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Η ηθική των μεταμοσχεύσεων

Η συμβολή των υπηρεσιών υγείας στην προαγωγή  
της υγείας

Ενδονοσοκομειακές λοιμώξεις. Νομική προσέγγιση

Διαχείριση μολυσματικών απορριμμάτων στα νοσοκομεία

Οι δράσεις του EB1089 στον αυτόματο καρκίνο του μαστού  
ποντικών της φυλής C<sub>3</sub>H/Sy

Καταγραφή επαγγελματικών παθήσεων στην Ελλάδα

Αποκατάσταση βουβωνοκήλης

The ethics of transplantation

Contribution of health services  
in health promotion

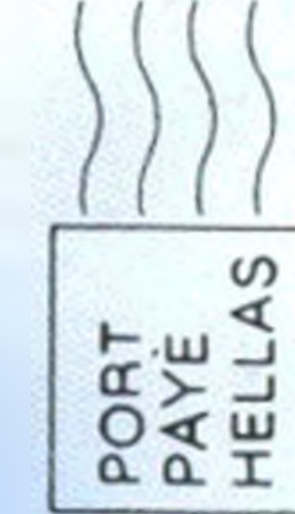
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The effects of EB1089 on spontaneous mammary  
carcinoma C<sub>3</sub>H/Sy of mice

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## Περιεχόμενα

### Ειδικό άρθρο

Βασικές θέσεις επί της ηθικής των μεταμοσχεύσεων. Μητροπολίτης Ιγνάτιος 7

### Ανασκοπήσεις

Η συμβολή των Υπηρεσιών Υγείας και Ασφάλειας της Εργασίας στην προαγωγή της Δημόσιας Υγείας. Ε.Χ. Αλεξόπουλος 13

Προστασία των ασθενών από τις ενδονοσοκομειακές λοιμώξεις. Η νομική προσέγγιση. Φ. Ομπέση 20

Διαχείριση μολυσματικών απορριμμάτων στα νοσοκομεία. Ε. Αποστολοπούλου 26

### Ερευνητικές εργασίες

Οι δράσεις του EB1089 στον αυτόματο καρκίνο του μαστού ποντικών της φυλής C<sub>3</sub>H/Sy Δ. Σαχπαζίδου, Π. Στραβοράβδη, Θ. Τόλιου, Γ. Γερομιχαλός 33

Καταγραφή επαγγελματικών παθήσεων στην Ελλάδα. Μία πρόβλεψη βασισμένη στα δεδομένα καταγραφών ευρωπαϊκών χωρών. Ε.Χ. Αλεξόπουλος, Φ. Χαριζάνη, Α.Α. Μπαρμπαρή, Χ. Κουτής 37

### Κλινική μελέτη

Τεχνικές αποκατάστασης της βουβωνοκήλης χωρίς τάση. Θ. Διαμαντής, Ι. Ζιούνας 44

Οδηγίες για τους συγγραφείς 51

## Contents

### Special article

Basic principles on the ethics of transplantations. Archbishop Ignatios 7

### Reviews

Contribution of workplace health and safety services in public health promotion. E.Ch. Alexopoulos 13

Patient's protection against hospital infections. The legal approach. Ph. Obessi 20

Hospital waste management. E. Apostolopoulou 26

### Original papers

The effects of EB1089 on spontaneous mammary carcinoma (SMC) of C<sub>3</sub>H/Sy mice. D. Sahpazidou, P. Stravoravdi, Th. Toliou, G. Geromichalos 33

Occupational diseases report in Greece. A prediction through comparison of registries in other European countries. E.Ch. Alexopoulos, F. Charizani, A.A. Barbari, Ch. Koutis 37

### Clinical study

Groin hernia repair. Tension-free techniques. Th. Diamantis, J. Ziounas 44

Instructions to authors 51

# The effects of EB1089 on spontaneous mammary carcinoma (SMC) of C<sub>3</sub>H/Sy mice

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**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<sub>3</sub>H/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 promising antitumor agent, provided that monitoring of doses will be attended.

**Περίληψη** Οι δράσεις του EB1089 στον αυτόματο καρκίνο του μαστού ποντικών της φυλής C<sub>3</sub>H/Sy. Δ. Σαχπαζίδου,<sup>1</sup> Π. Στραβοράβδη,<sup>1</sup> Θ. Τόλιου,<sup>2</sup> Γ. Γερομιχαλός.<sup>3</sup> <sup>1</sup>BSc, PhD, <sup>2</sup>MD, PhD, <sup>3</sup>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)<sub>2</sub>D<sub>3</sub>, έχει μελετηθεί εκτενώς, κυρίως ως προς την αντικαρκινική της δράση. Οι ισχυρές όμως παρενέργειες που προκαλούνται (υπερασβεστιαϊμία) 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 EB1089 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 EB1089 είναι ότι εμφανίζει σαφώς χαμηλότερη υπερασβεστιαϊμική δράση από το μητρικό μόριο της δραστικής μορφής της βιταμίνης D<sub>3</sub>. Η δράση του EB1089 δεν έχει μελετηθεί μέχρι σήμερα πειραματικά σε αυτόματους όγκους. Στην παρούσα πειραματική εργασία ερευνήθηκε η αντικαρκινική δράση του EB1089 σε αυτόματο καρκίνο του μαστού, σε ποντίκια του είδους C<sub>3</sub>H/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 EB1089 στο μέγεθος του όγκου μελετήθηκαν και τα επίπεδα ασβεστίου στο αίμα των ποντικών. Χρησιμοποιήθηκαν συνολικά 16 θηλυκά ποντίκια C<sub>3</sub>H/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 EB1089 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 EB1089 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 EB1089 εμφανίζει ισχυρή αντικαρκινική δράση στον αυτόματο καρκίνο του μαστού ποντικών C<sub>3</sub>H/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, EB1089

## Introduction

The hormonally active form of vitamin D,  $1\alpha,25(\text{OH})_2\text{D}_3$ , 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.<sup>1</sup>

EB1089, develops lower calcemic activity than  $1\alpha,25(\text{OH})_2\text{D}_3$ . It inhibits among others, the growth of breast cancer cell lines and promotes tumor regression in experimental rat mammary tumors.<sup>1,2</sup>

We report here a pilot study on the effects of systemic administration of EB1089 on spontaneous mice mammary carcinomas (SMMCs) of C<sub>3</sub>H/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.<sup>3</sup> Among hormones, estrogens and prolactin play the major role both in its induction and growth.<sup>4</sup> 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.<sup>5</sup> 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 C<sub>3</sub>H/Sy mice, obtained from the Experimental Department of the "Theagenio" Cancer Hospital, were used. C<sub>3</sub>H/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".<sup>6,7</sup> It was also suggested by the producers of EB1089 (for Leo Pharmaceuticals, Dr. L. Binderup). The mean number of doses administered was 21, ranging 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  $axb^2/2 \text{ cm}^3$ , 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,  $1\alpha,25(\text{OH})_2\text{D}_3$ , is a hormone with important roles in inhibition of cell proliferation, induction of cellular differentiation and apoptosis,<sup>1</sup> inhibition of angiogenesis<sup>8</sup> and modulation of immune responsiveness.<sup>9</sup> It also influences the secretion of prolactin and growth hormone in the pituitary<sup>10</sup> and has diverse effects on thyroid and adrenal cells. Vitamin D receptors have been identified in most breast cancer cell lines and tumors.<sup>11,12</sup> Unfortunately, administration of  $1\alpha,25(\text{OH})_2\text{D}_3$  to animals also leads to dangerous elevation of calcium blood levels.<sup>13</sup> Therefore attempts are currently being directed towards identification of chemically modified vitamin D<sub>3</sub> 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 <sup>3</sup> )		Blood Ca <sup>2+</sup> level (mg/dL)***
					(at 2.5 weeks)**	(at death)	
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 <sup>3</sup> )	Blood Ca <sup>2+</sup> 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

1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> 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.<sup>2</sup> In both tests EB1089 showed strong inhibitory effects with lower calcemic action than the 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub>. 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<sub>3</sub>H/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.

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