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The Metabolite N-4-Methoxyphenylretinamide is a Major Determinant of Fenretinide Induced Decline of Plasma Insulin-like Growth Factor-1

R. Torrisi, F. Pensa, V. Fontana, A. Costa and A. Decensi

INSULIN-LIKE GROWTH factor (IGF-1), a potent mitogen for breast cancer cells, has been implicated in the promotional steps of breast carcinogenesis [1]. We have recently shown that the administration of the synthetic retinoid, fenretinide (N-(4-hydroxyphenyl)retinamide, 4-HPR), induces a significant decline in circulating levels of IGF-1 in breast cancer patients, which was more pronounced in younger women [2]. It is possible that the decrease of available IGF-1 in plasma might represent at least one of the mechanisms through which the retinoid interferes with the carcinogenic process. This study augments our previous work in that it endeavours to investigate the effects of plasma concentrations of 4-HPR and of its major metabolite N-(4-methoxyphenyl)retinamide (4-MPR) on plasma IGF-1.

The 20 stage I ($T_{1-2}N_0$) breast cancer patients enrolled in the study were part of a large randomised trial of contralateral breast cancer chemoprevention with 4-HPR [3]. All patients had been randomised to receive 4-HPR (R.W. Johnson Pharmaceutical Institute, Springhouse, Pennsylvania, U.S.A.) 200 mg daily, orally for 5 years with a monthly 3-day drug holiday. Median age was 53.5 years (range 39–67) and mean body mass index (BMI), expressed as weight (kg) divided by squared height (m^2), was 24.32 ± 0.6. Sixteen patients were postmenopausal and 4 premenopausal.

IGF-1 levels were measured by radioimmunoassay on plasma obtained at randomisation and after a mean interval of 11 ± 0.6 months, as previously described [2]. At baseline, plasma IGF-1 levels (mean ± S.E.) were 154.8 ± 11.4 ng/ml and decreased to 132.8 ± 8.7 ng/ml after treatment ($P=0.006$, Student t-test). Plasma levels of 4-HPR and 4-MPR were determined by high-performance liquid chromatography (HPLC), on blood samples obtained after a median time on treatment of 32 months (range 6–45), as previously described [4]. Since plasma levels of the drug and the metabolite have been shown to be stable during treatment for as long as 5 years [4], we assumed that the levels of 4-HPR and 4-MPR at the time of IGF-1 determination, i.e. after 1 year of treatment were comparable with those obtained at the time of our assay. Plasma levels of 4-HPR were 387.2 ± 26.9 ng/ml and those of 4-MPR 285.2 ± 21.7 ng/ml. When the difference (△) in IGF-1 levels between follow-up and baseline values was employed as a dependent variable, simple linear regression analysis showed a significant inverse relationship of △IGF-1 with 4-MPR, but not with 4-HPR levels ($r=0.47$, $P < 0.05$ and $r=0.08$, $P=$not significant, respectively).

Multiple regression analysis using △ IGF-1 as a dependent variable and age, menstrual status, BMI, 4-HPR and 4-MPR concentrations as covariates showed a significant regression of △IGF-1 on metabolite and, to a lesser extent, on drug concentrations (F test=5.50, $r^2=0.66$, $P<0.01$). The dependence of △IGF-1 on 4-MPR and 4-HPR levels was further explained by a significant interaction with age (see legend to Figure 1). In fact, when patients were categorised according to median 4-MPR concentration, in younger women the decline of IGF-1 (i.e., negative △) was greater in patients with low 4-MPR levels, while in older women a negative △ was observed in patients with higher 4-MPR concentrations (Figure 1b). An opposite trend was observed when patients were categorised according to median 4-HPR concentration (Figure 1a). Interestingly, the occurrence of a negative △ appears to be constant with age in patients with higher levels of 4-MPR while a trend towards a positive △IGF-1 with increasing age is observed at lower 4-MPR concentrations. Menstrual status and BMI did not affect △IGF-1 (data not shown).

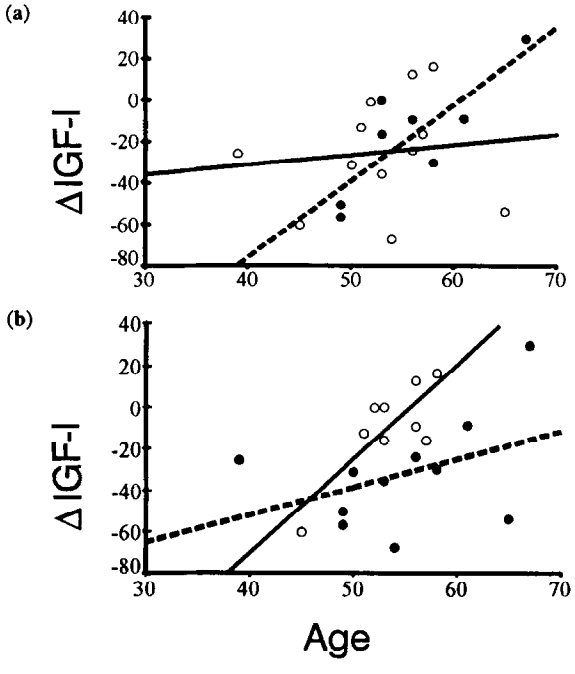

Figure 1. (a) Age-dependent behaviour of the difference (△) of plasma IGF-1 (ng/ml) in patients with plasma 4-HPR above (●,---) or below (○,—) the median value =370 ng/ml, β=0.01, 95% confidence interval =0.0008–0.02, P=0.04; (b) age-dependent behaviour of the △ IGF-1 in patients with plasma 4-MPR above (●,---) or below (○,—) the median value =290 ng/ml, β= −0.04, 95% confidence interval=0.07–0.01, P=0.01 (see text for detailed explanation). Data are expressed as expected values.

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Our results seem to suggest that 4-MPR is the major determinant of plasma IGF-1 decline (negative △). This is in keeping with our previous observations of a dominant role of 4-MPR in determining another principal effect of fenretinide administration, namely, the decline of plasma retinol levels [5]. We may also speculate that the reversal of effect induced by 4-HPR and 4-MPR concentrations on △IGF-1 as a function of age, is due to age-related differences in the metabolism and in tissue distribution of the two compounds, which are partially different both in mice [6] and in human mammary gland [7]. 4-MPR appears to be less extensively metabolised than 4-HPR and selectively concentrated in adipose tissue from which it may be slowly released [7]. In humans, 4-MPR has a longer half-life than 4-HPR [4], potentially exerting a prolonged effect in circulation, while having the same potency as 4-HPR in *in vitro* differentiation assays [8]. In addition, the metabolism to 4-MPR has recently been shown to be critical to the antiproliferative effect of 4-HPR on the growth of breast cancer cell lines [9]. Thus, the preferential effect of 4-MPR on IGF-1 may have a pharmacological explanation or, alternatively, be the result of a selective biological action elicited by 4-MPR itself, supporting a leading role for 4-MPR in determining some of the main biological effects induced *in vivo* by treatment with fenretinide.

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Fotemustine and Tamoxifen Combination Therapy in Metastatic Malignant Melanoma. A Phase II Study

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SEVERAL STUDIES have shown that the addition of tamoxifen to chemotherapy may enhance the response rate in patients with metastatic malignant melanoma [1–4]. Fotemustine, a nitrosurea, has activity against melanoma as a single agent [5–7], with a response rate of 24.2% in the largest study with 153 patients [5]. Tamoxifen enhances *in vitro* the cytotoxic effect of fotemustine on melanoma cell lines expressing oestrogen receptors [8]. In patients with metastatic melanoma, high-dose tamoxifen may result in a higher complete response rate compared with low-dose tamoxifen [9]. We, therefore, initiated a phase II study of high-dose tamoxifen and fotemustine in patients with metastatic melanoma.

Eligibility criteria included histologically confirmed metastatic melanoma, measurable progressive disease, age 18–75 years, WHO performance status ≤ 2, life expectancy ≥ 3

Table 1. Patients' characteristics

	No. of patients
Male/female	8/5
Median age (range)	50 years (33–72)
Median WHO performance (range)	1 (0–1)
Previous therapy	
Radiotherapy	3
Chemotherapy	4
Immunotherapy	2
Number of metastatic sites	
1	5
2	1
≥ 3	7
Metastatic sites	
Cerebral	3
Visceral	10
Non-visceral	10

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