Variation in transplacental transfer of tyrosine kinase inhibitors in the human perfused cotyledon model

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Background: The use of tyrosine kinase inhibitors (TKi) during pregnancy in humans remains rare, and little data are available on their transplacental passage. Erlotinib and gefitinib are the first-line targeted therapy in case of stage IV non-small-cell lung cancer with an EGFR-activating mutation. There are no data available regarding the comparative use of these TKi in pregnant patients. We aimed to compare the transplacental transfer of gefitinib, imatinib and erlotinib, using the ex vivo method of human perfused cotyledon, and to determine the placental accumulation of TKi.

Materials and methods: Term placentas were perfused after delivery with gefitinib, imatinib and erlotinib at targeted maternal concentrations around the steady-state plasma trough concentration (i.e. 500, 1000 and 1500 ng/ml, respectively). Samples from fetal and maternal circulations were collected in order to monitor TKi concentrations. Main transfer parameters such as fetal transfer rate (FTR), clearance index (CI) and placental uptake were assessed.

Results: Mean FTR of gefitinib, imatinib and erlotinib were 16.8%, 10.6% and 31.4%, respectively. Mean CI of gefitinib, imatinib and erlotinib were 0.59, 0.48 and 0.93, respectively. Placental uptake in cotyledon was 0.030% %, 0.010% and 0.003% for gefitinib, imatinib and erlotinib, respectively, corresponding to a mean mass of 27.7 µg for gefitinib, 15.7 µg for imatinib and 6.8 µg for erlotinib.

Conclusion: The results suggest that TKi cross the placenta at therapeutic level. Particularly, erlotinib crosses the placenta at a higher rate than gefitinib or imatinib. All of them have a very low placental uptake. These data may suggest that gefitinib should be preferred to erlotinib for the treatment of pregnant woman with lung cancer harboring an EGFR-activating mutation, during the second and third trimesters of pregnancy.

Key words: lung cancer, pregnancy, tyrosine kinase inhibitors, placental transfer

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introduction

Cancer is the second leading cause of mortality in young women [1]. About 1/1000 pregnant patients are diagnosed with a malignancy [2], melanoma, breast and gynecological cancers being the most common primary tumor types. Nonsmall-cell lung cancer (NSCLC) in pregnancy is rare, and has poor prognosis.

Chemotherapy may be indicated in pregnant patients, with the need to balance both the maternal and fetal prognoses. While chemotherapy is contraindicated during the first trimester, several drugs may be used during the second and third trimesters of pregnancy [3]. Among molecular targeted agents, the use of tyrosine kinase inhibitors (TKIs) during pregnancy in humans remains rare. Few in vivo data regarding the transplacental passage of TKIs are available, except for imatinib. Indeed, the majority of the clinical reports are relative to imatinib, a drug prescribed for over 10 years [4–7].

The international guidelines for the treatment of metastatic NSCLC-harboring EGFR-activating mutations recommend the use of the TKIs erlotinib or gefitinib, as they increase the progression-free survival with fewer side-effects than cytotoxic chemotherapy [8]. However, no data are available regarding the comparative use of these TKIs in pregnant patients. Indeed, the use of gefitinib or erlotinib in pregnancy is limited to few case reports [9–11].

Given the lack of objective data that may guide the selection of a TKI for the treatment of pregnant patients during the second half of pregnancy, we aimed to comparatively assess the transplacental transfer of gefitinib and erlotinib with imatinib used as a TKI reference, using the ex vivo gold standard method of human perfused cotyledon [12], and to determine the placental accumulation of TKIs.

material and methods

material

Term placentas (37–40 weeks of gestation) from uneventful pregnancies were immediately collected after vaginal delivery or cesarean section and perfused. The study was conducted after having obtained patient’s written consent, and approval from the local ethics committee (CPP Paris Ile-de-France 3, N-18-05, Paris, France).

Imatinib, erlotinib and gefitinib were provided from LC laboratories, and their pharmacological characteristics are summarized in Table 1. The targeted maternal concentrations of TKIs were around the steady-state plasma trough concentration, i.e. 500 ng/ml for a 250 mg/day gefitinib treatment, 1000 ng/ml for a 400 mg/day imatinib treatment and 1500 ng/ml for a 150 mg/day erlotinib treatment [13–15].

methods

Placental perfusion and analytic methods are described in supplementary File S1, available at Annals of Oncology online.

Standard placental parameters were calculated according to the formulas of Challier [16]. Placental transfer was estimated from two transport parameters: the fetal transfer rate (FTR) and the clearance index (CI) (formulas are detailed in supplementary File S1, available at Annals of Oncology online). Calculating the CI allowed us to compare results during the different experiments, and to evaluate the index of transplacental transfer from one placental unit to another. The placental uptake was evaluated in each cotyledon to assess the TKIs distribution in placenta tissue during the placental transfer.

data and statistical analysis

For the analysis of the respective transplacental transfers of gefitinib, imatinib and erlotinib, a Mann–Whitney U-test was applied. The significance level was set as P < 0.05. The correlation assay was a Pearson test, with a confidence interval of 95%.

results

transplacental transfer of antipyrine

Fourteen placentas were successfully perfused and validated. For this study, four to five placentas were perfused for each drug. The plateau of mean antipyrine FTR across the 14 placentas evaluated was reached after 15 min of perfusion, and was 29.1 ± 11.8% (supplementary File S2, available at Annals of Oncology online).

| Table 1. Characteristics and transplacental passage parameters of tyrosine kinase inhibitors |
|---------------------------------|-----------------|-----------------|-----------------|
|                                 | Gefitinib       | Imatinib        | Erlotinib       |
| Half-life (h)                   | 48              | 18              | 36.2            |
| Molecular weight (kDa)          | 446.9           | 493.6           | 429.9           |
| Protein binding (%)             | 90              | 95              | 93              |
| Log P                           | 3.2             | 3               | 2.7             |
| pKa                             | 4.7/7.6         | 1.5/2.6/3.7/8.1 | 5.4             |
| Molecule state at pH = 7.4      | NI+I            | NI+I            | NI              |
| No. of perfused placentas       | 4               | 5               | 5               |
| Fetal transfer rate (%), mean ± SD | 16.8 ± 6.4   | 10.6 ± 5.9      | 31.4 ± 13.7     |
| Clearance index, mean ± SD      | 0.59 ± 0.04     | 0.48 ± 0.14     | 0.93 ± 0.03     |
| Maternal TKI mass (µg)          | 750             | 1500            | 250             |
| TKI mass/g of cotyledon (µg/g), mean ± SD | 4.8 ± 1.5  | 5.6 ± 2.0       | 1.0 ± 0.3       |
| Total TKI cotyledon mass (µg), mean ± SD | 27.7 ± 13.5 | 15.7 ± 3.7      | 6.8 ± 3.0       |
| Placental uptake (%), mean ± SD | 0.030 ± 0.010   | 0.010 ± 0.002   | 0.003 ± 0.001   |

NI, nonionized; I, ionized; TKI, tyrosine kinase inhibitor; SD, standard deviation.
The mean FTR of antipyrine were 24.2 ± 5.5%, 29.8 ± 7.6% and 36.0 ± 14.0% for imatinib, gefitinib and erlotinib, respectively. Transplacental passage parameters were calculated from the values collected over the 15 min of perfusion. All individual TKi maternal concentrations were stable during the experiments (data not shown). The kinetics of TKi CI during the perfusion procedure is represented in Figure 1A. For each TKi, the mean CI increased up to 15 min of perfusion, and then reached a plateau up to 90 min. The mean FTR of gefitinib, imatinib and erlotinib were 16.8 ± 6.4%, 10.6 ± 5.9% and 31.4 ± 13.7%, respectively. Data on TKi transplacental transfer are summarized in Table 1. The mean CI of gefitinib, imatinib and erlotinib were 0.59 ± 0.04, 0.48 ± 0.14 and 0.93 ± 0.03, respectively. As show in Figure 1B, the differences of transplacental transfer between gefitinib versus erlotinib, and imatinib versus erlotinib were both significant ($P = 0.016$ and $P = 0.011$, respectively).

**comparision of transplacental transfer of TKi**

All individual TKi, maternal concentrations were stable during the experiments (data not shown). The kinetics of TKi CI during the perfusion procedure is represented in Figure 1A. For each TKi, the mean CI increased up to 15 min of perfusion, and then reached a plateau up to 90 min. The mean FTR of gefitinib, imatinib and erlotinib were 16.8 ± 6.4%, 10.6 ± 5.9% and 31.4 ± 13.7%, respectively. Data on TKi transplacental transfer are summarized in Table 1. The mean CI of gefitinib, imatinib and erlotinib were 0.59 ± 0.04, 0.48 ± 0.14 and 0.93 ± 0.03, respectively. As show in Figure 1B, the differences of transplacental transfer between gefitinib versus erlotinib, and imatinib versus erlotinib were both significant ($P = 0.016$ and $P = 0.011$, respectively).

**placental uptake of TKi**

In order to determine the TKi distribution in placenta, we analyzed the TKi mass in the perfused cotyledon tissues (Figure 2A). After the perfusion, the mass of TKi per gram of cotyledon was 4.8 ± 1.5 µg/g for gefitinib, 5.6 ± 2.0 µg/g for imatinib and 1.0 ± 0.3 µg/g for erlotinib. Thereby, the mean total mass of TKi, remaining in the cotyledon was found to be 27.7 ± 13.5 µg for gefitinib, 15.7 ± 3.7 µg for imatinib and 6.8 ± 3 µg for erlotinib. The maternal masses of TKi, were 750 µg for gefitinib, 1500 µg for imatinib and 2250 µg for erlotinib. Taking into account these results, we determined the placenta-to-maternal ratio of 0.030 ± 0.010%, 0.010 ± 0.002% and 0.003 ± 0.001% for gefitinib, imatinib and erlotinib, respectively. As shown in Figure 2B, there is a significant negative correlation between TKi CI and placental uptake (Pearson $r = −0.9083$, $P < 0.0001$), predicating that the more the TKi crosses this barrier, the less it accumulates in placenta.

**discussion**

Gefitinib and erlotinib are important therapeutic options to treat EGFR-mutated NSCLC [8]. Since no comparative clinical data exist in pregnant patients, we aimed to comparatively assess the transplacental transfer of gefitinib and erlotinib, and chose to explore as well imatinib as a TKi reference. We found that transplacental transfer of erlotinib is superior to that of gefitinib. In vivo studies evaluating the transplacental kinetics of drugs in humans raise numerous ethical concerns regarding fetal and maternal safety. Placental drug transfer can be evaluated in vivo only by measuring drug concentrations in maternal blood, and in fetal or in umbilical cord blood at the time of delivery. Results from animal studies can be extrapolated to humans only with
caution, because the placenta is the most species-specific mammalian organ. The ex vivo placental perfusion model is the only experimental technique which allows to study human placental transfer, with the dual maternal-fetal circulation and placental tissue integrity [17].

To our knowledge, this is the first study on TKis, placental transfer carried out in an ex vivo human perfused cotyledon model. The main mechanism of placental transfer is passive diffusion. In these conditions, the transfer rate across the barrier is determined by the physicochemical properties of the drug, such as lipid solubility, polarity, molecular weight, protein binding, ionization. The addition of a marker that undergoes only passive diffusion, such as antipyrine, into the maternal circulation can be used to measure tissue integrity/membrane permeability. Since antipyrine does not bind to proteins, and does not accumulate in placental, its transfer rate depends only on fetal and maternal flows which should be constant during the perfusion. Hence, a CI value close to 1 demonstrates a mechanism of placental transfer by passive diffusion without placental accumulation [16].

Our results suggest that TKis cross the placenta at targeted therapeutic level (500, 1000 and 1500 ng/ml for gefitinib, imatinib and erlotinib, respectively). Particularily, erlotinib crosses the placental barrier at a higher rate (CI 0.93) than gefitinib or imatinib (CI 0.59 and 0.48, respectively). Although belonging to the same therapeutic class, gefitinib and erlotinib exhibited different placental transfer. Some physicochemical properties (Table 1) do not really allow to predict their behavior (molecular weight, protein binding). Bronte et al. [18] emphasize on the difference of lipophilicity to explain some of the difference in pharmacokinetic and pharmacodynamic properties of the two compounds. This hypothesis is in contradiction with our study of placental transfer. Indeed, gefitinib has a lower CI than erlotinib, while it is more lipophilic. However, we can observed that erlotinib's pKa is 5.3, the sole value of pKa in contrast to the two other TKis. At pH = 7.4 (corresponding to the pH of maternal circulation), this molecule is then totally nonionized, which is not the case for gefitinib and imatinib, which are partially ionized (see pKa values in Table 1). The more molecules are ionized, the less they cross the placental barrier [19]. This could explain the transplacental passage differences between these three TKis.

To our knowledge, this is the first study concerning TKIs placental accumulation. The results show masses of 27.7, 15.7 and 6.8 µg of gefitinib, imatinib and erlotinib in placental tissue, respectively. It corresponds to 0.03%, 0.01% and 0.003% of the maternal TKis masses.

As expected, the more the TKI crosses the placental barrier, the less it accumulates in cotyledon tissue. Berveiller et al. [12] have determined the placental transfer and uptake of two taxanes, paclitaxel and docetaxel. Their CI were 0.1 and placental uptake were about 4%. Those results confirm that the uptake is inversely proportional to CI.

TKIs have a low placental uptake, but repercussion on the placental functions remains unknown.

In the clinical setting, the treatment of advanced NSCLC is a complex issue. Indeed, the gold standard first-line therapy is a platinum-containing doublet: paclitaxel–carboplatin, or pemetrexed–cisplatin ± bevacizumab (in nonsquamous histological subtypes) [20]. While the use of paclitaxel during the second and third trimesters of pregnancy has been shown to be safe [21], recent pharmacokinetic data in pregnant patients suggest a decreased exposure compared with nonpregnant patients, and thereby raises some concerns regarding a potential decreased efficacy [22, 23]. The use of pemetrexed during pregnancy has not been reported yet, and should probably be avoided due to its structural similitude with methotrexate, a teratogenic antifolate agent [3]. Finally, the use of carboplatin in pregnant women raises the issue of drug dosing, since no population pharmacokinetics model has addressed the issue of carboplatin AUC calculation in pregnant women [23, 24]. Otherwise, the use of cisplatin at doses ≤80 mg/m² is considered safe during the second and third trimesters of pregnancy [24].

In summary, the EGFR TKis could be used in pregnant patients with EGFR-mutated NSCLC.

These results support a new published case report [25] with pharmacological data on gefitinib. A woman, suffering from lung cancer diagnosed during her pregnancy, has been treated by gefitinib 250 mg/day from 28 weeks of gestation to the delivery at 35 weeks of gestation. She delivered a healthy baby. The concentrations of gefitinib at delivery, 17 h after the last oral intake, were 127.1 ng/ml in maternal plasma and 16.9 ng/ml in amniotic fluid. The ratio fetal/maternal was 0.13, corresponding to the FTR found with the ex vivo perfusion model (FTR = 16%). The concordance of in vivo and ex vivo results confirms the validity of this model for the study of placental transfer of drugs.

In conclusion, the transplacental transfer of erlotinib is superior to that of gefitinib. Transplacental passage and accumulation of gefitinib and imatinib are similar. These data may suggest that gefitinib should be preferred to erlotinib for the treatment of pregnant woman with NSCLC-harboring EGFR-activating mutations during the second and third trimesters of pregnancy.

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disclosure

OM has acted as consultant for Novartis, Astra-Zeneca and Roche.

references

Results: A significantly greater growth inhibition was seen in BRAF<sup>TM</sup> and PIK3CA<sup>M</sup> cells upon maximal MEK (P = 0.004) and AKT inhibition (P = 0.038), respectively. KRAS<sup>M</sup> and BRAF/PIK3CA/KRAS<sup>WT</sup> cells were not significantly more likely to be sensitive to MEK or AKT inhibition. Significant incremental growth inhibition of the combination of MEK + AKT over either MEK or AKT inhibition alone was seen when MEK + AKT was inhibited maximally and not when sub-maximal

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