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HEPATIC AND INTESTINAL BIOTRANSFORMATION AND TRANSPORT OF XENOBIOTICS DURING PREGNANCY AND LACTATION

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ABSTRACT

The liver is the main place for phase II metabolism and transport of xenobiotics mediated by Mrp2, leading to elimination of conjugated metabolites into bile. In the gastrointestinal tract, phase II enzymes and Mrp2 are preferentially localized in the proximal region of the small intestine, particularly at the tip of the villus. In pregnant rats, conjugating enzymes (e.g. UGT and GST) and Mrp2-mediated transport of xenobiotics are decreased when compared to normal females, whereas these systems are preserved in small intestine, suggesting a complementary role for this tissue. After delivery, these same enzymes increase their expression and activity, being maximal during the last week of lactation. Mrp2 protein in liver also recovers and reaches the control level by the end of the lactation period. Post-partum rats exhibit a significant increase in development of the digestive tract in association with induction of phase II enzymes and Mrp2 expression and activity. Although the factors involved in regulation of protein expression may not be the same for conjugating enzymes and Mrp2 in the different experimental situations or tissues studied, their common localization and co-regulation provide evidence that they may work in cooperation. This is not surprising if we consider that most of the substrates for MRPs are the products that result from phase II reactions. Here, we provide a brief description of regulation of major phase II enzymes and Mrp2 during pregnancy and lactation, as particularly seen in the rat.

Keywords: Liver; intestine; Phase II reactions; Mrp2.

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Introduction

During pregnancy and lactation important hormonal changes occur which mainly involve increased levels of estrogens, progestogens and lactogenic factors. These hormones act on metabolizing systems in maternal liver producing a variety of effects which may have physiological, pharmacological and toxicological consequences. This results in changes in the capability for drug biotransformation and elimination. For lipid-soluble drugs, in particular, these consequences are extended to fetuses and also to newborns since nearly all drugs administered to mothers are detectable in maternal milk [1]. Most of the studies of xenobiotic metabolism during pregnancy and lactation performed in experimental animal models were concentrated on oxidative and reductive biotransformations, rather than in conjugation reactions. It is generally accepted that gestation depresses rat hepatic metabolism associated to the endoplasmic reticulum and decreases the content of cytochrome P450 in rats [2]. Because of the increase in liver weight, it is not clear whether the absolute capability of these animals for drug metabolism is really decreased.

The phase II or post-oxidative biotransformation system comprises a wide spectrum of enzymes that catalyze the incorporation of polar groups into hydrophobic molecules, thus decreasing hydrophobicity and eventually toxicity of the parent compounds **(Figure 1).** The multidrug resistant proteins (MRPs) represent a family of transporters that exhibit high expression in tumoral cells but are also expressed normally in epithelial cells. Mrp2, one of the members of the family, is preferentially localized in the apical membrane of hepatocytes, renal tubular cells and enterocytes and is involved in secretion of a wide spectrum of conjugated compounds [3, 4, 5,]. Here we report studies conducted to determine the influence of pregnancy and lactation on expression of hepatic and intestinal phase II reactions mainly involving UDP-glucuronosyltransferase (UGT) and Glutathione-S-transferase (GST). Because most Mrp2 substrates are conjugated derivatives of endogenous and exogenous compounds (drugs, carcinogens, etc), we compiled also studies on the expression of Mrp2 in liver and intestine from pregnant and lactating rats.

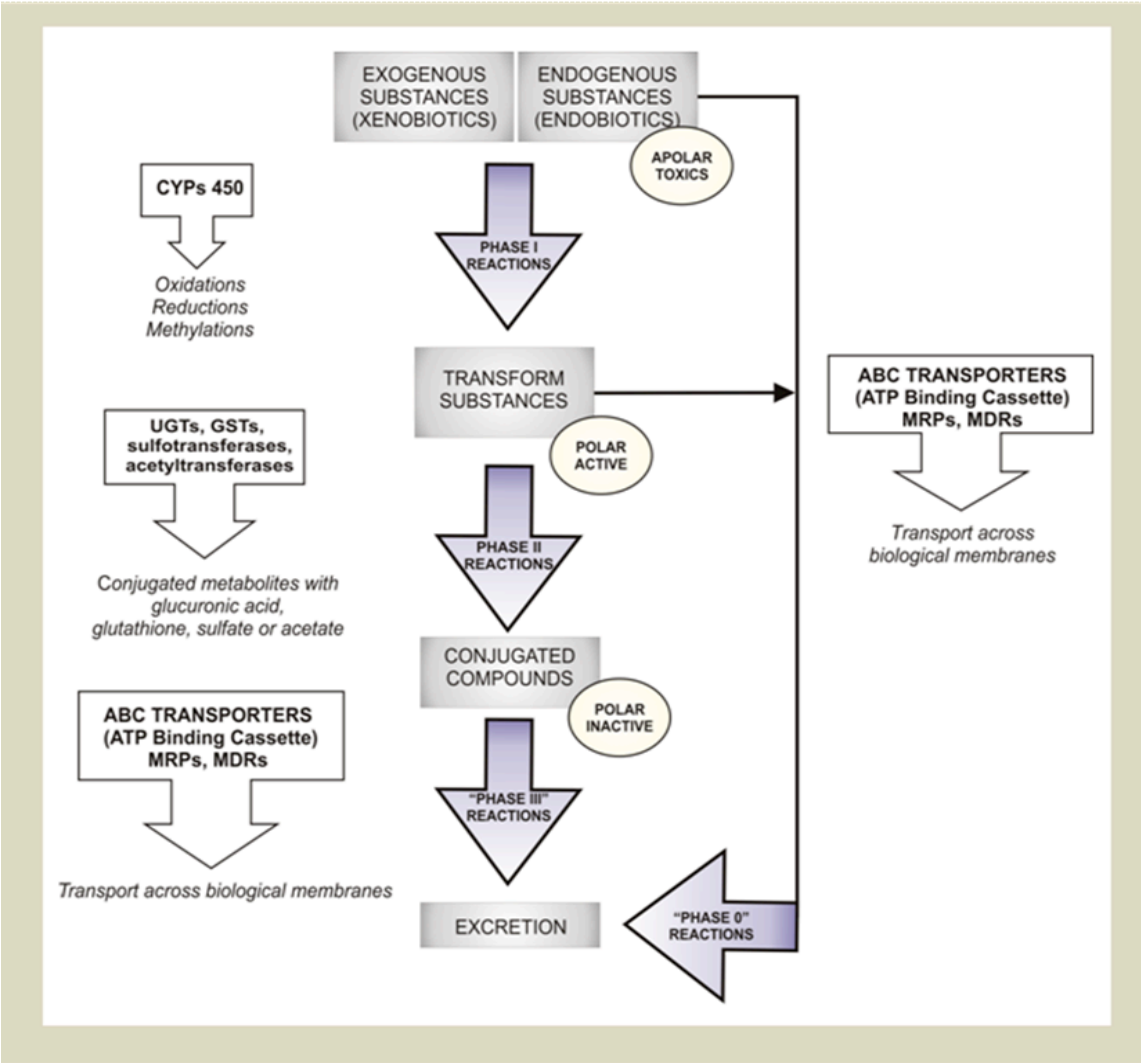

Figure 1. Biotransformation and transport systems.
Phase I reactions consist of oxidation, reduction and methylation that convert original compounds into more polar derivatives. Phase II involve conjugation reactions; substances (eventually Phase I reaction products) are coupled with a conjugating agent (e.g. glucuronic acid, glutathione, sulfate, acetate) and further converted to more hydrophilic, non toxic, derivates. Water soluble products are finally pumped out of the cells through the cell membranes with participation of specific transporters like the ABC members multidrug resistance-associated proteins (MRPs) and multidrug resistance proteins (MDRs) and with parallel ATP consumption.

Hepatic and intestinal metabolism and transport of xenobiotics.

We are daily exposed to many xenobiotics that are naturally present in the environment in addition to a wide variety of pollutants, drugs and food additives. They may enter the body

through inhalation, dermal absorption and incorporation into the gastrointestinal tract [6]. In addition to naturally occurring xenobiotics, there are hundreds of dietary contaminants that are intentionally added to food, which are daily incorporated into the gastrointestinal tract. Lipid-soluble chemicals readily cross biological membranes and distribute into fluid compartments and particularly in highly perfused tissues. After that, the chemicals may undergo redistribution into tissues that may act as storage reservoirs. In order to facilitate their elimination, the enzyme systems produce metabolites that are generally more water-soluble. Most drugs undergo first-pass metabolism in which biotransformation in hepatocytes and/or enterocytes prevents the distribution of intact chemicals throughout the body and facilitates their elimination from the body [7]. The liver represents the main place for drug biotransformation reactions, including phase I and II reactions, whereas the small intestine plays a less relevant role **(Figures 1 and 2)**.

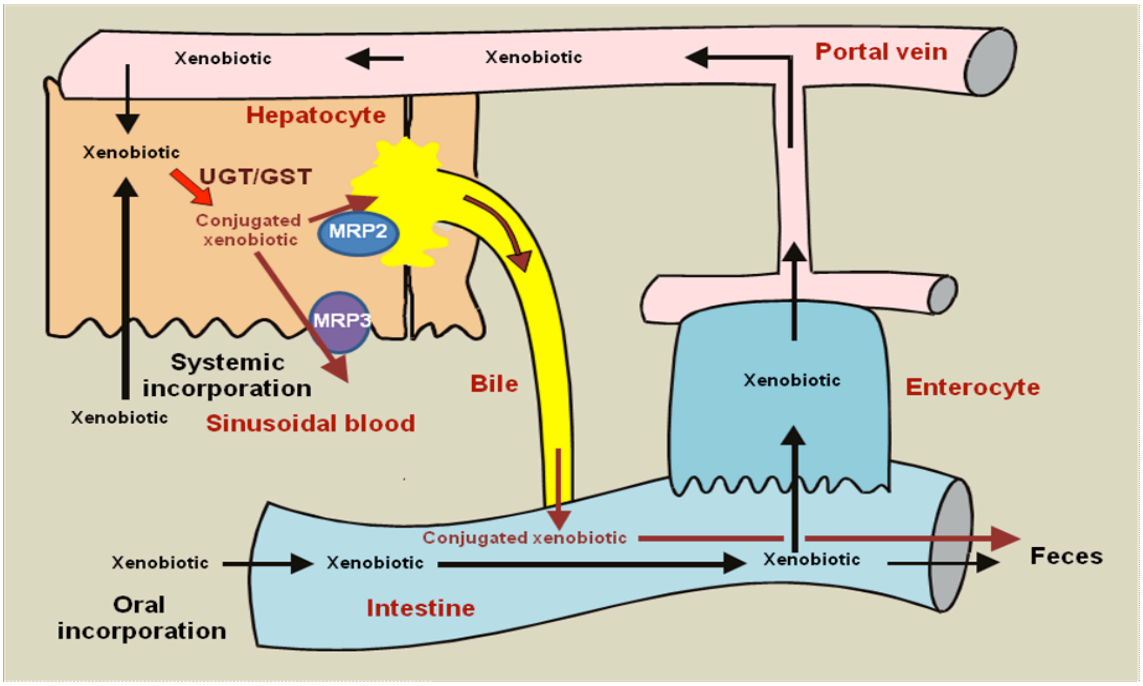

Figure 2. Fate of orally incorporated xenobiotics. Phase II biotransformation reactions and MRPs may act coordinately to metabolize hydrophobic xenobiotics entering the body via the gastrointestinal tract. Once into the intestinal cell, a hydrophobic xenobiotic can be transported without change to the portal vein, probably by simple diffusion. The xenobiotic then reaches the hepatocyte, the main place for phase II reactions and Mrp2-mediated transport, where it is biotransformed and secreted into bile. Once in the intestinal lumen, the conjugated xenobiotic may be eliminated together with the feces, hydrolyzed (e.g. by β–glucuronidase) and reabsorbed, or directly reabsorbed in the intact form. The presence of Mrp2 on the apical membrane of the enterocyte may contribute to prevent reabsorption (see Fig 3 for details). The kidney may be an alternative place for elimination of xenobiotics suffering phase II metabolism since it expresses conjugating enzymes and Mrp2. Glomerular filtration of conjugated derivatives may also account for renal elimination of xenobiotics. Basolateral MRPs (e.g. Mrp3) in hepatocytes can pump conjugated xenobiotics to blood thus initiating this

alternative route of elimination. The balance between transport through the apical (canalicular) or basolateral (sinusoidal) membrane in liver would determine the definitive fate of the ingested xenobiotic (feces vs urine).

In recent years, molecular biologists have introduced the possibility of studying the contribution of the different enzyme isoforms involved in biotransformation of xenobiotics as well as the molecular basis of regulation of their expression. It is now known that the small intestine may express phase I and II isoenzymes different than those present in liver, which could result from a different regulation of the genetic machinery. Several phase II or conjugating enzymes are good examples of such differences. The UGT isoenzymes UGT1A7, 1A8 and 1A10, belonging to UGT family 1, are predominantly expressed in the gastrointestinal tract, in contrast to most other UGT forms which are expressed in the liver [8]. In some cases different isoenzymes may share substrate specificity. For example, UGT1A6 that is mainly expressed in the liver, similarly to UGT1A7 is involved in conjugation of planar phenols. Thus, they may act independently as a double barrier to prevent absorption of natural occurring phenols of plant materials. Expression of different isoforms of phase II biotransformation enzymes between liver and intestine was also described for GST. While subunits belonging to GST family alpha and mu are predominantly expressed in the liver, pi subunits are poorly expressed in this tissue except when a malignant tumor is present [9]. In contrast, pi subunits present a significant constitutive expression in small intestinal tissue, in addition to alpha and mu classes [9]. Pi subunits are almost the unique classes of GST expressed in colon. Functional differences in terms of substrate specificity and response to inducers were associated to the different pattern of subunits between tissues.

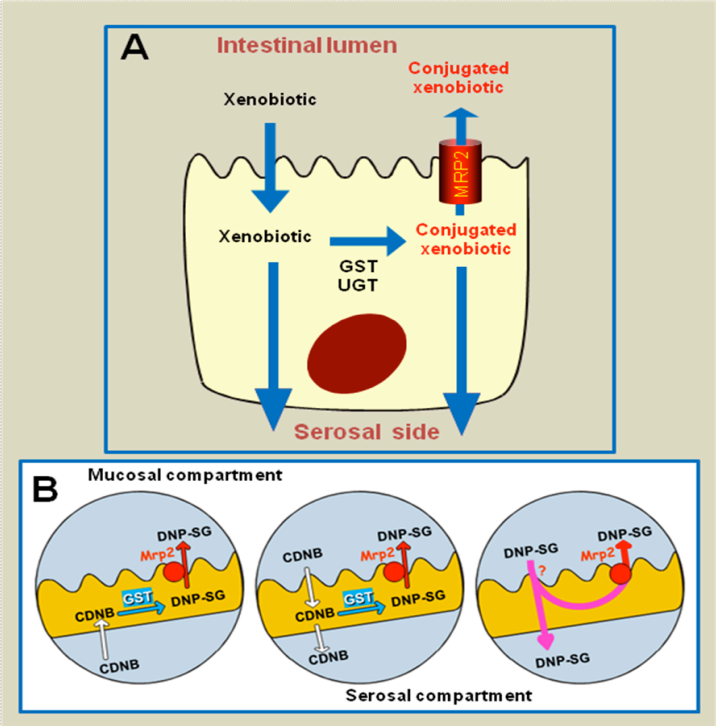

Figure 3. Phase II biotransformation and MRP2-mediated transport of xenobiotics in the enterocyte.
Hydrophobic xenobiotics freely enter the cell by diffusion through the basolateral or apical membrane in the enterocyte. They then suffer phase I and/or phase II biotransformation reactions resulting in more hydrophilic derivatives. Drug transporters mediate elimination of such derivatives from the cell. In the case of conjugation reactions (e.g. with glutathione), the product is further secreted into the intestinal lumen in a process mediated by Mrp2 (A). Alternatively, MRP substrates may be secreted through the basolateral membrane (B), and incorporated into blood circulation. Mrp3, one of the candidates to mediate this latter process, was demonstrated to be present in both hepatocytes and enterocytes. Panel B shows how these processes particularly affect the xenobiotic 1-chloro-2,4-dinitrobenzene (CDNB).

Contribution of the liver and intestine to first-pass metabolism depends not only on their respective enzyme activities but also on the capacity of transporters expressed at the plasma membrane level to secrete metabolized xenobiotics [10, 11]. While the coordinate action of phase I metabolizing enzymes and the transporter Multidrug Resistant Protein (MDR1) p-glycoprotein was extensively analyzed in previous reports [12, 13], little is known about the coordinate action of phase II systems and transporters specialized in conjugated xenobiotics, such as MRPs. Although the route of entry of phase II substrates in hepatocytes and enterocytes may be the basolateral membrane, the enterocyte presents an alternative way for substrates entering the cell from the lumen (dietary xenobiotics and drugs) **(Figs 2 and 3)**. After conjugation mediated by microsomal UGT or cytosolic GST or sulfotransferase, the conjugated derivatives could be secreted through the apical or basolateral membrane in a process mediated by MRPs.

Expression and activity of phase II enzymes and Mrp2 in pregnancy.

UDP-glucuronosyltransferase (UGT) specific activity is decreased in liver from pregnant rats when compared to non-pregnant controls, affecting substrates such as bilirubin, phenol derivatives, estradiol and estrone [2]. Although the expression of the different GST subunits was not evaluated in pregnancy, it was reported that GST activity towards 1-chloro-2,4-dinitrobenzene (CDNB) is increased at late lactation [2] Thus, differences between regulation of UGT and GST activities are apparent in pregnancy. We evaluated the expression (protein and mRNA) of the UGT isoforms involved in conjugation of phenols (UGT1A6), bilirubin (UGT1A1 and 5) and estradiol in the 17β-OH group (UGT2B1) at late pregnancy in the rat [14]. The western blot studies revealed a decrease (about 50%) in the level of all the isoforms tested. Interestingly, the levels of the respective mRNAs were not affected by pregnancy, suggesting post-transcriptional downregulation of UGT protein. The mechanism by which UGTs are downregulated in pregnant rats is still not clarified. Treatment of female rats with ethynylestradiol (EE) decreased UGT activity towards estradiol (3-OH and 17β-OH groups) in a similar magnitude as it was observed in pregnancy [15]. In an attempt to reproduce the decrease in bilirubin UGT activity and in maximal biliary excretion of the pigment observed in pregnant rats, Muraca et al [16]

administered a combination of estradiol and progesterone to ovariectomized rats leading to plasma levels of the hormones similar to those observed in pregnancy. Treatment with this appropriate combination of the steroids failed to reproduce the effects observed in pregnant rats. The authors suggested that other steroids might be involved or that other hormones might be required to allow the effect of estrogens and progestogens to became manifest, thus exerting a permissive effect. The reason by which the synthetic estrogen EE but not the natural estrogens in combination with progestogens, was able to mimic downregulation of UGTs in maternal rat liver is not clear, but it is interesting to note that EE administration is able to decrease expression of UGT proteins. Treatment of female rats with 5 mg of EE per Kg of body weight (twice daily) for 5 days, was able to decrease the level of the hepatic UGT isoforms 1A1, 1A5 and 2B1, in a similar magnitude observed for pregnancy [14]. However, it is not possible to generalize because of the occurrence of inter-species differences in the regulation of the expression and activity of metabolizing enzymes. For example, it was observed that elimination rates of drugs metabolized by certain isoforms of UGT, such as UGT1A4 and UGT2B7, are increased in pregnant women when compared to non-pregnant women [17, 18].

Biliary excretion of several Mrp2 substrates is also decreased in rats in the latter stages of pregnancy in accordance with a clear decrease in the level of Mrp2 in liver plasma membrane detected by western blotting but preserved level of Mrp2 mRNA detected by northern blotting [19]. The mechanism by which Mrp2 are downregulated in pregnant rats was not clarified, but it was observed that treatment of male rats with EE decreased Mrp2 protein level in liver and was able to mimic the dissociation between protein and mRNA [20]. These data would suggest that hormonal factors (e.g. sex steroids) affect Mrp2 expression post-transcriptionally, thus leading for example, to an impairment of protein synthesis, or to an increase in protein degradation. Alternatively, a redistribution of protein molecules to a compartment distinct from the canalicular membrane (e.g. intracellular vesicles) may explain the decreased levels observed in pregnant or EE treated rats. The question about whether decreased capacity to metabolize and secrete Mrp2 substrates into bile in pregnant animals could increase toxicity of several endogenous and exogenous compounds to the maternal liver, and in addition, increase the risk of exposure to the fetuses, is controversial. Taken glucuronidation and subsequent transport of bilirubin as a model substrate, one can speculate that depending on the way to express the data, different conclusions arise. For example, bilirubin UGT activity was decreased in pregnant rats when expressed per mg of protein or per g of liver. However, because the liver weight is increased in these animals, UGT activity did not change when expressed per whole liver [16]. Because body weight is also higher in pregnant rats, bilirubin conjugation is again decreased when the activity is normalized per body weight. Altered metabolism of bilirubin in pregnant animals may result from altered UGT activity or from a reduced availability of the cosubstrate of the reaction, i.e. UDP-glucuronic acid. Unfortunately, there is no information available on UDP-glucuronic acid levels in pregnancy. Dean et al [21] analyzed the pharmacokinetic of salicylate, which is mainly metabolized in liver by conjugation with glucuronic acid, in pregnant rats. They found that these animals showed a

significant decrease in body weight-normalized total clearance but no change in the absolute glucuronide or total clearance. However, because the biological half-life of salicylate was significantly increased in pregnant rats, the authors speculated that pregnancy is associated with a reduction in the capability of metabolizing salicylate in these rats.

In a recent study, we demonstrated that expression of Mrp2 protein as well as mRNA is preserved in small intestine during pregnancy [22]. The data clearly indicate a differential regulation of Mrp2 expression between liver and intestine during pregnancy. In the same study we analyzed the capacity of enterocytes to secrete GS-DNP, the conjugated derivative of CDNB, through the apical membrane, a process that was postulated to be mainly mediated by Mrp2. Because it is not possible to obtain inside-out vesicles from the apical membrane of the enterocyte, different strategies, such as Caco-2 cells, isolated intestinal sacs, Ussing chambers and preloading of BBMVs have been used to study intestinal secretion mediated by ABC transporters. We analyzed transport of GS-DNP in the proximal small intestine using the everted intestinal sac model. The parent compound of GS-DNP, CDNB, was added to the mucosal compartment of the intestinal sac, and the conjugated derivative generated endogenously was detected in the same compartment **(Fig 3)**. We observed that secretion of GS-DNP from the cell to the mucosal compartment, most probably mediated by Mrp2, was the rate-limiting step of the overall process and that it was preserved in pregnant rats. This was consistent with preserved expression of Mrp2, reinforcing the postulate that Mrp2 is at least partially responsible for GS-DNP transport. Because UGTs and Mrp2 are down regulated in liver during pregnancy, secretion of conjugated derivatives across the apical membrane of the proximal intestinal cells may represent an alternative pathway to prevent toxicity of xenobiotics, particularly those entering the body via the intestinal tract.

Expression and activity of phase II enzymes and Mrp2 in lactating mothers.

The activity of the conjugating enzymes, UGT and GST, are significantly increased in liver [23, 24] and small intestine [25] of female post-partum rats. Fourteen and 21 days after delivery, lactating rats exhibit an increase in the activity of UGT towards p-nitrophenol and in GST activity towards CDNB, primarily due to an increase in expression of specific isoforms. By western blotting we demonstrated that hepatic GST activity was increased mainly at expenses of the Mu subunits GSTM1 and GSTM2 while in intestine GST activity was increased mainly at expenses of the Pi subunit GSTP1. The increase in the activity of UGT towards p-nitrophenol observed in liver correlated well with an increased expression of the UGT1A6 also detected by western blotting. A northern blot study demonstrated that the upregulation was at transcriptional level. Because the liver weight is significantly increased in post-partum animals one can speculate that the absolute activity is also increased in these rats. In addition, we observed that hepatic content of UDP-

glucuronic acid is also increased in post-partum rats [23], suggesting an increased capability for UGT-mediated reactions in vivo. Administration of ovine prolactin to ovariectomized rats was able to reproduce GST and UGT changes, strongly suggesting that this hormone was the main factor involved in their regulation post-partum [24, 25]. To check whether Mrp2 follows a parallelism with phase II enzymes in lactating rats we studied expression of the pump in the post-partum liver [19] and intestine [22]. We found that hepatic Mrp2 protein, which was downregulated during pregnancy, slowly recovered along the lactation period reaching the same values observed in normal rats. Intestinal levels of Mrp2 protein and mRNA, which were preserved in pregnant rats, increased significantly with respect to controls, reaching a maximum at the latter stage of lactation (about 100% increase). The in situ analysis of Mrp2 levels by immunohistochemistry brought additional information. We observed that in agreement with the increase in the size and weight of the intestine in post-partum rats (about 130% with respect to control females), the villus exhibited a substantial increase (about 100%) in height. We did not analyze quantitatively the in situ expression of Mrp2, however, no substantial differences were observed in the intensity of the immunostaining between groups. We speculated that because the size of the villus, and thereby the villus surface, changed substantially between control and post-partum rats, the increased expression of Mrp2 protein observed postpartum in western studies results from preferential development of the regions where Mrp2 is better expressed (i.e., the upper villus), rather than derive from a specific increase in Mrp2 expression. Analysis of CDNB metabolism and transport in the everted sac model revealed that Mrp2-mediated secretion of GS-DNP into the mucosal compartment was also increased in jejunum from lactating rats in agreement with the increased expression of Mrp2. In additional experiments using the same model, we analyzed whether GS-DNP added to the mucosal compartment is transported to the serosal compartment in intestinal sacs from jejunum, ileum and colon of control, pregnant and post-partum rats. This study brought information about whether GS-DNP, once secreted into the lumen, may be reabsorbed, thus decreasing the efficiency of the secretory process. The data indicated that transport of GS-DNP was similar among the different regions of the intestine in control and pregnant rats but was significantly decreased in jejunum from post-partum animals. It is possible that the increased expression of Mrp2 in the proximal region of the intestine in lactating rats contributes not only to facilitate secretion but also to prevent reabsorption of the conjugated derivative of CDNB from the lumen.

Food intake is greatly increased in post-partum rats (2-4 fold) with respect to normal females, particularly at the latter stage of lactation (14 to 21 days after delivery). This implies adaptation of the intestinal tract to satisfy the increased need for absorption of nutrients, e.g. by increasing the mucosal surface. The absorption and interaction of potentially toxic compounds with intestinal cells, particularly dietary xenobiotics, is also expected to increase. In consequence, increased expression of phase II enzymes and Mrp2 may act coordinately to prevent the absorption of dietary xenobiotics. Interestingly, the increase in expression of phase II enzymes and Mrp2 was maximal during the last week of the lactation period [22] in agreement with the maximal increase in food intake and

intestinal hypertrophy. Because prolactin was not involved in these changes, we speculated that the factors involved in UGT, GST and Mrp2 regulation during lactation are different, since phase II enzymes, but not Mrp2, were induced by administration of the lactogenic hormone.

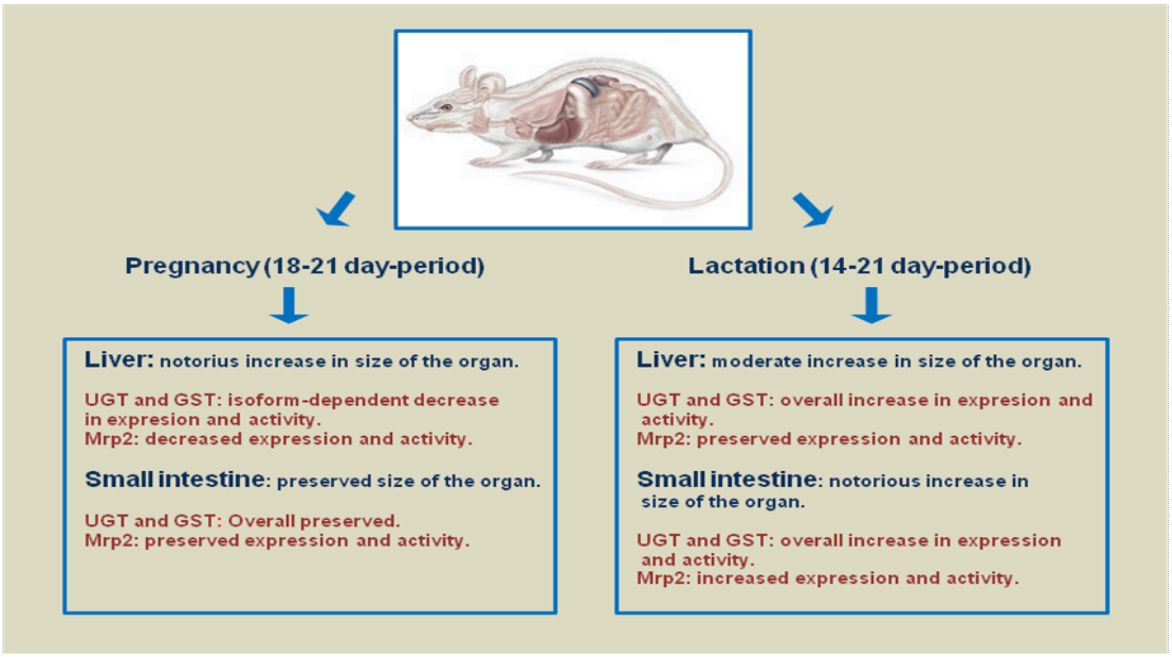

Figure 4. Main variations in Phase II reactions and Mrp2 in liver and intestine from pregnant and postpartum rats. Phase II enzymes UGT and GST variations are representative of overall changes in conjugation reactions. For details on specific changes in the different isoforms of UGT or GST as well as in regulation of Mrp2 expression and activity see references 2, 6, 14, 16-23.

Conclusions

Literature on regulation of biotransformation and transport of xenobiotics during pregnancy and lactation is scarce, and unfortunately, no significant updates were provided in the last years on this particular. Most of the evidence, supporting significant regulation of these systems in liver and intestine, comes from experiments performed in rats. Fig 4 summarizes major changes specifically observed in phase II reactions and Mrp2 during pregnancy or lactation in the rat. Due to potential differences in regulation of human vs rodent genes, any extrapolation of these results to pregnant or lactating women should be cautiously done.

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Aldo Mottino is Full Professor at UNR and a Researcher at CONICET. Our most recent interest is the role of the small intestine as a membrane barrier against the absorption of xenobiotics, particularly associated with Mrp2 expression and activity, as well as its hormonal modulation.