

Letter to the Editor**Correspondence re: M. Follen *et al.*, A Randomized Clinical Trial of 4-Hydroxyphenylretinamide for High-Grade Squamous Intraepithelial Lesions of the Cervix. *Clin. Cancer Res.*, 7: 3356–3365, 2001¹**

Clinical trials of 4-HPR² in several organ sites are ongoing, and the paper by Follen *et al.* (1) reports the results of a trial in patients with high-grade squamous intraepithelial lesions of the cervix. 4-HPR, administered for 6 months at 200 mg/day with a 3-day/month drug holiday was not active compared with placebo. I appreciated the decision of the authors to monitor the drug plasma levels, and I read with interest their hypothesis that the lack of activity was attributable to low drug serum levels. As pointed out in the discussion section, the 4-HPR concentrations effective in suppressing the *in vitro* growth of tumor cells range from 1 to 10 μM , which correspond to 400–4000 ng/ml, whereas the mean serum levels of 4-HPR in the participants in the trial were 34.8 ng/ml. We found, in patients participating in a breast prevention trial and treated with 4-HPR at the same dose and with the same schedule, 4-HPR plasma levels of 350 ng/ml (2,3), which are concentrations 10 times higher.

To understand the reasons for such differences and to provide information for 4-HPR monitoring in future trials, we reviewed the published data on similar analyses. Table 1 reports the levels of 4-HPR and of its metabolite 4-MPR found in plasma or serum of subjects participating in six different clinical trials in which the drug was administered at 200 mg/day with a 3-day/month drug holiday and in which blood samples for drug measurement were collected at a single point. Table 1 also reports the results of a study by Doose *et al.* (4) on 4-HPR bioavailability that clearly showed the influence of meals and meal composition on 4-HPR plasma levels. In the study, at variance with the clinical trials, the dose of 4-HPR was 300 mg, and drug levels were measured at different intervals after a single drug administration. Doose *et al.* showed that administration of 4-HPR after a high-fat breakfast increased 4-HPR peak levels three times (from 198 to 596 ng/ml) compared with administration of the same dose in the fasting state. They also showed that a high-fat meal caused the highest increase in 4-HPR absorption compared with high-protein and high-carbohydrate meals.

Among the six clinical trials with monitoring of 4-HPR levels, two were performed in Italy (2,5) and four in the United States (1,6–8). In a prevention trial run in Italy, in women operated on for early breast cancer, 4-HPR was taken after dinner, and blood samples were collected 12 h after drug intake (2). In these subjects, we found mean 4-HPR and 4-MPR plasma levels of 363 and 369 ng/ml, respectively. Similar levels were

found in other subjects participating in the same trial whose blood was collected at different times after the beginning of treatment starting from 5 months up to 5 years (3). In another trial run in Italy, patients with bladder cancer who took the drug after dinner had 4-HPR plasma levels similar to those found in our study (297 ng/ml; Ref 5). However, the coefficient of variation was higher, possibly because of the wide range of the interval between drug intake and blood sampling (18 ± 11 h). The 4-HPR levels found in the four trials carried out in the United States were much lower. In the trial run by Thaller *et al.* (6), Conley *et al.* (7), and Kurie *et al.* (8), they were three times lower, whereas in the trial by Follen *et al.* (1) they were 10 times lower. No information was provided on the modality of drug administration or on the interval between drug intake and blood sampling in the paper by Kurie *et al.* (8) and by Follen *et al.* (1). In the trial by Thaller *et al.* (6) the drug was taken after dinner, similarly to the two trials run in Italy (2,5), whereas in the trial by Conley *et al.* (7) it was taken after breakfast. The interval between drug intake and blood sampling was ~ 24 h in both studies. The longer interval than in the two studies run in Italy might in part account for the lower drug levels. The coefficient of variation of drug levels in the four studies was high (≥ 0.6), suggesting high variability in the drug intake-blood sampling interval. It might also reflect high variability in meal composition as a consequence of the different demographic origins of the trial participants. In the Doose *et al.* study (4), subjects received tests meals of which the composition was well defined. In our study (2), all of the participants lived in the northern part of Italy, where alimentary habits are rather uniform and where olive oil, which might account for higher 4-HPR bioavailability, is a constant component of the meals.

In conclusion, from the analysis of the results on 4-HPR levels measured in different clinical trials, we suggest that the observed differences might be attributable to differences in meal composition and in the interval between drug intake and blood sampling. Therefore, we draw attention to the importance of the modalities of drug administration and of meal composition in 4-HPR bioavailability, and of the interval from drug intake in monitoring drug levels. From this analysis we also conclude that the highest 4-HPR plasma concentrations achieved with the 200-mg daily dose are ~ 1 μM , *i.e.*, the least effective in suppressing tumor cell growth *in vitro*. For this reason we agree with the conclusion of the authors that higher doses should be tested in future clinical trials. The suggestion seems reasonable taking into account that 4-HPR was not toxic at 200 mg/day and is supported by the fact that high-dose 4-HPR was more effective than low dose in preventing mammary tumors in rats (9).

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² The abbreviations used are: 4-HPR, N-(4-hydroxyphenyl)retinamide; 4-MPR, N-(4-methoxyphenyl)retinamide.

Table 1 4-HPR and 4-MPR plasma or serum levels (mean ± SD in ng/ml) in humans after administration of 200–300 mg/day

Dose (mg/day)	No. of subjects	Sex	Age range (yr)	Drug administration modality	Length of treatment	Interval from last drug intake	4-HPR (C.V.) ^a	4-MPR (C.V.) ^a	Ref./kind of subjects/In plasma (P) or serum (S)
300	13	M	(19–50)	While fasting	1 day	at peak ^b	198 ± 75 (0.38)	91 ± 31 (0.34)	4 Healthy subjects (P)
				After HF ^c breakfast	1 day	at peak	596 ± 265 (0.44)	211 ± 94 (0.44)	
				After HF meal	1 day	at peak	669 ± 178 (0.27)	217 ± 49 (0.22)	
200	10	F	(35–65)	After HP ^c meal	1 day	at peak	319 ± 69 (0.22)	159 ± 61 (0.38)	2 3 Early breast cancer patients (P)
				After HC ^c meal	1 day	at peak	231 ± 64 (0.28)	101 ± 37 (0.37)	
				After dinner	5 months	12 h	363 ± 140 (0.38)	369 ± 193 (0.53)	
				After dinner	5 months	14 ± 2 h	348 ± 133 (0.38)	349 ± 173 (0.49)	
200	31	M/F	(46–81)	After dinner	12 months	18 ± 11 h	297 ± 200 (0.67)	339 ± 194 (0.57)	5 Bladder cancer patients (P)
200	14	M	(46–70)	After dinner	28 days	≤24 h	127 ± 107 (0.84)	141 ± 87 (0.62)	6 Prostate cancer patients (S)
200	32	F	(36–74)	After breakfast	25 days	20–24 h	132 ± 82 (0.62)	113 ± 85 (0.75)	7 High risk subjects for breast cancer (P)
200	34	M/F	(31–67)	n.d. ^d	2–6 months	n.d.	104 ± 64 (0.61)	201 ± 136 (0.68)	8 Smokers with bronchial squamous metaplasia and/or dysplasia (S)
200	20	F	(18–42)	n.d.	6 months	n.d.	35 ± 27 ^e (0.77)	53 ± 33 (0.63)	1 Patients with CIN ^f 2/3 (S)

^a C.V. = coefficient of variation.

^b At peak: corresponding to 3–4 h for 4-HPR and 6–12 h for 4-MPR.

^c HF, high-fat; HP, high-protein; HC, high-carbohydrate.

^d n.d., not described.

^e The SD was calculated from the results of the authors, assuming that all assessable patients in the 4-HPR group (*i.e.* 20) had serum drug measurement.

^f Cervical intraepithelial neoplasia.

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