2-Naphthol Transplacental Transfer in Human Placenta: A Review

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ABSTRACT: ***To evaluate the transfer of 2-naphthol (2-NPH) in fullterm human placental tissues. Six placentas were examined. The ex-vivo dual closed-loop human placental cotyledon perfusion model was utilized. 2-NPH was introduced to the perfusate in the maternal compartment. Samples were obtained from the maternal and fetus up to 360 min measurement. The mean fetal weight was 2880 ± 304.2 g. Mean perfused cotyledon weight was 26.3 (±5.5) g. All unperfused placental tissue samples contained NPH with a mean level of 7.98 (±1.73) g\g compared to a mean of 15.58 (±4.53) g\g after 360 min perfusion. A fast decrease in maternal 2-NPH concentration \swas found; from 5.54 g\g in the first 15 min and 13.8 g\g in 360 min. The fetal side rose from 0.65 g\g in the first 15 min to 1.5 g\g in 360 min. The transfer rate of NPH was significantly lower than that of antipyrine. 2-NPH has the capacity to quickly traverse the placenta from the maternal to the fetal compartment within 15 min. The placenta appears to have a function in restricting the flow of 2-NPH in the fetal compartment.***

KEYWORDS: Human, 2-Naphthol, Placenta, Transfer.

1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a broad category of chemicals that are commonly present in our environment and regarded as powerful air pollutants since several of its compounds have been discovered as carcinogenic, mutagenic, and teratogenic. They are released into the environment as a consequence of incomplete combustion of organic materials and carbon-containing fuels such as wood, coal, and diesel. Naphthalene (NAP), a volatile polycyclic regarded as one of the simplest PAH compounds., found in significant amounts indoors and outdoors due to solvent-related emissions, renovations, home goods, and pesticides. The first metabolites are epoxides which are cyclic ether with three ring atoms. They are very unstable and undergo spontaneous rearrangement into 1-naphthol or 2-naphthol (1 and 2-NPH), which conjugate to either glucouronides or sulphates and are eliminated in the urine [1].

Animal studies have revealed that NAP is carcinogenic and embryotoxic. The fetus is particularly vulnerable to the impacts of hazardous substances because of its young and undeveloped defensive systems. Hence, exposure during pregnancy may have a negative seffect on fetal growth and development and possibly raise the vulnerability to illnesses later in life. In-vitro studies on human scrod blood cells indicate that NAP, in particular its metabolites, modifies cord blood gene expression. The result is down-regulation of genes involved in the differentiation of immunocompetent cells, and overexpression of oncogenes in cell proliferation and cell cycle progression, eventually promoting cell proliferation. NAP itself does not appears to harm the fetus. Once ingested, it quickly disseminated throughout the body with minimal build up in any tissue. However, its metabolites (NPH) are hazardous. Therefore, NAP is not believed to be cytotoxic or genotoxic without metabolic activation. The precise quantity of metabolites that may cross the placental barrier has not been established. The metabolizing senzymes produced in the placenta and the vast diversity of transporters may have major effects on the transfer of sxenobiotics and their impact on the baby. The placental barrier may restrict the transport of hazardous substances shielding the fetus against their detrimental consequences. Several processes are proposed, including absorption, storage, and reflux [2].

In this research, we seek to evaluate the transfer of 2-NPH in full term human placental tissues. The result of this research may assist in the formulation of maternal environmental health related guidelines.

2. LITERATURE REVIEW

S Avigdor in his study discloses about the activity of QR was evaluated in first trimester placental tissue using colorimetric methods. There were no significant differences in the mean enzyme activity of women who smoked more than 20 cigarettes per day during pregnancy and of non-smokers (0.50 +/- 0.09 compared with 0.51 +/- 0.15 mumol/mg protein/10 min, respectively). Among the polycyclic aromatic hydrocarbons (PAH) examined, dimethyl benzanthracene (DMBA) enhanced QR activity in a dose-dependent manner in the first trimester placental explants at the 10- to 100-microM range after 6 h of incubation (440 percent increase) with the highest concentration. The impact of additional PAH of various potency added at 50-microM concentrations revealed that benz (a) anthracene (BA), dibenzo (a,h)anthracene (DBHA), dibenzo(a,c)anthracene (DBCA), or chrysene (CH) produced a substantial 2- to 3-fold increase in the enzyme activity after 6 h of incubation. At 24 h 50-microM DBCA impact was similarly stimulatory, whereas the 10-microM DMBA effect nearly approached statistical significance. However, no differences were found in the response of placental tissues to PAH between cigarette smokers and nonsmokers at 6 and 24 h. The current results show that placental QR activity is enhanced by exposure to PAH in vitro, but it does not seem to be altered by in vivo exposure to cigarette smoking. Thus, the early placenta seems to have a considerable capacity to inactivate carcinogens/mutagens locally, thus restricting their transmission to the embryo [3].

B Balakrishnan in his study dicusses about investigating transplacental transport and biotransformation of genistein in the human placenta. Human placentae derived from healthy term singleton births were used in a dual re-circulating model of ex-vivo placental perfusion. Four placentae were perfused for 180min after addition of genistein (10ng/mL) to the maternal perfusate. Antipyrine and FITC dextran were employed as positive and negative controls respectively to verify integrity of the circuits. Concentrations of genistein and its conjugates were measured by liquid chromatography-mass spectrometry. The transfer percentage for antipyrine and genistein was 25.6+/-1.40 percent and 22.1+/-1.61 percent correspondingly while the transfer index for genistein was 0.90+/-0.04 after 180min of perfusion. 12.0+/-2.40 percent of genistein in the fetal compartment and 7.36+/-4.73 percent of genistein in the maternal compartment were in the conjugated form. Genistein may pass through the human placenta at ecologically relevant amounts. Placental metabolizing enzymes conjugate a tiny portion of genistein into the glucuronide/sulphate form, which is devoid of estrogenic activity [4].

E R Barnea in his another study discloses the estrogen phenol A-ring metabolism in the first trimester placenta using radioenzymatic methods. In untreated explants grown for 16 h, estrogen hydroxylase (EH) but not catechol-O-methyl transferase (COMT) activity was raised substantially 1.8-fold (P less than 0.05). (P less than 0.05). Cultures produced in the presence of chemoprotectors, 25 microM of 1-phenylazo-2-naphthol (Sudan I) and coumarin but not 2(3)-tert-butyl-4-hydroxyanisole (BHA) induced a substantial increase in EH activity, 1.8- and 2.2-fold, respectively (P less than 0.05). (P less than 0.05). This was accompanied with a substantial, P less than 0.05, increase in the COMT activity by 25 microM of all three chemoprotectors, BHA, Sudan I, and coumarin, 2.7-, 2.3-, and 2-fold respectively. The carcinogens benzo(a)pyrene and 20-methylcholanthrene at 50 microM concentration, however, showed no impact upon both enzymes' activity. Finally, the two enzymes's activity were associated under the experimental circumstances examined. Except for zero time when no connection was discovered (r2 = 0.3), in all other experimental circumstances, a substantial (r2 = 0.75) association was seen. In conclusion, EH and COMT enzyme activity seem to undergo a coordinated induction in cultured placental explants in the first trimester. The consequences of catechol metabolism for embryonal development are addressed [5].

3. DISCUSSION

3.1 Placental Perfusion:

Six placentas were collected from women having elective Caesarean surgery or vaginal birth at term. The placentas were transferred to the laboratory within 30 min. We utilize techniques provided by Schneider et al. In summary, a suitable cotyledon was chosen and the fetal vasculature were catheterized using a French catheter. The perfusate which has been utilized consisted of a synthetic tissue culture medium M199 Medium Sigma, circulate through the fetal side at a flow rate of 4 ml/min supplied by a digitally controlled pump at a pressure not higher than 60 mmHg. For the delivery of maternal perfusate, three catheters were directly inserted into the intervillous

area at a depth of 0.5–.0 cm. Perfusion of the intervillous space was started at a flow rate of 12 ml/min. Maternal perfusate was equilibrated with 95 percent O2 and 5 percent CO_2 and fetal perfusate was equilibrated with 95 percent N and 5 percent CO2 after 20 min both circuits were closed. The perfusate on the maternal side was replaced by fresh perfusate contains 2-NPH Sigma–Aldrich and antipyrine at 0.05 g\l. The concentration of 2-NPH utilized for perfusion is based on the recommendation of the Human Biomonitoring Commission of the German Federal Environment Agency, where an upper margin of reference value of exposure for adult non- smokers to 2-NPH in urine should be less than 20 g/l. The perfusion was then maintained for six hours. Samples were collected from the maternal and fetal circulations at 15, 30, 60, 120, 240, and 360 min and kept at −80 ◦C.

The glucose and lactate levels were assessed using Rx Monza clinical analyzer, Randox, labs; UK and -human chorionic gonadotropin (-hCG) secretion was quantified using DRG diagnostics kits, DRG Instrument GMBH, Germany 2-NPH transfer and antipyrine transfer were assessed by HPLC [6].

3.2 Placental tissue extraction:

Three samples from the placenta, weighing 1 g each, were taken from both unperfused and perfused cotyledon and kept at –20 ◦C until analysis. Tissues samples were homogenized (Ultra Turrax® IKA,Werke, Germany)for 1 min at 15,000 rpm on ice. With addition of 2 mL ofACN –20 percent acetonitrile: water. The homogenate were then centrifuged at 13,000 rpm and the supernatant were collected and stored at −20 ◦C for further analysis using HPLC [7].

3.3 HPLC analysis:

All reagents used were HPLC grade Merck, Germany HPLC method as described. was utilized for antipyrine The dried extract was dissolved in 200 l of the mobile phase. Chromatographic separation were conducted using HPLC system Waters Alliance, USA.

For 2NH detection, a stock solution of concentration of 1 g/ml, 100 g/ml, and 1 mg/ml of 2NPH in acetonitrile HPLC grade were prepared and kept at −20 ◦C until the test. For each run 20 l was injected [8].

Table 1
shows glucose consumption, lactate production, HCG secretion as evidence of placenta viability and metabolic activity.

	Maternal	Fetal
Glucose consumption(μmol/g/min)	0.42 ± 0.04	0.35 ± 0.0.04
Lactate production (μmol/g/min)	0.49 ± 0.14	0.31 ± 0.06
HCG (mU/g/min)	40.8 ± 7.3	

3.4 Calculations:

Statistical analysis was conducted using the one-way or twoway ANOVA. Values of p less than 0.05 were regarded as statistically significant. The transfer percentage from maternal to fetal circulation was estimated using the following formula [2]:

100 × Fc × Fv/[(Fc × Fv) + (Mc × Mv)], where Fc = fetal concentration, Mc = maternal concentration, Fv represents fetal perfusate volume, and Mv = maternal perfusate volume.

FM ratio, computed given the figures, is the ratio of fetal concentration to mother concentration. Transfer index was determined by dividing the transfer percentage of 2-NPH with that of antipyrine. All data are given as a mean ± sd. Glucose consumption was estimated using the following formula:

Glucose consumption = glucose level of perfusate at 360 min − glucose level at starting time and adjusted with time and tissue weight. Lactate production was measured using the same formula [9].

4. DISCUSSIONS

A total of 6 term placentas were perfused. The mean pregnant women age was 27.6 ± 6.0 years, with a mean maternal weight at delivery of 83.1 ± 18.3 and mean fetal weight was 2880 ± 304.2 g. The mean weight of the perfused cotyledon was 26.3 ± 5.5 g. The perfusate pH varied from 7.2 to 7.4, and the fluid change between the fetal and maternal compartments was less than 3 ml/h. Evidence of placenta viability and metabolic activity during the perfusion was guaranteed by the continuous glucose and lactate production (Table 1. The glucose intake is estimated according to the method given by Di Santo: Glucose consumption = glucose level of perfusate at 360 minutes − glucose level at start time and adjusted with time and tissue weight. All unperfused placental tissue samples contained NPH with a mean level of 7.98 ± 1.73 g\g compared to a mean of 15.58 ± 4.53 g/g after 360 min of perfusion. The first decrease in the content of NPH in the maternal compartment was fast. It decreased 5.54 g/g in thefirst 15 min and 13.8 g/g in 360 min. However, the rise in NPH concentration on the fetal side was considerably slower. The rise in the first 15 min was as low as 0.65 g/g and rose only to 1.5 g/g in 360 min (Fig. 1). (Fig.1). The transfer rate of NPH was significantly lower than that of antipyrine. The greatest transfer index was at 120 min (Table 2) [10].

Pregnant women exposed to PAH during pregnancy exhibit a significant increase in the amount of chromosomal abnormalities in neonatal cord blood samples. NPHs (both 1 and 2 isomers) are used as biomarkers for polycyclic aromatic hydrocarbons exposure. In our research we chose 2-naphthol since it appears to be related to atmospheric contaminants. In particular, those relates to Cigarette consumption. Animal studies have revealed that NAP and its metabolites, are embryotoxic and abortifacient [2].

Interestingly, NPH was identified in placental tissue samples obtained prior to perfusion, suggesting a substantial NAP ambient exposure during pregnancy. The build-up of 2-NPH seems linked to the gestational age.Singh observed higher amounts of PAH, including NPD, in pre-term placenta compared to term placenta. This may be related to structural changes occurring during the early second half of pregnancy primarily the partial removal of the cytotrophoblast layer which decrease the placenta barrier thickness. Stabenau indicated that the buildup of NAP has larger impact on the functions of the liver that have higher metabolic activities, and those that have gaseous exchange function. The placenta is known to have a high metabolic activity. Hence, the presence of NPH in unperfused placental tissue and its fast buildup seen in our research could perhaps placental function. However, all neonates of pregnant women involved in this research had a normal birth weight and no structural anomalies. Nevertheless, there is growing evidence that prenatal exposure to PAH may have long term consequences on neonates and infants. Rosa et al. indicated that prenatal exposure to PAH be associated with development of asthma later in life.More worrying are the studies indicating linked chromosomal abnormalities in the infant and possible risk for cancer formation later in life [3].

Our findings show NPH presence in the fetal compartment within 15 min. This may be related to lipophilic nature and low molecular weight of NPH. PAH components with structures comparable to NPH quickly transfer from diet to milk via the mammary epithelium. Several variables may affect the rate of transfer of any xenobiotic drug, including placental membrane permeability, placental blood flow, pH variations between the maternal and fetal circulations, and protein binding characteristics of the molecule itself. In addition, the placenta possesses \sxenobiotic-metabolizing ability to metabolize a variety of foreign chemicals which may have an effect on the exposure of the fetus to foreign compounds. Coordinated induction of estrogen hydroxylase (EH) and catechol-O-methyl transferase (COMT) by xenobiotics in first trimester human placental explants, and COMT enzyme activity seem to undergo a coordinated induction in cultured placental explants in the first trimester. The limiting element in sulphation reaction appears to be the activity of sulphotransferase (ST) towards 2-naphthol [6].

In our research we found that NPH transfer through the placenta was at a rate considerably lower than that of antipyrine throughout the duration of perfusion. From 60 min the transmission achieved a steady state. This indicates that the mechanism of transfer of NPH may differ from that of antipyrine, which is transported through the placenta by simple diffusion. This may indicate NPH is delivered by active transport through the host of transporters expressed in the placenta [7].

Furthermore, the pace at which NPH rose in the fetal compartment was at a considerably lower rate than its decrease from the maternal compartment. This may be related to the buildup of NPH in placental tissue indicated by the substantially higher NPH levels observed in the perfused placenta cotyledon compared to levels in unperfused placental tissues. This result supports the protective role of the placenta against feto-toxic chemicals. Animal studies have revealed that NPH accumulates in various bodily tissues such as the liver, lung, and muscle at varying rates. However, its elimination from the body appears to more fast from organs that have high metabolic activity such as the liver. This is thought to be linked to the activity of cytochrome P450 enzymes, which seem to play the main role in metabolizing foreign toxins and chemicals from the placenta. They are present throughout pregnancy [8].

Similarly, additional PAHs are processed by a set of Cytochrome P450 oxidoreductase enzymes include CYP 1A2, 3A4.

Table 2
Shows the transfer percentage and transfer index of 2-naphthol.Transfer percentage and transfer index were calculated up to 3 h of perfusion, data are presented as mean ± SEM ($n = 6$).

Perfusion time (min)	Antipyrine Transfer percentage	2-Naphthol Transfer percentage	Transfer index
15	12.05 ± 2.79	4.46 ± 1.33	0.38 ± 0.12
30	25.55 ± 7.41	11.87 ± 2.36	0.46 ± 0.26
60	40.35 ± 6.32	11.82 ± 1.60	0.30 ± 0.09
120	30 ± 3.89	16.68 ± 2.67	0.55 ± 0.05
240	52.10 ± 6.18	15.89 ± 3.26	0.29 ± 0.06
360	48.15 ± 3.10	17.01 ± 3.26	0.35 ± 0.05

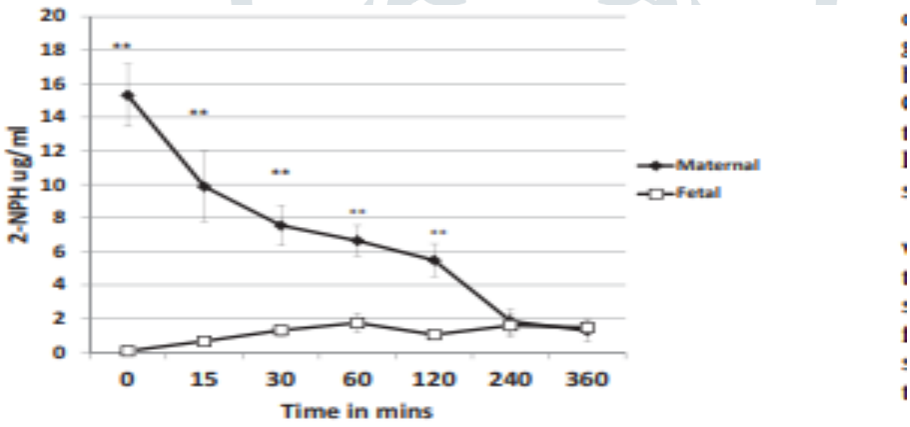

Fig. 1. Shows the mean (±sem) transfer of 2-naphthol (μg/ml) across the placentas showing the rapid drop from the maternal compartment and slow increase in the fetal compartment ($n = 6$).

5. CONCLUSION

Our research indicates that pregnant women are exposed to high levels of ambient NAP, and its metabolites accumulate in term placentas. NPH has the capacity to quickly traverse the placenta rom maternal to fetal compartment within 15 min. The placenta seems to have a function in restricting the passage of NPH in the fetal compartment by serving as a chemical barrier Our results recommend further research to better understand how the placenta metabolizes the 2-naphthol.

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