzerland). 2α-, 16α-, 16β-, 19-, and 11α-TOH were obtained from Steraloids Inc. (Newport RI, USA). HEPES was obtained from ICN Biomedicals, Inc. (Eschwege, Germany). 7HC-sulphate (7HC-SULF) was a kind gift from Mr. P. Mutch, GlaxoWellcome (Herts, UK). All reagents and materials were of the highest purity that is commercially available.

**Animals**

Male Wistar (HsdCpb:WU) rats weighing ca. 350 g were purchased from Harlan (Horst, The Netherlands). Rats were housed in a temperature- and humidity-controlled room on a 12-h light/dark cycle with food (Harlan chow no 2018, Horst, The Netherlands) and tap water *ad libitum*. Animals received a single dose of BNF (40 mg/kg body weight), DEX (40 mg/kg body weight) or olive oil (0.5 ml, vehicle) between 10 and 12 a.m. by oral cavage, and 24 h later the animal was sacrificed. Animals were exposed during 36 h to PB via drinking water, which was supplemented with 0.5% PB (w/v) at 6 pm and 36 h later the rats were sacrificed. The animal ethical committee of the University of Groningen approved the use of animals for these experiments.

**Preparation of precision-cut slices**

Under isoflurane/N₂O/O₂ anesthesia, the small intestine and colon were excised from the rat and put in ice-cold, oxygenated Krebs-Henseleit buffer (containing 10 mM HEPES and 25 mM D-glucose, pH 7.4). Different segments of the small intestine were used for further preparation: duodenum (D; 0-10 cm from the stomach), jejunum 1 (J1; 25-40 cm from the stomach), jejunum 2 (J2; 40-50 cm from the stomach) and ileum (I; last 5 cm of the small intestine). In some experiments only one part of jejunum (J) was tested, in those cases, slices were prepared from jejunum 2. For colon slices, the whole organ was used. Each segment was divided into 3 cm segments, flushed with ice-cold Krebs-Henseleit buffer, tightly closed at one side, filled with 3% (w/v) agarose solution in 0.9% NaCl (37°C) and cooled in ice-cold Krebs-Henseleit buffer, allowing the agarose solution to gel. Subsequently, the filled segment was embedded in 37°C agarose solution using a pre-cooled (0°C) tissue embedding Unit (Alabama R&D, Munford, AL USA). After the agarose solution had gelled, precision-cut slices (thickness about 400 μm and weight about 2 mg) were cut using a Krumdieck tissue slicer as described before [15].

**Incubation of precision-cut slices**

The slices were incubated individually in a 12-wells-culture-plate (Greiner bio-one GmbH, Frickenhausen, Austria) in 1.3 ml Williams Medium E (with Glutamax-I), supplemented with D-glucose (final concentration 25 mM), gentamicin (final concentration 50 μg/ml) and amphotericin B (final
Induction of drug metabolism along the rat intestine

concentration 2.5 \mu g/ml). The culture plates were placed in a pre-warmed cabinet (37°C) in plastic boxes. Slices were incubated under humidified carbogen (95% O₂ and 5% CO₂) and shaken back and forth 90 times per minute.

**In vitro induction studies**

Precision-cut slices prepared from duodenum (D), jejunum 1 (J1), jejunum 2 (J2), ileum (I) or colon (C) of non-treated rats were pre-incubated (in triplicate) for 0 and 24 hours with BNF (final concentration 50 \mu M), DEX (final conc. 100 \mu M), PB (final concentration 2.5 mM) or 0.5% DMSO as control. After 0 or 24 hours of pre-incubation, slices were either harvested in N₂(l) for mRNA analysis or transferred to fresh medium (pre-warmed and pre-gassed) and incubated for 3 hours with TT (final conc. 250 \mu M), 7EC (final conc. 500 \mu M) or 7HC (final conc. 500 \mu M), by addition of 13 \mu l of a 100x stock solution in methanol to the 1.3 ml medium. TT was used as a substrate to test induction by DEX; 7EC and 7HC metabolism were studied as a measure for induction by BNF, whereas TT and 7HC metabolism were studied as a measure for induction by PB. Each experiment was performed in 3-4 rats in triplicate.

**In vivo induction studies**

After *in vivo* treatment of rats through oral administration of DEX, BNF, PB or olive oil, precision-cut slices were prepared from duodenum (D), jejunum 1 (J1), jejunum 2 (J2), ileum (I) or colon (C) and incubated for 3 hours with TT (final conc. 250 \mu M), 7EC (final conc. 500 \mu M) or 7HC (final conc. 500 \mu M) as described for *in vitro* induction studies. Each experiment was performed in 3-4 rats in triplicate using 3 slices per experiment.

**Sample analysis**

Metabolite analysis: After TT incubation, slice and medium were collected together and stored at –20°C until further use. The analysis was performed as described in an earlier study [16]. As it was previously shown that 7EC and 7HC and their metabolites are not significantly retained in the tissue, analysis was performed on medium samples only as has been described earlier [15]. Total 7EC metabolism, which occurs via phase I metabolism and subsequent conjugation with either glucuronide or sulphate, was calculated from the total 7HC, 7HC-GLUC and 7HC-SULF formation. All experiments were performed in at least 3 rats in triplicate.

RNA isolation and analysis of gene expression levels: Pieces of tissue from several regions of the intestinal tract of non-treated rats were snap-frozen in N₂(l) and analyzed as described before [16]. The matching primers are listed in table I.

**Table 1: Primer information of the rat genes under study**

<table>
<thead>
<tr>
<th>Gene</th>
<th>GenBank number</th>
<th>Forward Primer (5’-3’)</th>
<th>Reverse Primer (5’-3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Villin</td>
<td>XM_001057825</td>
<td>GCTCTTTAGTGCTCCAACC</td>
<td>GGGGTGGGTCTTGAGTATT</td>
</tr>
<tr>
<td>GAPDH</td>
<td>XR_008524</td>
<td>CGCTGGTGCTGAGTATGCG</td>
<td>CTGTGGTAGAGCCCTTCC</td>
</tr>
<tr>
<td>AhR</td>
<td>NM_013149</td>
<td>GGGCCAAGAGCTTCTTTTGATG</td>
<td>GCAAATCCTGGCCATCTTCTGA</td>
</tr>
<tr>
<td>PXR</td>
<td>NM_052980</td>
<td>GATGATCATGTCTGATGCTG</td>
<td>GAGGTTGAGAGCTCCAGATGCTG</td>
</tr>
<tr>
<td>CAR</td>
<td>AB105071</td>
<td>ACCAGATCTCCCTTCTCAAG</td>
<td>CTCGTAATGGACCAGTA</td>
</tr>
</tbody>
</table>
Agarose gel electrophoresis and dissociation curves confirmed homogeneity of the PCR products. Only for CAR, a minor secondary product was formed when mRNA from colon was analyzed. All experiments were performed in 3-4 rats. The cycle number at the threshold (Ct) is inversely related to the abundance of mRNA transcripts in the initial sample. Mean Ct of duplicate measurements was used to calculate the difference of Ct for the target gene and the reference villin gene (ΔCt), which was compared to the corresponding delta Ct of the control experiment (ΔΔCt). Data are expressed as fold-induction of the gene of interest according to the formula $2^{-\Delta\Delta Ct}$.

**Protein determination**
After incubation with 7EC and 7HC, slices were stored at $-20^\circ$C until further use as has been described in an earlier study [8].

**Statistics**
Statistical significance was determined using Student’s $t$-test. For analyzing statistical significance in mRNA expressions data were calculated on $\Delta\Delta$Ct.

**Results**

**Gene expression levels along the intestinal tract**
Villin expression tended to increase from duodenum to jejunum 1, and then decreased from jejunum 2 to colon in non-treated rats (figure 1).

**Figure 1:** Levels of villin (A), GAPDH (B), PXR (C), CAR (D) and AhR (E) mRNA expression along the intestinal tract (D: duodenum, J1: jejunum 1, J2: jejunum 2, I: ileum, C: colon). Average expression level of D is set at 1. Results are mean ± SEM of 3-4 rats. Significant differences towards D are indicated with * p < 0.05.

* Significantly different from D
# Significantly different from J2
GAPDH was constant along the small intestine, but was significantly lower in colon. The basal mRNA expression of PXR along the intestinal tract showed a similar expression profile as AhR. The expression levels related to villin decreased from duodenum to jejunum 2 (significantly for AhR expression, p < 0.05) and then increased, significantly, up to and including the colon. The expression levels of CAR related to villin were constant along the intestinal tract.

**Metabolic rates along the intestinal tract**

The metabolic rates of 7EC were similar in duodenum and jejunum and were approximately 3-fold lower in ileum and colon with a significant difference between duodenum and ileum (p < 0.05) (figure 2). The 6β-hydroxylation rate of testosterone was 21.5 pmol/mg protein/min in duodenum and was much lower in ileum (4.9 pmol/mg protein/min, p < 0.05) and in colon (1.4 pmol/mg protein/min, p < 0.05). The androstenedione formation rate was highest in duodenum (195 pmol/mg protein/min), followed by jejunum (119 pmol/mg protein/min), whereas the levels in ileum and colon were 43 and 55 pmol/mg protein/min respectively. CYP2B mediated reactions (16α and 16β hydroxylation of testosterone) were equal in duodenum and jejunum (16α-TOH: 8.1; 16β-TOH: 7.0 pmol/mg protein/min) and were barely detectable in ileum and in colon.

**Figure 2:** Metabolic activity along the intestinal tract. Precision-cut slices from small intestine were prepared at distinct regions (D: duodenum, J: jejunum 2, I: ileum, C: colon) and incubated for 3 hours with testosterone. The formation rates of 7HC (A), 6β-TOH (B), androstenedione (C), 16α-TOH (D) and 16β-TOH (E) are depicted. Results are mean ± SEM of 3 rats. Significant differences toward D are indicated with * p < 0.05.
Induction studies

**BNF exposure in vitro and in vivo**

Both *in vivo* and *in vitro* 7EC metabolism was increased by BNF in all regions of the intestine, with the exception of the colon, in which no induction was found *in vivo* compared with olive oil treated controls (figure 3). The induction of the metabolic rate of 7EC conversion by BNF in duodenum was higher *in vivo* (47-fold) than after exposure *in vitro* (5-fold).

*Figure 3: Induction of 7EC metabolism in precision-cut slices prepared from small intestine (D: duodenum, J1: jejunum 1, J2: jejunum 2, I: ileum, C: colon) after in vitro (A) and in vivo (B) exposure to BNF for 24 h. In the in vitro induction studies, slices were pre-incubated for 24 hours with 0 (0.5% DMSO) or 50 μM β-naphthoflavone (BNF) and subsequently incubated for 3 h with 7EC (A). In the in vivo induction studies, animals were orally exposed for 24 h with nothing (control), olive oil (vehicle of BNF) or BNF. Subsequently, slices were prepared and incubated for 3 h with 7EC (B). Results are mean ± SEM of 3-4 rats. In each experiment, 3 slices were incubated per treatment. Significant differences toward the activities of control slices (B) are indicated with # *p < 0.05. Significant differences toward the activities of '0.5% DMSO' slices (A) or olive oil slices (B) are indicated with * *p < 0.05.*

BNF exposure for 24 h *in vitro*, induced 7EC metabolism significantly in all parts of the intestinal tract, compared with 24 h DMSO-treated control incubations (figure 3A). Compared with control incubations, 7EC metabolism was induced 5-fold in duodenum. The induction was similar in jejunum 1 (5.6-fold) and seemed higher in jejunum 2 (78-fold, however the latter fold-induction was due to very low control values), ileum (8-fold) and colon (18-fold). After exposure with BNF, the original pattern of 7EC metabolism (figure 2A) changed and now the activity in colon was comparable to jejunum and duodenum and higher than ileum.

*In vivo*, BNF induced the metabolic conversion of 7EC over 30-fold in small intestine and 18-fold in colon (figure 3B). 7EC metabolism along the intestinal tract was also induced by olive oil (the vehicle of BNF), but to a lesser extent (small intestine: 3.5-fold, *p < 0.05*; colon: 15-fold). In all regions of the small intestine, induction by BNF was at least 2-fold higher than by olive oil alone. Although the activity of 7-EC metabolism was significantly induced in all regions of the intestinal tract (*p < 0.05*), the pattern of 7EC metabolism over the intestinal
tract changed after treatment with BNF. In this case, the activity was comparable in duodenum, jejunum and ileum. The fold-induction compared with non-treated samples along the small intestine was the highest in ileum (150-fold). In colon, however, 7EC metabolism was induced by olive oil (19-fold) and was not induced further by BNF. After both in vitro and in vivo exposure to BNF, 7HC-glucuronidation and 7HC-sulphation were not affected. In addition, olive oil did not affect these conjugations either.

**DEX exposure in vitro and in vivo**

In duodenum, 6β-TOH formation was induced after in vitro exposure (6-fold compared with DMSO treated controls) as well as after in vivo exposure (3-fold, p < 0.05) to DEX (figure 4). Androstenedione formation was slightly, but not statistically significantly induced in vitro (1.6-fold), whereas in vivo the same fold-induction was found being significantly different from control tissues.

![Figure 4: Induction of testosterone metabolism in precision-cut slices prepared from small intestine (D: Duodenum, J1: jejunum 1, J2: jejunum 2, I: ileum, C: colon) after in vitro (A, C) and in vivo (B, D) exposure to DEX for 24 h. In the in vitro induction studies, slices were pre-incubated for 24 hours with 0 (0.5% DMSO) or 100 μM DEX and subsequently incubated for 3 h with testosterone forming 6β-TOH (A) and androstenedione (C). In the in vivo induction studies, animals were orally exposed for 24 h with nothing (control), olive oil (vehicle of DEX) or DEX. Subsequently, slices were prepared and incubated for 3 h with testosterone forming 6β-TOH (B) and androstenedione (D). Results are mean ± SEM of 3-4 rats. In each experiment, 3 slices were incubated per treatment. Significant differences toward the activities control slices (B, D) are indicated with # p < 0.05 and toward the activities of '0.5% DMSO' slices (A,C) or olive oil slices (B,D) are indicated with * p < 0.05.](image-url)
Also in ileum in vitro, DEX induced 6β-TOH formation (figure 4A, 5-fold compared with control incubations, p < 0.05) and in jejunum a significant induction was observed (jejunum 1: 2.0-fold, p < 0.05, and jejunum 2: 1.5-fold, non-significant). In contrast, in colon no induction response was found compared to DMSO controls, but 6β-TOH formation was induced up to 5-fold by DMSO. In vivo, DEX significantly induced 6β-TOH formation in duodenum, jejunum 1 and ileum (3-fold, 3-fold and 4-fold respectively, p < 0.05) and not in colon, which is qualitatively similar to the results obtained in vitro. Quantitatively, induction was similar in all parts of the intestinal tract, with exception of jejunum 2. Olive oil (the vehicle of DEX) showed no effect on 6β-TOH formation in the small intestine (figure 4B), but in colon it increased the 6β-TOH formation rate by 5.5-fold (p < 0.07).

**Figure 5**: Induction of 2β-TOH (A), 16α-TOH (B) and 16β-TOH (C) formation in precision-cut slices prepared from small intestine (D: duodenum, J1: jejunum 1, J2: jejunum 2, I: ileum, C: colon) after in vivo exposure to DEX for 24 h. Results are mean ± SEM of 3-4 rats. In each experiment, 3 slices were incubated per treatment.

Both in vivo and in vitro, DEX induced androstenedione formation (figure 4C and D) in duodenum as well as jejunum 2 after in vitro (1.6-fold and 2-fold, respectively) and in vivo exposure (1.5-fold with p < 0.05 and 1.9-fold respectively).

After in vitro exposure, 16α-TOH, 16β-TOH and 2β-TOH could not be quantified, since the formation of 16α-TOH and 16β-TOH decreased during 24 h of slice incubation to undetectable levels with or without DEX. Furthermore, the detection of 2β-TOH by HPLC was disturbed by the presence of DEX. After in vivo exposure, DEX levels were sufficiently low in slices and did not interfere with quantification of 2β-TOH. 2β-hydroxylation of testosterone after in vivo exposure to DEX was induced from levels below the detection limit to clearly detectable levels in duodenum, jejunum and ileum, but not in colon (figure 5A). 16α- and 16β-hydroxylations of testosterone appeared, although not statistically significant, down-regulated by olive oil in duodenum and jejunum. This down-regulation was less prominent when incubations occurred in the presence of DEX (figure 5B and 5C).

**PB exposure in vitro and in vivo**

In slices, induction of 16α-TOH and 16β-TOH formation by PB could not be detected. Experiments in vitro in both jejunum 1 and colon, showed no induction of 6β-TOH and appeared to be in line with in vivo treatment (figure 6). Androstenedione formation, however, was slightly but significantly induced in duodenum (1.3-fold) and jejunum (jejunum 1: 2.1-fold and jejunum 2: 1.2-fold (p < 0.05)) after in vivo administration, but in vitro no induction was
Induction of drug metabolism along the rat intestine

detected. Furthermore, the rates of 16α- and 16β-hydroxylation were slightly but non-
significantly increased (2.2-fold and 2.6-fold respectively) along the intestinal tract (figure 7).
Only for 16β-TOH formation in duodenum the induction was significant (p < 0.05).

Figure 6: Induction of testosterone metabolism in precision-cut slices prepared from small intestine (D: Duodenum, J1: jejunum 1, J2: jejunum 2, I: ileum, C: colon) after exposure in vitro (24 h, A, C) and in vivo (36 h B, D). In the in vitro induction studies, slices were pre-
incubated for 24 hours without or with PB (2.5 mM) and subsequently incubated for 3 h with testosterone forming 6β-TOH (A) and androstenedione (C). In the in vivo induction studies, animals were orally exposed for 36 h with nothing (control) or PB. Subsequently, slices were prepared and incubated for 3 h with testosterone forming 6β-TOH (B) and androstenedione (D). Results are mean ± SEM of 3-4 rats. In each experiment, 3 slices were incubated per treatment. Significant differences toward the activities of control slices are indicated with * p < 0.05.

Figure 7: Induction of 16α-TOH (A) and 16β-
TOH (B) formation in precision-cut slices prepared from intestine (D: Duodenum, J: jejunum 2, I: ileum, C: colon) after in vivo exposure to PB for 36 h. Results are mean ± SEM of 3-4 rats. In each
extperiment, 3 slices were incubated per treatment. Significant differences toward the activities of control slices are indicated with * p < 0.05.
Chapter 5

Discussion

Induction of DMEs in the intestine can result in a marked variation in the bioavailability of drugs and cause an imbalance between toxification and detoxification processes [1]. In vivo gradients of drug metabolizing activity are demonstrated along the intestinal tract [6,17], but knowledge on the regional differences in induction are scarce. Recently, we showed the applicability of rat precision-cut jejunum and colon slices for induction studies up to 24 h [8,14]. This method allows investigation of induction processes in the different regions of the intestine under identical conditions. In the present study, we used intestinal precision-cut slices to compare the extent of induction of drug metabolizing activity in duodenum, jejunum, ileum and colon in vitro with that in vivo.

First, we investigated the gradient of villin (marker for epithelial cells) and GAPDH (marker for all cell types) along the rat intestinal tract. Whereas villin expression tended to decrease from duodenum to colon, GAPDH was constantly expressed along the small intestine. Only in colon tissue, the expression level of GAPDH was lower compared with duodenum. Assuming that the expression of villin is related to the number of enterocytes, these observations are in line with the general finding that the number of enterocytes per quantity of tissue decreases in distal direction along the intestinal tract, whereas the mucus secreting goblet cells increase [8].

To investigate the inducibility of DMEs in the different regions of the intestine, we investigated the expression levels of NRs involved (PXR, CAR and AhR) in the enterocytes. These expression levels were expressed relative to the villin expression to take into account the different number of enterocytes per amount of tissue. The protein expression level of AhR has been reported to decrease along the small intestine [6]. In the present study, however, AhR (related to villin) only decreased up to jejunum 2 in our study and then non-significantly increased in ileum and colon. When AhR was expressed per GAPDH, still the expression level increased in distal direction (data not shown), indicating that both per tissue as well as per enterocyte the AhR expression increases in distal direction. This expression level of AhR in colon relative to small intestine has not been reported earlier. The higher expression of PXR in colon compared with small intestine is in line with data of Zhang et al. [10]. CAR expression did not show a gradient along the rat intestinal tract, which was not published before to the best of our knowledge. These results indicate that the enterocytes in different regions of the intestine express these NRs to a different extent. At first sight, this would be expected to result in corresponding gradients of expression of the DME genes that are regulated via these NRs. However, in the present study, we found decreasing gradients of several CYP-mediated conversions in distal direction. This is in line with several reports by others [7,8,18], whereas AhR and PXR expression showed increasing gradients. It suggests that the NR expression is not rate limiting in the transcription of DMEs and indicates that other factors cause the DME gradients. Natural ligands of these NRs are components of the diet [19,20], and it can be assumed that lower concentrations of natural inducers are available in the distal part compared with the proximal part of the intestinal tract.

In vivo, it was found that olive oil (the vehicle usually applied for oral dosing of DEX and BNF) influenced DME expression along the tract to some extent. Oral administration of olive oil, but also corn, sesame or soybean oil have been reported to influence CYP levels in rat
Induction of drug metabolism along the rat intestine

For example, CYP2C11 protein levels decreased by 31% after corn oil or olive oil intake [21] or 6β-TOH formation levels were 2.5-fold higher in rat liver after corn oil intake [22]. No such data, unfortunately, has been published for rat intestinal tissue to date, but influence of oil on intestinal DMEs can also be expected based on these data [21,22]. In vitro, BNF induced 7EC metabolism in all regions of the intestinal tract. The fold-induction was the highest in ileum and colon, which is in line with the observed AhR expression profile. After in vivo exposure, in addition to the up-regulation of 7EC metabolism caused by olive oil, BNF further enhanced the activity level of 7EC metabolism in small intestine and colon. In distal direction along the small intestine, the fold-induction increased after in vivo administration, but was lower in colon. This is in line with an earlier report showing induction of ethoxyresorufin metabolism in small intestine (83-fold) and colon (3.2-fold) by BNF administered via the diet for 7 days [23]. From the in vitro studies, we tend to conclude that colon is more sensitive for inducing stimuli than the small intestinal regions. After in vivo administration of BNF, however, the colon cells responded the least, which suggests that a lower inducer concentration reaches the colon cells in vivo. Furthermore, no effect on glucuronidation rates was detected after BNF exposure either in vivo or in vitro, confirming the published in vivo data of other investigators [24].

After in vitro exposure to DEX, the induction of the 6β-TOH formation rate was the highest in duodenum, ileum and colon, which is in line with the observed expression levels of PXR. This suggests that induction is related to the expression level of PXR. Induction, however, is also dependent on the intracellular inducer concentration. Since the expression levels of uptake and efflux transporters [25-29], as well as DMEs are not constant along the tract, differences in intracellular concentrations of inducers can be expected also to cause variation in induction response. After in vivo exposure, the induction of 6β-TOH formation (CYP3A) was constant along the intestinal tract in contrast to induction of 2β-TOH formation (CYP3A), which was more induced in ileum compared to duodenum and jejunum. The fold-induction of androstenedione formation decreased along the intestinal tract. This suggests that the enzyme involved in androstenedione formation, mainly 17β-HSD, is regulated via another mechanism than CYP3A, which is responsible for 6β-TOH formation and is regulated via PXR [9].

PB exposure did not induce 6β-TOH formation in duodenum, jejunum and colon after exposure in vivo or in vitro, which is in agreement with data of other investigators [4], but is in contrast to findings in another study showing the induction of CYP3A1/2 protein expression after 3 days of intraperitoneal treatment with PB [3]. In the current study, 6β-TOH formation was only induced in ileum (2-fold) after in vivo exposure. Furthermore, androstenedione formation was only induced in duodenum and jejunum after in vivo exposure. Since CAR is generally known to induce CYP2B [9], induction of 16α-TOH formation (CYP2B) along the entire intestinal tract was observed as expected. This indicates that all regions are in principle sensitive for PB mediated induction. The difference between these in vitro and in vivo findings may be a matter of inducer concentration or exposure time, in accordance with what was found in human hepatocyte studies [30]. To confirm this hypothesis, PB exposure should be administered at various concentrations and according to other time frames both in vivo and in vitro.
Chapter 5

**In vitro** and **in vivo** exposure studies differ in many aspects. For example, when DEX (40 mg/kg) or BNF (40 mg/kg) are orally dosed in the administered dosage form, a concentration of approximately 70 mM DEX and 100 mM BNF is applied. **In vitro** studies with intestinal slices showed that only 50 μM BNF already results in maximal induction responses [8]. Since the volume of the dose **in vitro** was 0.5 ml and the volume of rat intestinal fluid is only 0.7-0.8 ml [31], it can be estimated that the concentration of DEX and BNF to which the enterocytes in the proximal intestine are exposed is approximately 1000-fold higher **in vivo** than **in vitro**. Furthermore, **in vitro**, slices were exposed during the same incubation period to a high concentration of inducer, independent of the original location of the tissue. **In vivo**, after oral administration, a peak concentration is reached in the proximal part of the intestine, which decreases in time and distal direction due to absorption and transport. This suggests that the exposure time **in vivo** is largely determined by the transit time. For dietary fibers, the transit time has been reported to be approximately 2 h in small intestine, 1-2 h in cecum and 2-4 h in colon/rectum [32]. Furthermore, intestinal DMEs may be further induced by BNF via the blood after absorption of the inducer, as has been reported after intraperitoneal administration of BNF or DEX [3,5]. Despite the large differences in exposure (both time and concentration), the induction responses were qualitatively similar after both **in vitro** and **in vivo** exposures to BNF and DEX.

To summarize, the **in vitro** responses to BNF and DEX were comparable to those found **in vivo** in all segments of the small intestine with the exception of colon tissue. This shows that intestinal precision-cut slices of all regions of the intestine are a valuable model to study drug-drug interactions **in vitro**, thereby reducing the number of animals needed for this type of research. In colon, 7EC metabolism was not induced after **in vivo** exposure to BNF, but was induced **in vitro** and suggests that lower concentrations of inducing agents reach the colon **in vivo**. In addition, the induction patterns along the intestinal tract are largely in line with the expression levels of the accompanying NR. The discrepancy between the induction **in vivo** by PB and the lack of effect of PB **in vitro** should be further addressed.

To conclude, all segments of the intestinal tract are sensitive to inducing stimuli, albeit to a different extent. Regional differences in induction **in vivo** can at least partly be explained by the different exposure to inducers, but regional differences in expression of NR also may play a role.

References


Induction of drug metabolism along the rat intestine

in various organs of rats by 3-methylcholanthrene or beta-naphthoflavone. Cancer Lett 97: 137-43


Chapter 5


