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SHORT COMMUNICATION

Relationship among pharmacokinetics and pharmacodynamics of fenretinide and plasma retinol reduction in neuroblastoma patients

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Abstract
Purpose Fenretinide (4-HPR), a synthetic retinoid currently used in clinic for cancer therapy and prevention, markedly lowers plasma retinol levels, an effect associated with nyctalopia. Our aim was to investigate the relationship between 4-HPR pharmacokinetics, plasma retinol reduction and incidence of nyctalopia.
Patients and methods Children with neuroblastoma, participating in a phase I trial, were treated with oral 4-HPR, once a day for 28-day courses followed by a 7-day drug interruption, with escalating dose levels from 100 to 4,000 mg/m^2 per day. Blood samples were collected at baseline and up to 48 h after the 1st (50 patients) and 28th (41 patients) administration, and the plasma concentrations of 4-HPR and retinol were measured by HPLC.
Results After the first administration, nadir retinol concentrations were reached at 16–20 h post-dosing; the extent of retinol reduction was related to 4-HPR dose and plasma concentrations as well as to pretreatment retinol concentrations. After repeated treatments, nadir retinol concentrations (10–20% of baseline values) were maintained during the 24 h dosing interval and were similar at all doses; the extent of retinol reduction was significantly ($r = 0.97$, $P < 0.0001$) related to pretreatment retinol concentrations. After a single dose, the relationship between 4-HPR pharmacokinetics and pharmacodynamics indicated a counterclockwise hysteresis suggesting the presence of an effect compartment. At steady state, the hysteresis collapsed suggesting that the 4-HPR concentrations in plasma and in the effect compartments were in equilibrium. Nyctalopia was not related to the administered dose, but was significantly associated ($P = 0.05$) with lower nadir retinol concentrations (0.11 ± 0.012 vs. 0.17 ± 0.015 μM).
Conclusions During 4-HPR chronic treatment, plasma retinol reduction is not proportional to the dose. Plasma retinol levels of 0.11 μM could be considered as a safety biomarker in children with neuroblastoma. Finally, since initial retinol levels strongly predict the extent of retinol reduction, retinol decrease could be used to monitor 4-HPR compliance.

Keywords Fenretinide · Retinol · Pharmacodynamics · Safety biomarker · Retinoids · Neuroblastoma

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Introduction

Fenretinide or *N*-(4-hydroxyphenyl)retinamide (4-HPR), a synthetic analog of all-*trans*-retinoic acid, has emerged as a promising tumor preventive and therapeutic agent due to its efficacy in preneoplastic [1] and neoplastic [2] conditions and good tolerability [2–5]. Prolonged disease stabilization and regression of some lesions were reported in two phase I

studies in neuroblastoma patients [4, 5]. Unlike other retinoids, 4-HPR rarely induces dermatologic or metabolic toxicity, but causes alterations in dark adaptation and impaired night vision, a side effect also named nyctalopia.

A relevant pharmacologic effect of 4-HPR associated with nyctalopia that we first documented in rats [6] and humans [7, 8] is a marked reduction in circulating levels of both retinol (vitamin A) and its specific transport protein, retinol-binding protein (RBP4).

In mice, 4-HPR is effective in blocking the formation of a toxic vitamin A-based fluorophore [9], and therefore the lowering effect of 4-HPR on retinol might represent a potential therapeutic option in lipofuscin-based retinal diseases. Recent evidences indicate that RBP4, in addition to its role in retinol transport, may also be involved in the development of metabolic syndrome and type 2 diabetes. In mice, 4-HPR normalized serum RBP4 levels and improved insulin resistance [10]. Two clinical trials, one in obese subjects with insulin resistance and one in subjects with geographic atrophy associated with dry age-related macular degeneration, are currently ongoing with 4-HPR (http://www.clinicaltrials.gov). Therefore, the interaction of 4-HPR with retinol is responsible for its toxicity, but it might also mediate its therapeutic effects in pathologic conditions in which the therapeutic goal is to lower retinol or RBP4 levels.

The purpose of this study was to define the association of the already described 4-HPR pharmacokinetics [11] with reduction in retinol levels and the resulting nyctalopia in neuroblastoma patients participating in a phase I trial [4]. The results obtained will, on one hand, improve the understanding of the relationship between 4-HPR and retinol and, on the other hand, will be useful to optimize the design of future clinical trials by maximizing 4-HPR efficacy while reducing the occurrence and severity of nyctalopia.

Patients and methods

Patient characteristics, design of the phase I study, and methods for pharmacokinetics analysis and nyctalopia monitoring and scoring have been previously reported [4, 11] 4-HPR was administered with the following schedule: orally, once a day, for 28 consecutive days with a 7-day drug interval after each course for a maximum of six courses. Patients received doses ranging from 100 to 4,000 mg/m^2 per day. Blood samples were collected at time 0 and 1, 3, 4, 6, 9, 12, 16, 20, 24, 36 and 48 h thereafter, after the 1st drug intake (50 patients) and after the 28th drug administration (48 patients). The concentrations of 4-HPR and retinol were simultaneously determined by high-performance liquid chromatography (HPLC) as previously described [10, 11].

The intensity of response after single and repeated administration was expressed as percentage reduction of retinol levels (% reduction), defined as $[1 - (R/R_0) \times 100]$, where R_0 and R are the retinol plasma concentrations at baseline and during treatment, respectively. The time course of plasma 4-HPR concentrations and plasma retinol reduction was then assessed to investigate the relationship between pharmacokinetics and pharmacodynamics. The lowest attained retinol levels after single and repeated administration were defined as nadir retinol levels, and retinol decrease was calculated as R_0: retinol at nadir.

Correlation analysis was performed between retinol decrease and 4-HPR dose, C_{max}, AUC_{24h} and retinol at baseline. In all the analyses, a P value of less than 0.05 was considered as statistically significant.

Results

The patients' characteristics have been previously reported [4, 11]. Pretreatment retinol levels were on average 1.24 ± 0.37 μM and were not related to sex, age or body mass index (BMI).

After the first administration, retinol plasma concentrations started to progressively decrease and the lowest retinol concentrations (nadir concentrations) were reached at 16–20 h post-dosing (45% of pretreatment values after the lowest dose and 20% after the highest dose). Afterward, retinol concentrations progressively increased. The time taken for retinol plasma concentrations to fall by one half, i.e., retinol $t_{1/2}$, did not differ among doses and was on average 14 ± 9 h. During multiple daily doses, retinol plasma levels were reduced to 10–20% of pretreatment levels after all tested doses and retinol plasma concentrations remained suppressed for the whole interval between drug administrations (24 h) (data not shown).

The estimated correlation coefficients (Fig. 1) showed significant, although weak, direct association between retinol decrease (i.e., retinol levels at baseline minus retinol levels at nadir) and dose ($r = 0.35$, $P = 0.0126$), retinol decrease and 4-HPR exposure ($r = 0.43$, $P = 0.0017$ for C_{max} and $r = 0.35$, $P = 0.0148$ for $AUC_{0–24h}$) but only after the first administration. On the contrary, plasma retinol decrease was directly and strongly correlated with baseline retinol levels after both single ($r = 0.78$, $P < 0.0001$) and repeated ($r = 0.97$, $P < 0.0001$) treatments.

The drug effect, i.e., the plasma retinol reduction was plotted against the corresponding 4-HPR plasma drug concentration and the results are reported in Fig. 2. Since similar results were observed at all doses, Fig. 2 reports only the average results observed after the lowest and

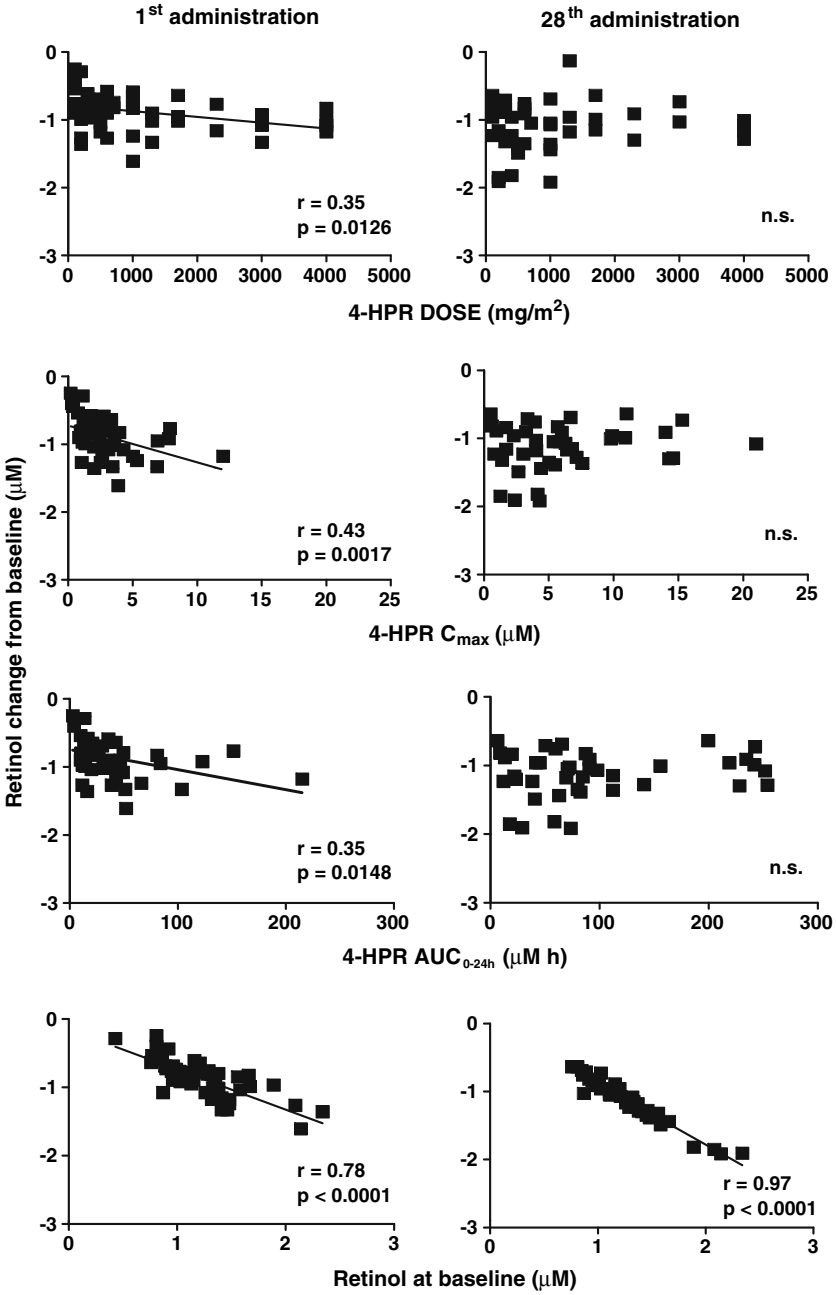

Fig. 1 Interrelationship of retinol plasma changes with 4-HPR dose, C_{max} and AUC_{0-24h}, and plasma retinol at baseline after the 1st and 28th administration of 4-HPR. Retinol changes were evaluated as plasma retinol at baseline minus those at nadir

highest doses, i.e., 100 (Fig. 2a, b) and 4,000 mg/m^2 per day (Fig. 2c, d), respectively. A characteristic counter-clockwise hysteresis loop was evident after the first administration (Fig. 2a, c); after chronic treatment, i.e., at day 28, the hysteresis loop collapsed (Fig. 2b, d).

Nyctalopia occurred to some degree during treatment in 10/41 patients [4]. As previously described [4], only the patient with grade 3 nyctalopia reported this side effect, whereas in the remaining nine cases nyctalopia was diagnosed only after accurate anamnesis. This side effect was not associated with basal retinol levels, 4-HPR dose or extent of exposure, but was associated with the nadir of plasma retinol during chronic treatment. Patients who developed nyctalopia had significantly lower mean plasma retinol concentrations at nadir during chronic treatment (0.11 ± 0.01 μM) compared with patients who did not develop nyctalopia (0.17 ± 0.01 μM; $P = 0.0497$ unpaired *t* test).

Discussion

It is well established that 4-HPR has profound effects on endogenous retinol levels [7, 8, 12, 13]. The results,

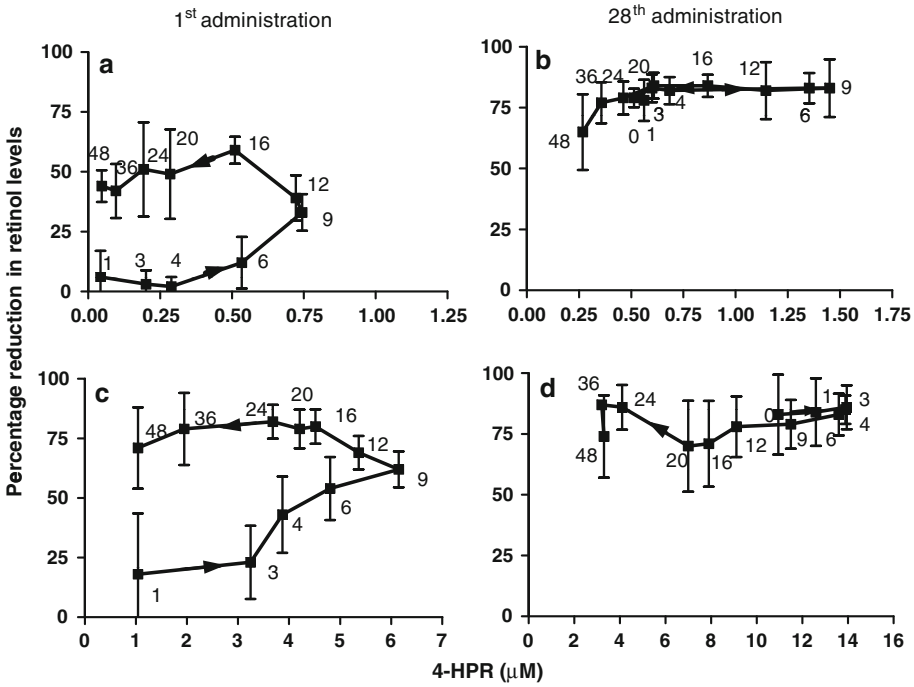

Fig. 2 Percentage reduction of retinol levels versus plasma 4-HPR after the 1st and 28th administration of 4-HPR in patients treated with 100 (**a**, **b**) and 4,000 (**c**, **d**) mg/m^2 per day. The results are the means and SD of four and three patients for 100 and 4,000 mg/m^2 per day dose, respectively. The time of sampling (h) is indicated next to each point and the *arrows* indicate the sequence of observations

however, were always obtained from blood samples collected at a single time point after 4-HPR administration. The present study foresaw serial drug and retinol plasma measurements, and is the first one to describe the time course of the pharmacodynamic effect of 4-HPR as retinol inhibitor and specifically focuses on the effects of acute and long-term drug administration.

The homeostatic control of plasma retinol within the physiological range relies on the vitamin A content of the liver, the main primary source of new retinol and of other extrahepatic tissues. The underlying mechanism for retinol level reduction by 4-HPR has been partly elucidated. In rats, 4-HPR inhibits the secretion of retinol-RBP4 from the liver [14] and this seems to be due to competition in the complex formation. We have in fact demonstrated that 4-HPR binds in the retinol-binding cavity of RBP4 and forms a tight complex. However, this complex, at variance with the retinol–RBP4 complex, lacks binding affinity to transthyretin (TTR) [15, 16].

The time taken for retinol plasma concentrations to fall by one half was independent of the dose and it was on average 14 h. Since theoretically all the retinol maybe regarded as being eliminated by five elimination half-lives, the nadir of plasma retinol was reached after 70 h, i.e., after the third drug administration. This information might be relevant to currently ongoing clinical trials in which retinol and/or RBP4 reduction should contribute to 4-HPR therapeutic activity, such as geographic atrophy associated with dry age-related macular degeneration, and for the treatment of obese subjects with insulin resistance (http://www.clinicaltrials.gov).

During repeated treatments, plasma retinol levels were reduced by 80–90% independently of the dose. In adults, retinol reduction following chronic 4-HPR treatment ranged from 44 to 76% after 200 mg/day [7, 8, 12, 13] and it was higher (80–90%) after higher doses (900 mg/m^2 bid) [17, 18]. Interestingly, in a trial in which the expected effect of 4-HPR on IGF-I was not observed [13], lack of compliance was excluded because retinol concentrations declined to an extent, which was in the same range previously reported with that dose. Taking into account that the 200 mg/day dose tested in adults is equivalent to 120 mg/m^2 in children, (i.e., the lowest dose tested in children), and that we considered the nadir retinol levels, the results in adults seem to be in reasonable agreement with those found in children.

As previously found in breast cancer patients treated with 200 mg/day 4-HPR for 5 years [19], we confirm here that in children treated daily with 4-HPR in the dose range of 100–4,000 mg/m^2, the reduction in retinol levels was strongly and directly correlated with pretreatment plasma retinol concentrations. The results of the present study also provide additional data indicating that the reduction was independent of the dose. Therefore, pretreatment retinol levels may predict retinol level reduction by 4-HPR; in fact, the higher the initial values, the higher is the absolute decrease, with the percentage decrease remaining the same.

This result seems to confirm the suggested 4-HPR interaction with retinol in retinol-secreting organs: individual differences in retinol levels are due to different rates of retinol secretion and 4-HPR seems to interact with these rates by perfectly substituting for retinol.

Interaction of 4-HPR with retinol in retinol-secreting organs and not in plasma is in agreement with the observed counterclockwise hysteresis, likely produced by the distribution of 4-HPR in an effect compartment (possibly the liver) and to the consequent delay in reaching pharmacologically active concentrations in this compartment after the first administration. The hysteresis collapsed after chronic treatment and this seems to be due to the time needed for the liver and plasma 4-HPR concentrations to reach equilibrium.

Nyctalopia occurred in 23% of the patients and, with only one exception, it was found only after an accurate anamnesis [4]. The lack of relationship between plasma retinol reduction and 4-HPR doses is consistent with the occurrence of nyctalopia, which was found at most dose levels and with similar severity across doses [4]. Nyctalopia was associated with plasma retinol levels, which were at nadir on average 0.11 μM. In women receiving 200 mg/day 4-HPR, moderate alterations of dark adaptability were associated with plasma retinol levels below 100 μg/L, corresponding to 0.35 μM [12]. The lower safety level found in children is likely due to underreporting by young patients.

To conclude, plasma retinol levels of 0.11 μM were found to be associated with nyctalopia and this concentration could be considered as a safety threshold below which the incidence of this adverse event starts becoming clinically relevant in children with neuroblastoma. From the results herein reported, it is clear that when 4-HPR-mediated plasma retinol reduction contributes to its toxic effect, it did not occur at the highest tested doses. Similarly, for studies in which retinol reduction should contribute to 4-HPR therapeutic activity, the maximum tolerated dose should not be needed. Finally, since initial retinol levels strongly predict the extent of retinol decrease, this could be used as a robust approach to monitor 4-HPR compliance.

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