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Toxicokinetics of the Food-toxin IQ in Human Placental Perfusion is not Affected by ABCG2 or Xenobiotic Metabolism

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ABSTRACT

Metabolizing enzymes and transporters affect toxicokinetics of foreign compounds (e.g. drugs and carcinogens) in human placenta. The heterocyclic amine, 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) is a food-borne carcinogen being metabolically activated by cytochrome P450 (CYP) enzymes, especially by CYP1A1/2. IQ is also a substrate for ABCG2 transporter. Placental transfer of ^{14}C-IQ was evaluated in 4–6 h *ex vivo* human placental perfusions in Finland and Denmark. In Finland placentas were perfused with ^{14}C-IQ alone (0.5 μM, $n = 6$) or in combination with GF120918 (inhibitor of ABCG2, 1 μM, $n = 6$) or Ko143 (specific inhibitor of ABCG2, 2 μM, $n = 4$) to study the role of ABCG2 inhibition in transfer while in Denmark perfusions were performed with ^{14}C-IQ alone. Critical parameters (leak from fetal to maternal circulation, pH values, blood gases, glucose consumption, the production of hCG hormone and transport of antipyrine) were analyzed during the perfusions. ^{14}C-IQ on maternal and fetal sides was determined by liquid scintillation counting. In Finland IQ and its metabolites in final perfusates were determined also by LC/TOF-MS. ABCG2 expression and EROD activity (CYP1A1/2) were analyzed from perfused tissues. ^{14}C-IQ was easily transferred through the placenta from maternal to fetal side in both laboratories. Neither significant EROD activity nor IQ metabolites were found in placentas from non-smoking mothers. Inhibition of ABCG2 by GF120918 (FM-ratio of IQ 0.95) or Ko143 (FM-ratio of IQ 0.94) did not affect ^{14}C-IQ transfer (FM-ratio of IQ in IQ only perfusions 0.97), which indicates that placental ABCG2 does not have a significant role in protecting fetus from IQ.

1. Introduction

The diet contains many chemicals of which most are believed to cross the placenta at least to some extent leading to fetal exposure during pregnancy. Some of the compounds may have severe immediate effects on pregnancy and the developing fetus while others may cause adverse effects emerging later in life, for instance as a higher cancer susceptibility. Currently the exposure level in the majority of fetuses is unknown and there are gaps in the information regarding the factors affecting the exposure level [1,2].

Heterocyclic amines (HCAs) are carcinogens to which people are exposed via meat and fish cooked at high temperatures [3]. IQ (2-amino-3-methylimidazo[4,5-f]quinoline) is not the most abundant HCA in food (1.9–77.4 ng of IQ/g processed meat, 0.2–7.7 μg total HCA/day/person) [4] but it is highly carcinogenic and genotoxic in several animal studies [3]. IQ is metabolically activated to DNA damaging metabolites mainly by cytochrome P450 (CYP) -enzymes such as CYP1A2, CYP1A1 and CYP1B1 [5] (Fig. 1). In monkeys, high concentrations of IQ have been shown to result in DNA-adducts in placenta and fetus in a dose- and gestational age-dependent manner [6]. In mice, IQ crossed the placenta and reached fetal tissues, especially liver and intestine [7]. Unfortunately, there is an absence of information on placental kinetics for IQ in the human.

Even though most compounds transit the placenta, not all compounds do. Thus, the placenta is not merely a passive barrier. Placenta contain metabolic enzymes and transporters that interact

Abbreviations: IQ, 2-amino-3-methylimidazo[4,5-f]quinoline; hCG, human chorionic gonadotropin; CYP, cytochrome P450; ABC, ATP-binding cassette; HCA, heterocyclic amine; EROD, ethoxyresorufin-O-deethylase.

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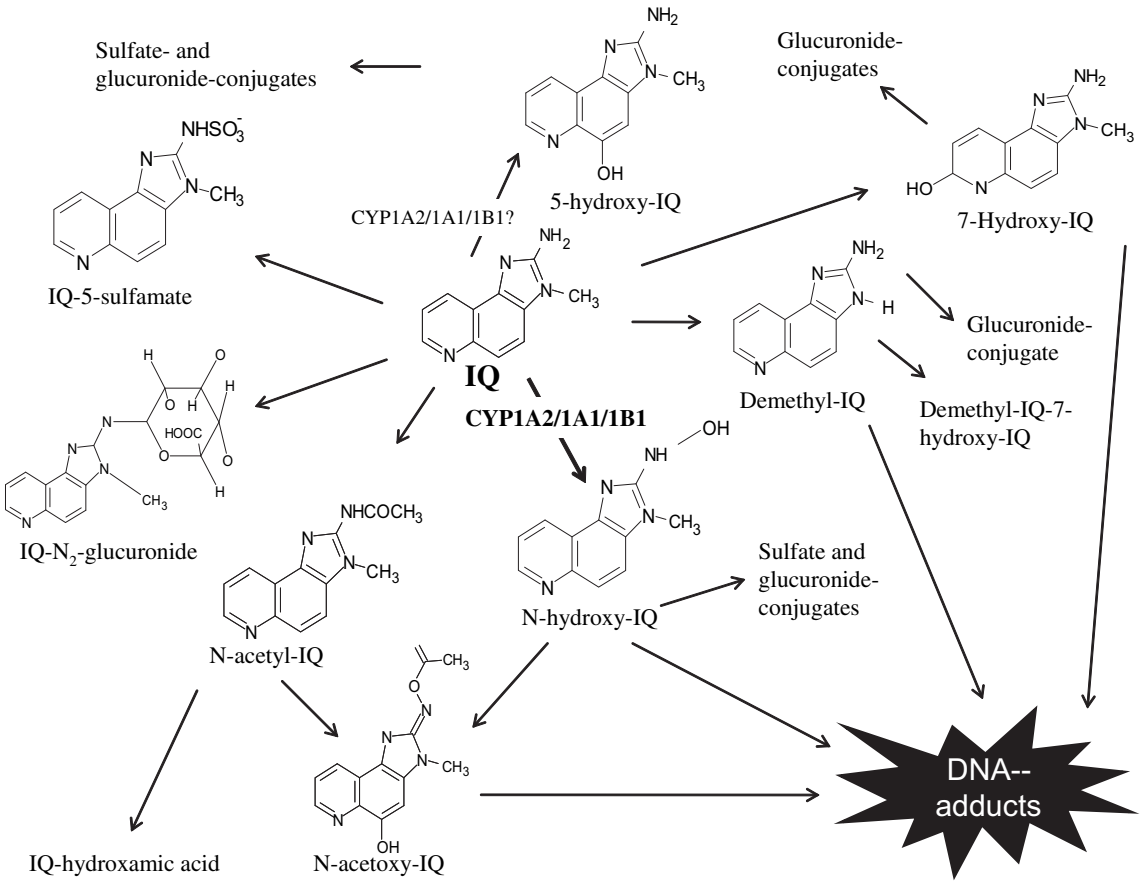

Fig. 1. Chemical structure of IQ and metabolic pathways leading to common metabolites. Possible metabolic routes for IQ based on data with animal and human studies. Metabolism of IQ to demethyl-IQ and 7-hydroxy-IQ is performed by human intestinal bacteria [34,47–50].

with xenobiotics [8–10]. Individual variation in fetal exposure to xenobiotics can be associated with differential expression of xenobiotic metabolic enzymes and transporters due to physiological changes during pregnancy, polymorphism and exposure to other xenobiotics [8–10]. One of the most important ATP-binding cassette (ABC) transporters for transport of xenobiotics is ABCG2 (breast cancer resistance protein, BCRP) [11]. ABCG2 is an efflux transporter expressed extensively in placental tissue, mainly in the apical side of syncytiotrophoblast membrane [12]. Based on human data, ABCG2 is significantly expressed in the placenta throughout the gestation [13–15]. In humans ABCG2 expression has high interindividual variability [16,17] which may alter the placental toxicokinetics for xenobiotics.

Inhibition of ABCG2 activity increases substantially the fetal uptake of the associated substrate as seen in the mouse [18]. In rats, both the placental and fetal expression of ABCG2 together participate in restricting the fetal exposure [19,20]. ABCG2 is associated with the transport of many xenobiotics including HCA, PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine) [21,22]. We have previously shown that ABCG2 protein restricts the transfer of PhIP in human placenta [17]. IQ is a substrate for ABCG2 transporter in mouse and in polarized canine kidney cell line MDCK-II transduced with human ABCG2 cDNAs [22]. So far, there are no human data available on the significance of ABCG2 in the toxicokinetics of IQ. In mice ABCG2 increases the secretion of IQ into the breast milk [22].

The aim of this study was to examine the placental kinetics of the food-toxin IQ using a human placental perfusion model in vitro. Because transporters and metabolizing enzymes affect toxicokinetics of IQ in other tissues [5,6,22], the metabolism of IQ and the role of ABCG2 on the transfer of IQ in human placenta were investigated.

2. Methods

2.1. Chemicals

^{14}C-IQ (2-amino-3-methylimidazo[4,5-f]quinoline, CAS registry number: 76180-96-6) was from Toronto Research Chemicals (Toronto, Canada). The unspecific ABCG2 inhibitor, elacridar (GF120918, CAS registry number: 143664-11-3) was from GlaxoSmithKline (Brentford, UK). The specific ABCG2 inhibitor, KO143 (3-(6-isobutyl-9-methoxy-1,4-dioxo-1,2,3,4,6,7,12,12a-octahydro-pyrazino[1′,2′:1,6]pyrido [3,4-b]indol-3-yl)-propionic acid tert-butyl ester) was a kind gift from Dr. Schinkel (The Netherlands Cancer Institute, Amsterdam, The Netherlands) [23]. Dimethyl sulfoxide (DMSO, Sigma, Steinheim, Germany) was used as a solvent for study chemicals.

2.2. Placental perfusions

Study protocol was approved by the ethics committee of the Northern Ostrobotnia Hospital District. Human placentas ($n = 16$ for successfully perfused) were collected from normal vaginal deliveries or elective caesarean sections from the Department of Gynecology and Obstetrics, University Hospital of Oulu, Finland. All collected placentas were full-term from healthy non-smoking mothers. Mothers donating their placenta signed an informed consent. Placentas were handled anonymously.

Dual re-circulating human placental perfusions were done as described previously [24,17]. Immediately after delivery the blood vessels of the placenta were flushed with heparinized (25 IE/ml, Heparin LEO, LEO Pharma AB, Malmö, Sweden) and oxidized (by carbogenic oxygen) Krebs–Ringer solution. A pair of peripheral vessels on the chorionic plate of one lobule was cannulated. If circulation of heparinized/oxidized Krebs–Ringer phosphate solution was adequate (the outflow of the perfusion medium from the vein equaled to the inflow of the medium into artery, flow ca. 1 ml/min), the cannulated lobule was attached to the perfusion chamber. In