ISSN 1109-4486

ΤΡΙΜΗΝΙΑΙΑ ΕΚΔΟΣΗ ΤΜΗΜΑΤΟΣ ΝΟΣΗΛΕΥΤΙΚΗΣ Α΄ ΤΕΙ ΑΘΗΝΑΣ

ΙΑΝΟΥΑΡΙΟΣ-ΜΑΡΤΙΟΣ 2003 ΤΟΜΟΣ 2 ΤΕΥΧΟΣ 1

ΤΟ ΒΗΜΑ ΤΟΥ ΑΣΚΛΗΠΙΟΥ

VEMA OF ASKLIPIOS

JANUARY-MARCH 2003 VOLUME 2 No 1

QUARTERLY EDITION BY THE 1st NURSING DEPARTMENT
OF ATHENS TECHNOLOGICAL EDUCATIONAL INSTITUTION

ΤΟ ΒΗΜΑ ΤΟΥ ΑΣΚΛΗΠΙΟΥ

ΙΑΝΟΥΑΡΙΟΣ-ΜΑΡΤΙΟΣ 2003 ΤΟΜΟΣ 2 ΤΕΥΧΟΣ 1

VEMA OF ASKLIPIOS

JANUARY-MARCH 2003 VOLUME 2 No 1

Περιεχόμενα

Contents

VEMA OF ASCLIPIOS 2003, 2(1):33–36

Original paper

The effects of EB1089 on spontaneous mammary carcinoma (SMC) of C_3H/Sy mice

D. Sahpazidou,[1]
P. Stravoravdi,[1]
Th. Toliou,[2]
G. Geromichalos[3]

[1]BSc, PhD
[2]MD, PhD
[3]MSc, "Theagenio" Cancer Hospital of Thessaloniki, Thessaloniki

Key words: Mice, mammary carcinoma, EB1089

Aim **EB1089, a vitamin D analogue, induces growth inhibition, differentiation and apoptosis in tumor cells *in vitro* and *in vivo*. In this preliminary report we present the effects of EB1089 on tumor size and blood calcium levels, on 16 C_3H/Sy mice bearing SMCs.** *Material-Method* **One experimental (n=7) and one control groups were formed when a palpable tumor was evident. The former group received 0.5 μg/kg EB1089 every other day, the latter received no treatment. Tumor size was measured at 2.5 weeks after treatment initiation, for both groups. Blood was taken before sacrifice for calcium measurements.** *Results* **Our results show that EB1089 statistically decreases the tumor size and prolongs survival, causing also calcium elevation.** *Conclusions* **The findings suggest that EB1089, might be a promicing antitumor agent, provided that monitoring of doses will be attended.**

Περίληψη **Οι δράσεις του ΕΒ1089 στον αυτόματο καρκίνο του μαστού ποντικιών της φυλής C_3H/Sy.** Δ. Σαχπαζίδου,[1] Π. Στραβοράβδη,[1] Θ. Τόλιου,[2] Γ. Γερομιχαλός.[3] *[1]BSc, PhD, [2]MD, PhD, [3]MSc, «Θεαγένειο» Νοσοκομείο Θεσσαλονίκης, Θεσσαλονίκη. Το Βήμα του Ασκληπιού 2003, 2(1):33–36. Σκοπός* **Η δραστική μορφή της βιταμίνης D, το $1α,25(OH)_2D_3$, έχει μελετηθεί εκτενώς, κυρίως ως προς την αντικαρκινική της δράση. Οι ισχυρές όμως παρενέργειες που προκαλούνται (υπερασβεστιναιμία) από τις δόσεις που απαιτούνται για την επίτευξη θεραπευτικού αποτελέσματος, καθιστούν τη χορήγησή της απαγορευτική. Τα τελευταία χρόνια πολλές μελέτες έχουν επικεντρωθεί στην τροποποίηση του δραστικού μορίου της βιταμίνης, ώστε να προκύψουν ενώσεις (ανάλογα της βιταμίνης D) με τις ίδιες θεραπευτικές δράσεις, αλλά με ηπιότερες έως ελάχιστες παρενέργειες. Το ΕΒ1089 είναι ένα τέτοιο νέο ανάλογο. Αναστέλλει τον πολλαπλασιασμό των καρκινικών κυττάρων και επάγει τη διαφοροποίηση και την απόπτωση, τόσο σε κυτταρικές σειρές, όσο και σε πειραματικούς όγκους. Το κυριότερο όμως χαρακτηριστικό του ΕΒ1089 είναι ότι εμφανίζει σαφώς χαμηλότερη υπερασβεστιναιμική δράση από το μητρικό μόριο της δραστικής μορφής της βιταμίνης D_3. Η δράση του ΕΒ1089 δεν έχει μελετηθεί μέχρι σήμερα πειραματικά σε αυτόματους όγκους. Στην παρούσα πειραματική εργασία ερευνήθηκε η αντικαρκινική δράση του ΕΒ1089 σε αυτόματο καρκίνο του μαστού, σε ποντίκια του είδους C_3H/Sy. Το είδος αυτό χαρακτηρίζεται από υψηλά ποσοστά εμφάνισης αυτόματου καρκίνου του μαστού, ο οποίος οφείλεται κυρίως σε ιό, γνωστό ως MMTV (mouse mammary tumor virus) και μεταδίδεται κατά τη γαλουχία.** *Υλικό-Μέθοδος* **Το χαρακτηριστικό αυτού του είδους της φυλής το καθιστά ενδιαφέρον πειραματικό μοντέλο μελέτης του καρκίνου του μαστού. Παράλληλα με την επίδραση του ΕΒ1089 στο μέγεθος του όγκου μελετήθηκαν και τα επίπεδα ασβεστίου στο αίμα των ποντικών. Χρησιμοποιήθηκαν συνολικά 16 θηλυκά ποντίκια C_3H/Sy, που χρησιμοποιήθηκαν αμέσως μετά την εντόπιση όγκου μαστού με ψηλάφηση. Τα πειραματόζωα χωρίστηκαν σε δύο ομάδες, την πειραματική με 7 ζώα και την ομάδα αναφοράς (μάρτυρες), με 9 ζώα. Τα ζώα της πρώτης ομάδας έλαβαν 0,5 μg/kg ΕΒ1089 κάθε δεύτερη ημέρα, έως τη κατάληξή τους από τη νόσο. Τα ζώα της ομάδας αναφοράς δεν έλαβαν θεραπεία. Όλα τα πειραματόζωα παρακολουθούνταν κάθε δεύτερη μέρα, ενώ οι όγκοι μετρήθηκαν στις 2,5 εβδομάδες από την εντόπισή τους και κατά τη νεκροτομή. Κατά τη νεκροτομή λαμβάνονταν επίσης αίμα για τον προσδιορισμό του ασβεστίου.** *Αποτελέσματα* **Τα αποτελέσματά μας έδειξαν ότι το ΕΒ1089 μειώνει πολύ σημαντικά το μέγεθος του όγκου (P=0,00046 σε 0,05% επίπεδο σημαντικότητας), αυξάνει σημαντικά το χρόνο επιβίωσης (P=0,00029 σε 0,03% επίπεδο σημαντικότητας), αυξάνει όμως και τα επίπεδα του ασβεστίου στο αίμα (P=0,026 σε 3% επίπεδο σημαντικότητας).** *Συμπεράσματα* **Συμπερασματικά, τα πρόδρομα αποτελέσματά μας δείχνουν ότι το ΕΒ1089 εμφανίζει ισχυρή αντικαρκινική δράση στον αυτόματο καρκίνο του μαστού ποντικών C_3H/Sy, επιμηκύνει το χρόνο επιβίωσης των ζώων, παράλληλα όμως αυξάνει τα επίπεδα ασβεστίου στο αίμα, αν και σε μη στατιστικά σημαντικά επίπεδα.**

Λέξεις κλειδιά: *Ποντίκια, καρκίνος του μαστού, ΕΒ1089*

Corresponding author: *D. Sahpazidou, "Theagenio" Cancer Hospital, 2 Al. Symeonidis street, GR-540 07 Thessaloniki, Greece*
e-mail: toliou@yahoo.com

Introduction

The hormonally active form of vitamin D, 1α,25 $(OH)_2D_3$, and its analogue EB1089 are novel putative anticancer agents with an interesting profile on the induction of growth inhibition, differentiation and apoptosis in a plethora of cancer cell lines and experimental tumors.[1]

EB1089, develops lower calcemic activity than 1α,25 $(OH)_2D_3$. It inhibits among others, the growth of breast cancer cell lines and promotes tumor regression in experimental rat mammary tumors.[1,2]

We report here a pilot study on the effects of systemic administration of EB1089 on spontaneous mice mammary carcinomas (SMMCs) of C_3H/Sy mice. SMMC constitutes one of the most widely used model systems for the study of breast cancer, in which a confluence of hormonal and viral agents are implicated.[3] Among hormones, estrogens and prolactin play the major role both in its induction and growth.[4] The other factor involved is the Mouse Mammary Tumor Virus (MMTV), which is believed to be ubiquitous in mice of the carrier strains and has the ability to modify the genetic machinery of the infected mammary cells.[5] Our purpose was to determine whether EB1089 can be used therapeutically to reduce the size of developing mammary tumors and/or prolong survival of the mice.

Material and method

Animals

A total of 16 female C3H/Sy mice, obtained from the Experimental Department of the "Theagenio" Cancer Hospital, were used. C_3H/Sy mice of our colony develop an incidence of 70–80% of spontaneous mammary carcinomas between 8–12 months of age. The animals were housed, just after lactation, in individual plastic cages under standard laboratory conditions, and received commercial palleted food and tap water "ad libidum".

Treatments

The mice were divided into two groups when a palpable mammary tumor was evident: (a) experimental animals (n=7) receiving EB1089 (Leo Pharmaceuticals) (tabl. 1), (b) controls (n=9) receiving no treatment (tabl. 2).

EB1089 stock solution was initially diluted in absolute ethanol (Baker, The Netherlands) to produce a working solution renewable every week. A proper volume of the working solution was diluted and administered in normal saline. After the detection of tumor, EB1089 was given intraperitoneally, every other day at a dose of 0.5 μg/kg of body weight. This dose was already used in other trials and found to be "non calcemic".[6,7] It was also suggested by the producers of EB1089 (for Leo Pharmaceuticals, Dr. L. Binderup). The mean number of doses administered was 21, raging from 11 to 30 (tabl. 1). Animals, in both groups, were sacrificed when they appeared moribund. The control animals were sacrificed 2.4 weeks (mean survival period) after tumor detection while the experimental animals 6.4 weeks (mean survival period) after tumor detection (tables 1 and 2).

For reasons of comparison, tumor size was measured in both groups 2.5 weeks after treatment initiation, using a micro-Vernier and the average tumor volume was calculated using the formula $axb^2/2$ cm^3, where a=length, b=width. The tumor size of the experimental animals was measured again when the animals were necrotomized (tabl. 1).

Blood samples were taken by cardiac puncture just before sacrifice in both groups, and calcium concentrations were measured by an automatic electrolyte system (Beckman Synchron Eli-se).

Statistical analysis

Data used for experimental analysis were expressed as mean SEM (standard error of the mean), while SD (standard deviation) values were also incorporated in tables 1 and 2. The significance of differences between drug-treated group and controls was assessed by Student's t-test and a difference was considered statistically significant when $P<0.05$.

Results

The systemic administration of 0.5 μg/kg of body weight of EB1089 every other day did not cause any deaths and serious side effects in the animals and was generally well tolerated.

Our results show that EB1089 causes: (a) a very statistically significant increase of the survival time ($P=0.00029$ at 0.03% level of significance), (b) a very statistically significant decrease in the tumor size ($P=0.00046$ at 0.05% level of significance) and (c) a statistically significant increase in blood calcium level ($P=0.026$ at 3% level of significance). Our results are analytically shown in tables 1 and 2.

Discussion

The biologically active form of the vitamin D, 1α,25$(OH)_2D_3$, is a hormone with important roles in inhibition of cell proliferation, induction of cellular differentiation and apoptosis,[1] inhibition of angiogenesis[8] and modulation of immune responsiveness.[9] It also influences the secretion of prolactin and growth hormone in the pituitary[10] and has diverse effects on thyroid and adrenal cells. Vitamin D receptors have been identified in most breast cancer cell lines and tumors.[11,12] Unfortunately, administration of 1α,25$(OH)_2D_3$ to animals also leads to dangerous elevation of calcium blood levels.[13] Therefore attempts are currently being directed towards identification of chemically modified vitamin D_3 analogues which have the above properties of

Table 1. Survival time, tumor volume and calcium levels in mice treated with EB1089.

Animal number	Age of tumor detection (weeks)	Age of death (weeks)	Nr of doses administered	Survival period (weeks)*	Average tumor volume (cm^3) (at 2.5 weeks)**	Average tumor volume (cm^3) (at death)	Blood Ca^{2+} level (mg/dL)***
1	38.0	44.0	17	6.0	5.00	21.4	14.76
2	44.0	53.0	30	9.0	0.75	6.0	12.64
3	48.0	56.0	27	8.0	2.00	23.3	14.08
4	43.0	47.0	11	4.0	6.00	5.0	–
5	53.0	60.0	22	7.0	2.20	8.0	–
6	39.5	44.0	13	4.5	2.20	2.8	–
7	40.5	47.0	25	6.5	2.20	24.5	13.69
Mean	43.7	50.1	21	6.43	2.91	13.0	13.79
SD			1.79		1.87		0.89
SEM			0.68		0.71		0.44

* Values are very statistically significant different (P=0.00029 at 0.03% significance level) vs survival data shown on table 2 (t=–6.109 with 14 degrees of freedom)

** Values are very statistically significant different (P=0.00046 at 0.05% significance level) vs data shown on table 2 (t=4.179 with 14 degrees of freedom)

*** Values are statistically significant different (P=0.026 at 3% significance level) vs data shown on table 2 (t=–2.600 with 10 degrees of freedom)

Table 2. Survival time, tumor volume and calcium levels in non-treated mice.

Animal number	Age of tumor detection (weeks)	Age of death (weeks)	Survival period (weeks)	Average tumor volume (cm^3)	Blood Ca^{2+} level (mg/dL)
1	32	35.0	3.0	24.50	9.56
2	36	39.0	3.0	12.50	13.87
3	58	60.0	2.0	8.60	10.23
4	63	66.5	3.5	13.50	12.69
5	81	84.0	3.0	12.50	10.71
6	58	60.0	2.0	11.90	8.83
7	65	67.0	2.0	14.90	13.14
8	69	70.0	1.0	1.69	–
9	33	35.0	2.0	15.75	11.21
Mean	55	57.4	2.39	12.87	11.28
SD	–	–	0.78	6.05	1.79
SEM	–	–	0.26	2.02	0.63

1α,25$(OH)_2D_3$ but are deprived of side effects on calcium metabolism.

A new vitamin D analogue is EB1089 which has been tested, among others, on breast cancer cell lines (MCF-7) *in vitro* and on experimental rat mammary tumors.[2] In both tests EB1089 showed strong inhibitory effects with lower calcemic action than the 1α,25$(OH)_2D_3$. No reports exists, however, confirming its efficacy on spontaneous mouse mammary carcinomas.

In the present study we examined the effects of systemic administration of EB1089 on C_3H/Sy mice bearing spontaneous mammary carcinomas. Our preliminary results showed that EB1089 causes a very statistically significant decrease in tumor size. Our present experiments, however, cannot possibly give answers about the mode of action of EB1089. Further studies, which are now underway using both immunohistological and biochemical methods, are necessary to clarify whether the decrease of tumor size is due to the induction of apoptosis or/and inhibition of tumor cell proliferation and neoangiogenesis. The very statistically significant increase of the survival time of treated mice, which was also noted, could be partially attributed to the decreased tumor size. The increase of calcium levels in the blood of experimental mice appear to be statistically significant compared to calcium blood levels of controls. It is important to note, however, that the mean value of calcium in the experimental group (13.79 mg/dL) falls within the range of calcium levels recorded for the control group (9.56–13.87 mg/dL).

In conclusion our preliminary results show that the synthetic analogue of vitamin D, EB1089, exerts strong antitumor effect on spontaneous mouse mammary carcinoma in doses of relatively low calcemic activity. This action makes EB1089 an interesting candidate for breast cancer management.

Acknowledgments

The authors thank Dr Lise Binderup from Leo Pharmaceuticals who provided us with EB1089. They also thank the technicians Mrs H. Lazaridou, Mrs P. Papadopoulou Mrs V. Hatzi and Mr P. Oustambasidis for their technical assistance.

References

1. Mathiasen IS, Lademann U, Jaattela M. Apoptosis induced by vitamin D compounds in breast cancer cells is inhibited by bcl-2 but does not involve known caspases or p53. *Cancer Res* 1999, 59:4848–4856
2. Colston KW, Mackay AG, James SY, Binderup L, Chander S, Coombes RC. EB1089: a new vitamin D analogue that inhibits the growth of breast cancer cells *in vivo* and *in vitro*. *Biochem Pharmacol* 1992, 44:2273–2280
3. Stravoravdi P, Toliou Th, Polyzonis M. A New Approach Combining Interferon and Bromocriptine LA to Prevent Spontaneous Mouse Mammary Carcinoma. *J Interf Cytok Res* 1991, 11:49–51
4. Welsch CW, Nagasawa H. Prolactin and murine mammary tumorigenesis: a review. *Cancer Res* 1977, 37:951–963
5. van Leeuwen F, Nusse R. Oncogene activation and oncogene cooperation in MMTV-induced mouse mammary cancer. *Semin Cancer Biol* 1995, 6:127–133
6. James SY, Mercer E, Brady M, Binderup L, Colston KW. EB1089, a synthetic analogue of vitamin D, induces apoptosis in breast cancer cells *in vivo* and *in vitro*. *Br J Pharmacol* 1998 125:953–962
7. Colston KW, Mackay AG, James SY, Binderup L, Chander S, Coombs RC. EB1089: A new vitamin D analogue that inhibits the growth of breast cancer cells *in vivo* and *in vitro*. *Biochem Pharmacol* 1992, 44:2273–2280
8. Oikawa T, Hirotani K, Ogasawara H, Katayama T, Wakamura O, Iwaguchi T et al. Inhibition of angiogenesis by vitamin D_3 analogues. *Eur J Pharmacol* 1990, 178:247–250
9. Bollag W. Experimental Basis of Cancer Combination Chemotherapy with Retinoids, Cytokines, 1,25-Dihydroxyvitamin D_3, and Analogs. *J Cellul Biochem* 1994, 56:427–435
10. Wark JD, Gurtler V. Vitamin D-induction of secretory responses in rat pituitary tumor (GH4Cl) cells. *J Endocrinol* 1988, 117:293–298
11. Eisman JA, Suva LJ, Sher E, Pearce PJ, Funder JW, Martin TJ. Frequency of 1,25-dihydroxyvitamin D_3 receptor in human breast cancer. *Cancer Res* 1981, 41:5121–5124
12. Eisman JA, Martin TJ, MacIntyre I, Moseley JM. 1,25-dihydroxyvitamin D_3 receptors in breast cancer cells. *Lancet* 1979, 2:1335–1337
13. Brown AJ, Dusso A, Slatopolsky E. Selective vitamin D analogues and their therapeutic applications. *Semin Nephrol* 1994, 14:156–174

Αλληλογραφία: *Δ. Σαχπαζίδου, «Θεαγένειο» Νοσοκομείο Θεσσαλονίκης, Αλ. Συμεωνίδη 2, 540 07 Θεσσαλονίκη*
e-mail: Toliou@yahoo.com