Placenta 31 (2010) 641–648

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

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

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ARTICLE INFO

Article history:
Accepted 6 May 2010

Keywords:
Human placental perfusion
Heterocyclic amine
IQ
Toxicokinetics
Metabolism

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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doi:10.1016/j.placenta.2010.05.002

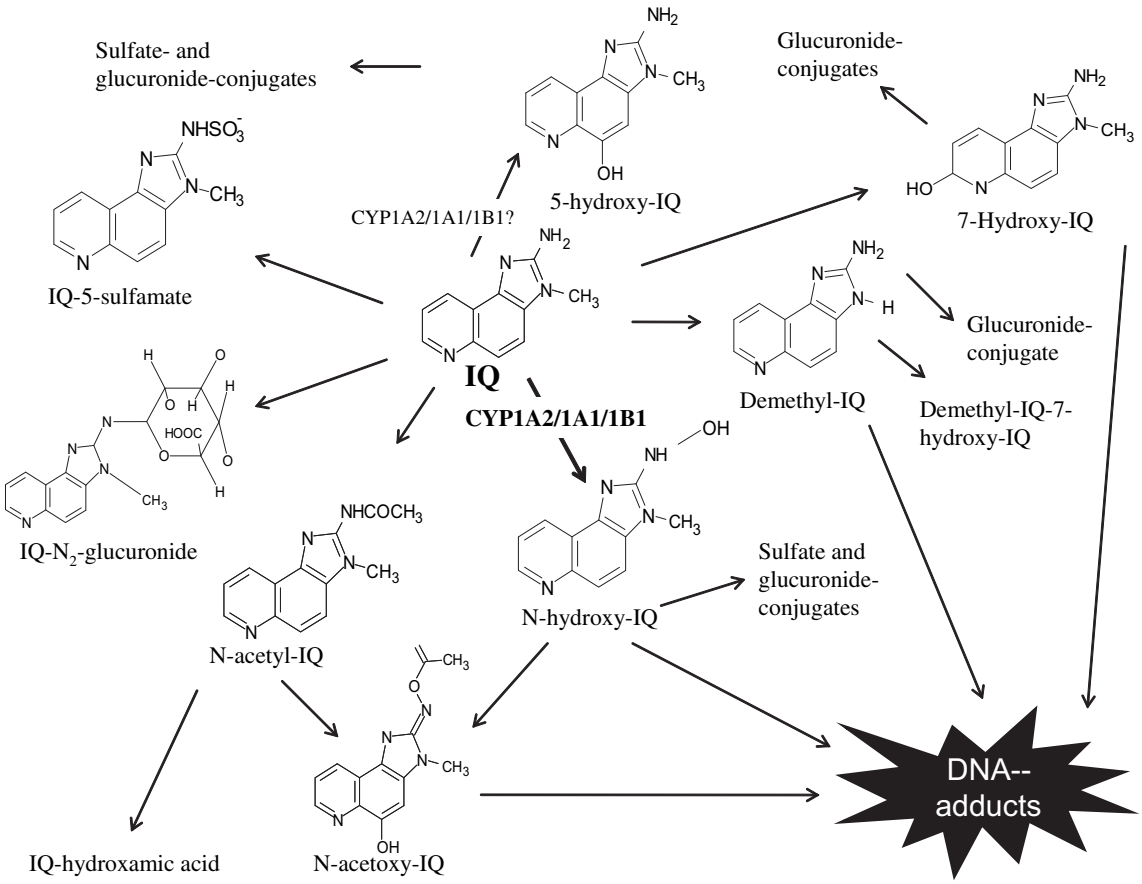

Fig. 1. Chemical structure of IQ and metabolic pathways leading to common metabolites. Possible metabolic routes for IQ based on data with animal and human studies. Metabolism of IQ to demethyl-IQ and 7-hydroxy-IQ is performed by human intestinal bacteria [34,47–50].

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

the maternal side, two cannulae were placed into the intervillous space through the basal plate. Cell culture medium (phenol red free RPMI 1640 medium, GIBCO, Paisley, UK) supplemented with 1% L-glutamine (Glutamax™, GIBCO, Paisley, UK), 1% non-essential amino acids (Euroclone, Siziano, Italy), human albumin (2 g/l) (Finnish Red Cross, Sanquin, Amsterdam, The Netherlands) and Dextran (2 g/l) (T-40, Sigma, Steinheim, Germany) was used as the perfusate (maternal 200 ml, fetal 120 ml). Heparin was added to maternal (25 KY/ml) and fetal (16.7 KY/ml) circulations.

After 30–45 min pre-perfusion, 0.5 μM ^{14}C-IQ (specific activity 370 MBq/mmol) was added to the maternal reservoir. The placentas were perfused with ^{14}C-IQ either alone ($n = 6$) or concurrently with ABCG2 inhibitor GF120918 (1 μM, $n = 6$) or Ko143 (2 μM, $n = 4$). ABCG2 inhibitors were added 30 min earlier than ^{14}C-IQ. Antipyrine (0.1 mg/ml, 99%, Aldrich-Chemie, Steinheim, Germany) was added on the maternal side as a reference substance. Flow rates were 9 ml/min on maternal and 2.5 ml/min on fetal side. Carbogenic oxygen (5% CO_2; 95% O_2) was led to maternal reservoir and gas mixture (95% N_2/5% CO_2) on fetal reservoir. Samples (1.6 ml) were collected from maternal and fetal reservoirs (before, 15 min, 30 min, 1 h, 1.5 h, 2 h, 2.5 h, 3 h, 4 h). One perfusion was performed without a placenta: IQ concentrations stayed constant throughout the perfusion on both sides confirming that IQ does not adhere to the perfusion equipment.

Tissue samples from unperfused (control) area at the beginning of perfusion and from perfused area at the end of perfusion were fixed in 10% phosphate buffered neutral formalin (FF-Chemicals, Haukipudas, Finland). Paraffinized tissue sections (5 μm) were stained with routine hematoxylin–eosine staining. Membrane protein extraction from snap frozen tissue samples stored in −70 °C and immunoblotting of transporter proteins were carried out as previously described [17].

2.2.1. Parameters followed during perfusions and criteria for success

The leak from fetal to maternal reservoir, antipyrine transfer, gas exchange, pH, glucose consumption and human chorionic gonadotropin hormone (hCG) production, were monitored during the perfusions. To confirm that feto-maternal barrier was intact perfusions with a leak of more than 3 ml/h were discarded [24]. Another criterion for a successful perfusion was antipyrine transfer by passive diffusion through placenta [24,25]. Antipyrine FM-ratio of at least 0.75 at the end of perfusion was considered as evidence of sufficient overlap of maternal and fetal circulations. During the perfusion, pH and blood gas exchange were monitored by blood gas analyzer (Stat Profile pHOx Basic, NovaBiomedical, Flintshire, UK) every half an hour from maternal arterial and venous flows and from the fetal reservoir. If needed, pH was adjusted by hydrochloric acid. Glucose concentration was measured by Super Glucocard II-analyzer (Arkray Inc., Kyoto, Japan) from both perfusates before and after perfusion to confirm glucose consumption. HCG concentrations in final perfusates on maternal side were analyzed using hCG + beta ELISA enzyme immunoassay kit (IBL Hamburg, Hamburg, Germany).

2.3. Analysis of ^{14}C-IQ by liquid scintillation counter

The amount of ^{14}C-IQ and its metabolites in perfusate samples and placental tissue homogenates were analyzed by liquid scintillation counter (1215 Rackbeta II, LKB Wallac). The samples were diluted to liquid scintillation cocktail (Optiphase 'HiSafe' 2, PerkinElmer, Shelton, USA). All samples were analyzed in duplicate and results were normalized by external standards. The recovery of ^{14}C-IQ from the perfusates and tissue was 81.6 ± 9.9%. Recovery was calculated by count values gained from scintillation counter and quantified with the help of external standard and standard curve. Recovery value contains detected ^{14}C-IQ in fetal and maternal compartments at the end volumes, in samples taken during perfusion and in perfused tissue compared to the amount of ^{14}C-IQ added to maternal side at the beginning of the perfusion.

2.4. Analysis of antipyrine

Analysis of antipyrine was done as previously described [26] with some modifications. Samples were added in 0.5 M ortho-phosphoric acid (85%, Merck, Darmstadt, Germany) with 50 μg/ml of the internal standard phenacetin (98%, CAS registry number: 62-44-2, Fluka, Sigma Aldrich-Chemie, Steinheim, Germany) and filtered with 0.2 μm syringe filter (Spartan 13/0.2 RC, Whatman, Dassel, Germany) before the analysis with HPLC (Shimadzu, SLC-10Avp) equipped with LichroCart®, LiChrospher® 100 RP18 (5 μm, Merck KgaA, Darmstadt, Germany) column. The eluent was: KH_2PO_4 (20 mM); acetonitrile (20%); triethylamine (0.05%). All samples were analyzed as duplicates and results were normalized by the values of the internal standard. The antipyrine concentrations were analyzed using a previously published method [26] from four perfusions with IQ alone. The chromatography conditions were similar but the samples were analyzed using external standards.

2.5. Enzyme, 7-ethoxyresorufin O-deethylase (EROD, CYP1A1/2) activity measurement

Microsomes were isolated by serial centrifugations from placentas perfused with ^{14}C-IQ alone ($n = 6$) or with the ABCG2 inhibitor GF120918 ($n = 6$). Enzyme activity was analyzed as previously described [27].

2.6. Analysis of IQ and its metabolites by liquid chromatography-mass spectrometry

IQ and its metabolites were analyzed from the final samples of perfusions with IQ alone or in combination with GF120918. Water was in-house freshly prepared with Direct-Q (Millipore Oy, Espoo, Finland) purification system and UP grade (ultra pure, 18.2 MΩ). The samples were thawed at room temperature, protein precipitated 1:1 with acetonitrile (HPLC grade, Merck, Darmstadt, Germany), shaken and centrifuged for 10 min at 16,100g (Eppendorf 5415D, Eppendorf AG, Hamburg, Germany) and pipetted to Max Recovery autosampler vials (Waters Corporation, Milford, Massachusetts, USA). Standard samples with concentrations 1 nM, 5 nM, 10 nM, 50 nM, 100 nM, 500 nM and 1 μM were spiked in perfusion medium and protein precipitated similarly as the samples. A Waters Acquity ultra-performance liquid chromatographic (UPLC) system (Waters Corp., Milford, MA, USA) with autosampler, vacuum degasser and column oven was used. The analytical column used was a Waters BEH Shield RP18 (2.1 × 50 mm, 1.7 μm, Waters Corp, Milford, MA, USA), together with a 0.2 μm on-line filter before the column. The eluents were 10 mM ammonium hydroxide (A, pH 10, BDH Laboratory Supplies, Poole, UK) and acetonitrile (B). The gradient elution from 2% to 10% in 2 min was employed, followed by 1 min linear gradient to 70% B and column equilibration. The flow rate was 0.5 ml/min and the column oven temperature was 35 °C. LC/TOF-MS data were recorded with a Micromass LCT Premier XE time-of-flight (TOF) mass spectrometer (Micromass Ltd., Manchester, England) equipped with a LockSpray electrospray ionization source. A positive ionization mode of electrospray was used with cone voltage of 40 V. The mass range of m/z 140–600 was acquired. The W-option of the reflector was used, and the DRE (dynamic range enhancement) option was set on. Leucine enkephalin ($[M + H]^+$ m/z 556.2771) was used as lock mass compound for accurate mass measurements and was delivered into LockSpray probe with a syringe pump. Masslynx 4.1 software was used for controlling the instrumentation and for data processing.

2.7. Statistics

Comparisons in single time points using one-way ANOVA followed by Tukey as a post-hoc test were performed with SPSS 16.0. Results were considered statistically significant at the $P < 0.05$ level. Data are presented as mean ± standard deviation (SD). Area under curve (AUC) values were calculated with Prism 4 (version 4.01), but statistical analysis was performed with SPSS.

3. Results

3.1. Parameters followed during placental perfusions

The leak (fetal reservoir volume loss) was less than 3 ml/h in all of the accepted perfusions considered successful (Table 1). All succesfully perfused placental lobules also consumed glucose and produced hCG hormone confirming metabolic activity of the tissue (Table 1). There were no major differences in the viability parameters between perfusion groups suggesting that the groups are comparable.

The morphology of the placental tissue after perfusion was analyzed according to Kaufmann (1985) [28]. The structure was retained well in all perfused tissues; villous trees and

Table 1
Leak, pH values, glucose consumption and hCG production in perfused cotyledons. Values are mean ± SD. $^*P < 0.05$, $^{**}P < 0.01$ compared to the perfusions done with IQ alone (one-way ANOVA using Tukey as a post-hoc).

	^{14}C-IQ alone ($n = 6$)	^{14}C-IQ with 1 μM GF120918 ($n = 6$)	^{14}C-IQ with 2 μM Ko143 ($n = 4$)
Leak (ml/h)	1.12 ± 0.34	1.30 ± 0.48	1.46 ± 0.82
pH			
At the beginning			
Maternal	7.38 ± 0.03	7.41 ± 0.03	7.48 ± 0.01**
Fetal	7.30 ± 0.04	7.34 ± 0.02	7.35 ± 0.01
At the end			
Maternal	7.43 ± 0.03	7.43 ± 0.05	7.45 ± 0.03
Fetal	7.42 ± 0.03	7.44 ± 0.03	7.38 ± 0.02
Glucose consumption (μmol/g of tissue/h)	21 ± 8	30 ± 17	49 ± 21*
hCG secretion (mIU/ml/g of tissue/h)	11.32 ± 7.34	20.61 ± 16.79	33.73 ± 38.64

syncytiotrophoblast were intact and no edema or syncytiotrophoblastic vacualization were found in any of the tissues (data not shown).

3.2. Placental toxicokinetics of ^{14}C-IQ

^{14}C-IQ was rapidly transferred from the maternal to the fetal side during 4-h perfusions of human term placenta. After 15 min ^{14}C-IQ was detectable in the fetal side in all perfusions, average concentration being 0.01 ± 0.004 μg/ml (Fig. 2A). After 2 h ^{14}C-IQ concentrations on both circulations were almost equal (maternal concentration 0.045 ± 0.015 μg/ml and fetal 0.037 ± 0.009 μg/ml). At the end of the perfusions concentrations of ^{14}C-IQ were in equilibrium; feto-maternal-concentration ratio (FM-ratio) of ^{14}C-IQ being 0.97 ± 0.12 (Fig. 2B). There was a slight perfusion to perfusion variation in FM-ratios ranging from 0.84 to 1.13 at the end of the perfusions (Fig. 2B). Also from the AUC-values (AUC_{4h} 170.65 ± 22.42; mean ± SD) at the end of the perfusions it is evident that there is a slight interindividual variation in placental IQ kinetics. The passively diffused reference compound antipyrine was transferred from the maternal to the fetal side as expected (FM-ratio at the end 0.91 ± 0.11) achieving equilibration between maternal and fetal circulations after 2 h (Fig. 2C). The transfer rate of ^{14}C-IQ was similar to that of antipyrine (Fig. 2D).

To confirm that radioactivity measurements of ^{14}C-IQ represent the kinetics of IQ, the amount of IQ and its metabolites in final maternal and fetal perfusates was analyzed using LC/TOF-MS. Results confirmed that fetal and maternal concentrations were equal at the end the FM-ratio for IQ being 1.19 ± 0.17 (range 0.998–1.423). Neither metabolites from perfusates nor significant 7-ethoxyresorufin-o-deethylase (EROD) activity from tissues were detected. IQ did not accumulate into tissues significantly, tissue retainment of ^{14}C-IQ being in the range of 1.95–8.28% in perfusions performed with IQ alone.

3.3. Interlaboratory comparison

As part of an on-going EU-funded projects (NewGeneris Project: Newborns and Genotoxic exposure risks, FOOD-CT-2005 016320; Reprotect: Development of a novel approach in hazard and risk assessment or reproductive toxicity by a combination and application of in vitro, tissue and sensor technologies, LSHB-CT-2004-503257) the placental transfer data for IQ of a Finnish laboratory was compared with the data from an independent Danish laboratory. The placental perfusions in Copenhagen were carried out according to previously published protocol using 2 g/L of albumin [29]. IQ crossed the placenta as easily in both laboratories without any statistically significant differences between the laboratories (Table 2). In Denmark placental perfusions were performed using two different IQ concentrations (0.5 μM, $n = 4$ and 1 μM, $n = 2$) suggesting that placental transfer of IQ is not dose-dependent (data not shown).

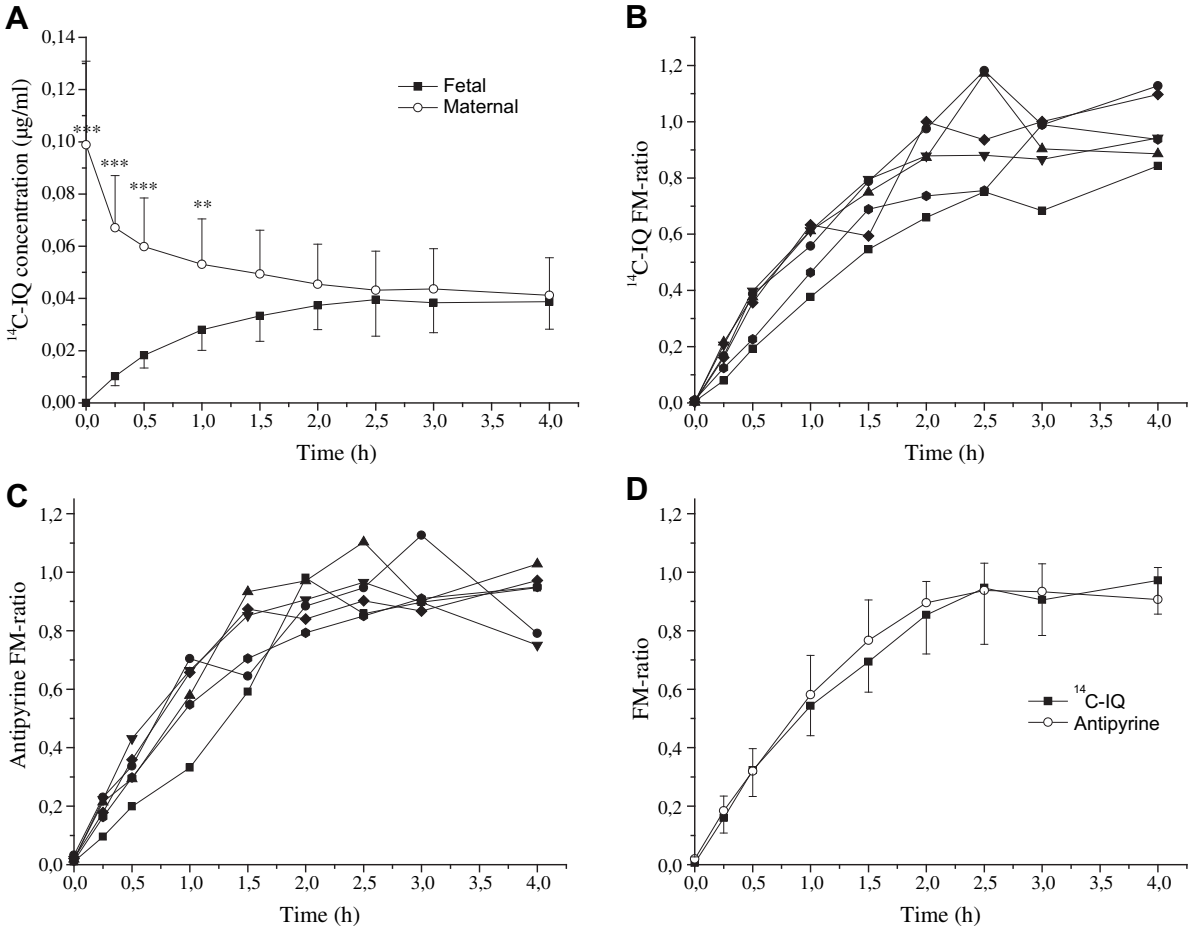

Fig. 2. Kinetics of ^{14}C-IQ and antipyrine in human placental perfusion. A. Concentrations of ^{14}C-IQ in maternal (open circle) and fetal (solid square) circulations ($n = 6$). B. Feto-maternal-concentration ratio (FM-ratio) of ^{14}C-IQ in individual perfusions ($n = 6$). C. FM-ratio of antipyrine in individual perfusions ($n = 6$, symbols in different perfusions correspond the symbols in Fig. 2B). D. FM-ratio of ^{14}C-IQ (solid square) and antipyrine (open circle). Values are mean ± SD. $^{***}P < 0.001$, $^{**}P < 0.01$ (one-way ANOVA using Tukey as a post-hoc).

Table 2
Comparison of placental transfer of ^{14}C-IQ between two independent laboratories. Values are mean ± SD. The values are not statiscally significantly different in any of the time points (one-way ANOVA using Tukey as a post-hoc).

Time (min)	FM-ratio 0.5 μM IQ ($n = 6$) Finland	FM-ratio 0.5 μM IQ ($n = 4$) Denmark
0–2	0.005 ± 0.003	0.012 ± 0.011
15	0.159 ± 0.051	ND
30	0.323 ± 0.090	0.339 ± 0.120
60	0.543 ± 0.102	0.570 ± 0.162
90	0.694 ± 0.104	0.665 ± 0.127
120	0.854 ± 0.133	0.735 ± 0.113
150	0.946 ± 0.193	0.752 ± 0.084
180	0.905 ± 0.121	0.752 ± 0.091
240	0.972 ± 0.115	0.792 ± 0.056
300	ND	0.843 ± 0.042
360	ND	0.834 ± 0.061

ND = not done.

3.4. ABCG2 inhibition and placental transfer of ^{14}C-IQ

Inhibition of ABCG2 had no effect on placental transfer of ^{14}C-IQ (Fig. 3A and B). Similarly to perfusions done with ^{14}C-IQ alone, ^{14}C-IQ was detected in the first sample after 15 min perfusion from the fetal side in all perfusions. Equilibration of ^{14}C-IQ concentrations occurred approximately after 2 h with both inhibitors similarly to perfusions performed with ^{14}C-IQ alone. FM-ratios of ^{14}C-IQ at the end of perfusions were 0.95 ± 0.05 and 0.94 ± 0.05 for GF120918 and Ko143 perfusions, respectively (Fig. 3A). Slight interindividual differences were seen in transfer within perfusion groups (Fig. 3B). Antipyrine transfer was similar in all three perfusion groups confirming that flow conditions were similar and groups were comparable (FM-ratios at the end of the perfusion: 0.95 ± 0.07 with GF120918 and 0.95 ± 0.03 with Ko143) (Fig. 3C and D). AUC-values for perfusions done with ^{14}C-IQ in combination with GF120918 or with Ko143 were $AUC_{4h} = 169.72 \pm 9.71$ and $AUC_{4h} = 160.7 \pm 18.87$ (mean ± SD), respectively. No statistically significant differences between groups or compared to perfusions done with IQ alone were found.

The amount of ^{14}C-IQ and its metabolites were analyzed by LC/TOF-MS in the samples of perfusions with ^{14}C-IQ in combination with GF120918. FM-ratio was 1.07 ± 0.10 (not statistically significantly different from the ratio with IQ only). As in perfusions done with IQ alone, no metabolites in perfusion media or significant EROD activities in placentas after the perfusions were found. Tissue retainment of ^{14}C-IQ in perfusions performed with IQ in combination with GF120918 or Ko143 was in the range of 1.66–7.69% and 0.57 –1.87%, respectively.

3.5. Expression of ABCG2 protein

As expected, there was a great interindividual variation in the expression of ABCG2 transporter protein (Fig. 4A). There were no statistically significant differences in ABCG2 expression between control samples taken before perfusion or samples taken from

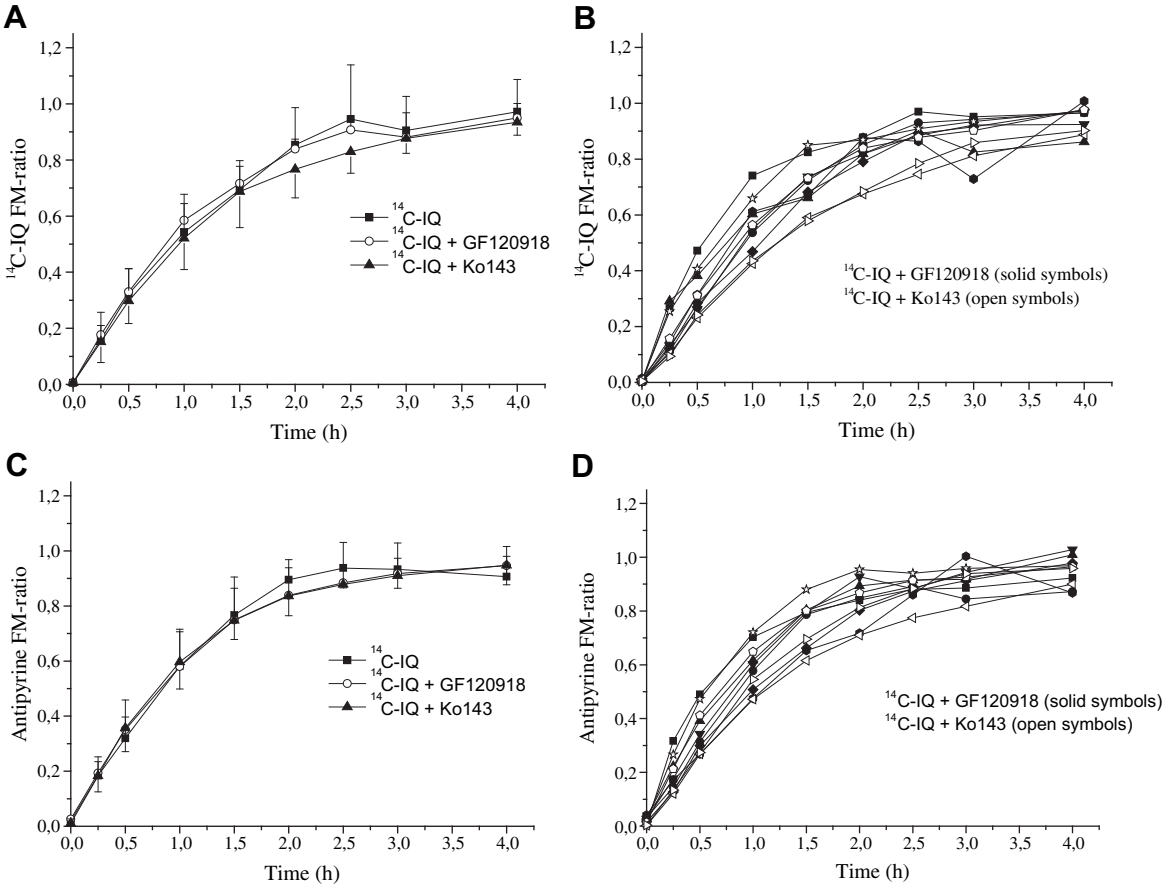

Fig. 3. Effects of ABCG2 inhibitors on placental transfer of ^{14}C-IQ. A. Feto-maternal-ratios (FM-ratio) of ^{14}C-IQ in perfusions done with 0.5 μM ^{14}C-IQ alone ($n = 6$, solid square), with 0.5 μM ^{14}C-IQ in combination with 1 μM GF120918 ($n = 6$, open circle) or 2 μM Ko143 ($n = 4$, solid triangle). B. FM-ratio of ^{14}C-IQ in individual perfusions with 1 μM GF120918 ($n = 6$, solid symbol) or 2 μM Ko143 ($n = 4$, open symbol). C. FM-ratio of antipyrine. D. Variation in FM-ratios of antipyrine in individual perfusions. Values are mean ± SD. No statistically significant differences (one-way ANOVA using Tukey as a post-hoc).

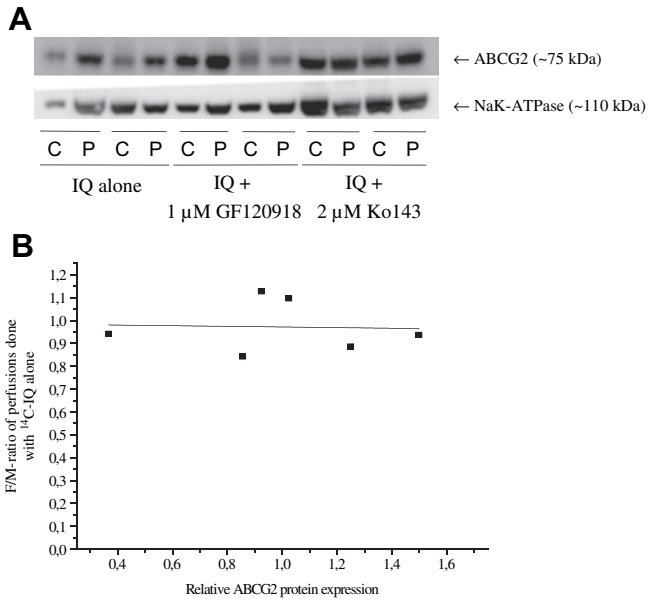

Fig. 4. The expression of ABCG2 protein in the studied placentas. A) A representative immunoblot on ABCG2 (75 kDa) expressions in membrane protein extracts (30 μg) from non-perfused (control = C) and perfused (P) areas. NaK-ATPase (110 kDa) was used as a loading control. B) No correlation was found between the relative expression of ABCG2 protein normalized by the loading control and the FM-ratio of ^{14}C-IQ in perfusions with IQ alone. All tissue samples were analyzed at least twice.

perfused areas. In addition, the expression of ABCG2 in the perfused tissue did not correlate significantly with ^{14}C-IQ FM-ratio in perfusions done with IQ alone (Fig. 4B) or in perfusions with ABCG2 inhibitors (data not shown).

4. Discussion

In this study the transfer of the food-toxin IQ in human placental perfusion was faster than that of another HCA, PhIP [17]. There were no significant differences in the placental transfer of IQ between two independent laboratories. Comparable results strengthen the view of relatively rapid transport. This study is the first to analyze IQ kinetics in human placental perfusion although placental transfer of IQ has been shown in animals. Josyula and coworkers [6] showed that IQ passes placentas of patas monkeys and Bergman [7] demonstrated the same to happen with radioactively labeled IQ in mice. The reports in the literature together with our results thus indicate the potential for significant fetal exposure to IQ through maternal exposure.

In our study, IQ crossed the placenta and equilibrated between maternal and fetal sides in all experiments but there was slight interindividual variation in the placental transfer. Differences between individuals may be due to the genetic variation or environmental factors. Putatively one source for variation could be the variation in the expression of ABCG2 transporter protein. The variation is partly due to the known polymorphisms of *ABCG2* gene [16] affecting the localization, expression and function of ABCG2 protein [8,30]. Kobayashi and coworkers [16] found variation in ABCG2 protein expression in human placenta due to allelic variants in position 421. However, the expression of ABCG2 protein did not correlate with the transfer rate of IQ in our study. To further test the effect of ABCG2 on the IQ transfer, perfusions were performed using ABCG2 inhibitors, which did not alter the placental transfer of ^{14}C-IQ. Previously we have shown that inhibition of ABCG2, using the same inhibitors as in this study, increased transfer of another HCA, PhIP in placental perfusion [17].

In the literature, inhibition of ABCG2 has been shown to decrease secretion of PhIP and IQ to breast milk in mice [22,31]. PhIP and IQ share many structural and physico-chemical similarities and they both have been shown to be substrates for human ABCG2 [3,21,22]. Observed differences in placental kinetics of IQ and PhIP may be due to differences in lipophilicity of compounds. PhIP is more lipophilic than IQ [32]. During *ex vivo* human placental perfusion highly lipophilic compounds tend to accumulate to placental tissue [33] which may partially explain the observed differences in the behaviour of PhIP and IQ. In the case of IQ, there might also be some differences in the affinity towards ABCG2 transporter compared to PhIP. Furthermore, it is possible that IQ may be a more potent substrate for transporters other than ABCG2. Information of possible protein binding of IQ is limited in the literature. However, Turesky and coworkers suggested low blood protein binding of IQ [34]. Interestingly, Holcberg and coworkers [35] reported that inhibition of ABCB1 (p-glycoprotein) did not affect the placental kinetics of ABCB1 substrate digoxin, in human placental perfusion. Controversies in the results may be due to different experimental models, e.g. *ex vivo* perfusion versus cell lines. Human placental perfusion system is close to the physiological situation in vivo but still it differs from an in vivo situation and stress/hypoxia during labor can theoretically change the expression and function of transporters and metabolic enzymes.

Several xenobiotics need metabolic bioactivation to become genotoxic. CYP1A1, known to metabolize IQ, is expressed and functionally active in human placenta throughout gestation [36–38]. Also, CYP1B1 mRNA is expressed in human term placenta [38,39]. On the other hand, CYP1A2 which is a more prominent metabolizer of IQ than CYP1A1 [5] seems not to be significantly active in human placenta. CYP1A2 mRNA has been found in early [36] but not in term human placenta [37,39]. Avery and coworkers [40], however, reported CYP1A2 protein expression and a low 7-methoxyresorufin-O-demethylation (MROD) activity in term human placentas.

In this study, we did not find significant EROD activity representing CYP1A1 activity in perfused placentas. Agreeing with this finding no IQ metabolites were found either. The detection method for the most common metabolites of IQ was the very sensitive LC/TOF-MS. Importantly, all of the placentas for the study were from non-smokers, who have a relatively low or nonexistent activity of placental CYP1A1 [36–38,40]. CYP1A1 is highly inducible by tobacco smoke in human term placentas while inducibility of CYP1B1 and CYP1A2 is uncertain [36–38,40,41]. HCAs may affect the activity of CYPs according to the literature [42,43]. Also, human CYP1A1/2 variants have been shown to affect IQ metabolism [44]. Therefore, it may be possible that some human placentas are able to metabolize IQ.

In patas monkeys, Josyula and coworkers [6] found IQ DNA-adducts in the placenta and fetus after maternal exposure. In mice IQ is not a potent transplacental genotoxin [43]. In our study isolated human placenta did not metabolize IQ. The human placenta has been shown to form metabolites of xenobiotics during pregnancy and also during human placental perfusion [45]. It should be noticed that fetuses express CYPs that may metabolize xenobiotics crossing the placenta [10,38]. According to Josyula and coworkers [6] part of the IQ DNA-adducts detected in fetal tissues resulted primarily from maternal liver activity but also that fetal tissues were capable of bioactivating IQ. In human fetus hepatic CYP1A1/2 enzymes are not expressed abundantly [10,39], but minor expression of CYP1B1 and CYP1A1 in fetal tissues has been reported in the literature [5,38]. Thus, fetal tissues may be able to bioactivate IQ although generally genotoxicants needing bioactivation are considered to be less potent in fetal tissues [46].

In conclusion, food-toxin IQ is easily transferred through the human placenta from maternal to the fetal circulation as shown by results from two laboratories. Most probably neither ABCG2 nor xenobiotic metabolism have significance in placental toxicokinetics of IQ in human placental perfusion.

Acknowledgements

We appreciate the collaboration of the nursing staff of the Department of Gynecology and Obstetrics, University Hospital of Oulu, Finland. Also the collaboration of Professor of Pharmacology and Toxicology Olavi Pelkonen is highly appreciated. We also thank GlaxoSmithKline for providing GF120918 and Dr. Alfred Schinkel for K0143. The project has been financially supported by the Academy of Finland, Thule Institute and EU networks: NewGeneris (FOOD-CT-2005 016320) and ReProTect (LSHB-CT-2004-503257).

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