Academic Sciences Asian Journal of Pharmaceutical and Clinical Research

Vol 6, Suppl 2, 2013 ISSN - 0974-2441

Research Article

EFFECT OF BIS-CARBOXY ETHYL GERMANIUM SESQUOXIDE ON N-NITROSO-N-METHYLUREA - INDUCED RAT MAMMARY CARCINOMA

J. VINODHINI [1] S. SUDHA[1*]

[1]**Department of Biotechnology, Karpagam University, Coimbatore, Tamilnadu, India**, **Email:** sudhasellappa@yahoo.co.in

Received: 6 March 2013, Revised and Accepted: 28 March 2013

ABSTRACT

Breast cancer is the most prevalent cancer in the world today next to lung cancer and is a major public health problem in developing countries. Ge-132, an organo metallic compound is thought to improve health as a potent antioxidant and demonstrated significant antitumor activity. The present study was undertaken to determine the antineoplastic activity of Ge-132 against N-methyl-N-nitrosourea (MNU) induced mammary carcinogenesis in Albino Wistar rats. A single intraperitoneal dose (50 mg/kg) of MNU was injected into each of 12 female rats (aged 40 days) to induce tumors. After MNU treatment, the rats were divided into two groups. One of the group (n = 6) received Ge-132 after the carcinogen treatment, and it continued until the termination of the study. Whereas another group served as carcinogen control and group I served as normal control animal. After 34 weeks, the rats were killed and tumors were removed for morphological and histopathological analyses. Ge-132 reduced the tumor volume and size of MNU-induced mammary tumors. Histopathological changes also confirmed the formation of tumor tubules and neovascularization after the treatment. Ge-132 administration significantly reduces the growth of MNU-induced mammary tumors, and therefore has strong potential as a useful therapeutic regimen for inhibiting breast cancer development.

Keywords: Ge-132, Breast cancer, Anticancer activity, N-methyl-N-nitrosourea,

INTRODUCTION

Breast cancer is the most frequent malignancy among women and is the second leading cause of cancer deaths in women[1-2]. It is a highly heterogeneous disease represented by tumors that have a diverse natural history, complex histology and a variable response to therapy[3]. Once metastasis has occurred, the survival rate is drastically reduced to a median of 2-3 years; therapy is then aimed at controlling symptoms, prolonging survival and improving quality of life [4]. The use of specific chemicals to prevent the development or retard the progression of carcinogenesis, a technique known as chemoprevention, offers a promising strategy for cancer prevention[5,6]. Cancer chemoprotective potential of synthetic or naturally occurring chemicals or medicinal plants continues to be a major area of scientific interest[7,8]. However, to cure cancer completely it is necessary to deliver drugs with a potent antitumor effect to tumors from the general circulation; thus one can eradicate all systemic and unidentified metastases to distant tissues.

Metal-based anticancer therapy arose from the discovery of platinum (II) and platinum (IV) complexes which inhibit cell division. Cisplatin is one of the most successful metal based anticancer drugs effective against a diverse range of tumour types, though it is associated with significant renal toxicity and induced or acquired resistance[9-11]. The search for novel anticancer drugs continues and other metals have been investigated for their anticancer potential, including germanium, a naturally occurring metalloid found in soil[10].

Germanium is a constituent of many medicinal plants such as ginseng, and it is considered to play an important role in the pharmacological effects of the plants[12]. Several types of organogermanium compounds have been investigated and found to possess anti-microbial, anti-viral, anti-tumor and immunomodulative effects[13] of which Ge-132 (carboxy ethyl germanium sesquioxide) has been clinically used[14]. However, the mechanisms underlying the anti-tumor abilities of Ge-132 on breast cancers are still not clear enough.

The carcinogen N-methyl-N-nitrosourea (MNU) induces hormone-dependent mammary tumors in rats. This model has previously been used to develop breast cancer therapy[15], because rat mammary glands develop neoplasm that closely mimics human breast disease and share several morphological similarities; for example, carcinogenesis initiation occurs primarily, as does human breast cancer, from the terminal ductal-lobular unit. Again, experimental conditions and requirements are well established and accepted in this model system. Furthermore, most of the lesions found in the human breast have a counterpart in rat pathology[16]. Histopathological and morphometric studies were taken as end-point biomarkers. In the present study we investigated the inhibitory effect of Ge-132 *in vivo* on development of MNU induced mammary tumors in rats.

MATERIALS AND METHODS

Chemicals and reagents

All the chemicals used were of analytical grade and purchased from Sigma (St. Louis, MO, USA), N-methyl-N-nitrosourea (Sigma, St. Louis, MO), *bis*-carboxy ethyl germanium sesquioxide (Alfa Aesar, USA).

Ethical clearance

The study was approved by Institutional Animal Ethical Committee (IAEC) constituted for the purpose of CPCSEA, Government of India.

Animals

Virgin female rats of the Wistar albino strain (40 days old), were procured from Karpagam University, Coimbatore, India were used. Rats (120 g body weight) were divided into four groups consisting of six animals each. They were maintained at 28±1 ◦C, relative humidity 60◦C (12-h light and 12-h dark cycle), and provided with standard food pellets (diet composition, wheat broken-moisture 9.0%, crude protein 11.5%, crude fat 1.9%, crude fibre 4.0%, Ash 0.2%, nitrogen-free extract 73.4%) supplied by Hindustan Lever Ltd, Mumbai, India and tap water *ad libitum*.

Experimental design and tumor induction

We studied MNU induced mammary tumors according to the method of Thompson[17], with slight modifications. By using this protocol, rats weighing 120 g were randomized into four different groups of six rats each. **Group I** served as normal control (received intraperitoneal injection of 0.5 ml normal saline daily), **group II** were injected intraperitoneally with MNU 50 mg/kg dissolved in 0.9% saline and these served as breast cancer control animals without any treatment, **group III** were treated with 50 mg/kg of MNU and then provided with Ge-132 at the concentration of 1500

mg/kg/day for 34 weeks, intraperitoneally, **group IV** animals received only Ge-132 (1500 mg/kg/day for 34 weeks) and these served as drug control animals.

Morphometric findings

The experimental rats were regularly monitored for food and water consumption, the apparent signs of toxicity, weight loss, or mortality. Palpation and the time of mammary tumours appearance was recorded began 6 weeks after MNU treatment and continued until termination of the study. Once a week, the rats were lightly anesthetized with ether and the tumours were measured in two dimensions with vernier calliper and the tumour volume was calculated based on an ellipsoidal tumour shape with the following formula:

$$v = 4/3\ \pi\ r_1^2 r_2$$

where r_1 is the minor radium, r_2 is the major radium[18].

Histological Changes

Thirty-four weeks after the experiment, animals from each group were randomly selected, and the mammary tissues were surgically excised from ether anaesthetized rats. The recovered tissues were then fixed in 10% buffered formalin, embedded in paraffin using a conventional automated system. The blocks were cut to obtain 5µm-thick sections and stained with haemotoxylin-eosin for histological evaluation under light microscope[16].

Statistical analysis

To analyse the differences between control group and the animals with mammary tumours induced by MNU injections, we used the unpaired Student's *t*-test. All comparisons with *p* values below 0.05 were considered significant.

RESULTS

Morphometric findings

During the entire term of the study, no treatment-related alteration in the daily intake of food and water was observed among the different groups of rats. The size and volume of palpable mammary tumors in the control and experimental group was determined. In the NMU group, 100% of animals had tumors, at the end of 34 wk. The multiple tumors of different sizes were frequently found in the same animal; most of the tumors were between 3 and 5 mm in size (Table 1). Ge-132 treatment on NMU induced rat characteristically attenuated the number of tumors of 2 and 4 mm in size, indicating slower tumor progression. Between 25 and 30 weeks, a highly significant ($p<0.05$) lowering of the percentage of rats developing tumors was also observed between 32 and 34 weeks, and a significant ($p<0.05$) reduction in tumor volume was observed when compared with the carcinogen control group (Table 2). No tumors were observed in the normal (Group I) and Ge-132 (Group IV) controls at any time point.

Histological changes

The cellular architecture was found to be altered and enlargement of the alveolus was seen with cells showing nuclear pleomorphism, characterized by nuclear enlargement, clumping of chromatids, a typical epithelial hyperplasia was observed and epithelial cells showed variation in nuclear size with irregular chromatin and prominent nucleoli in carcinogen treated animals(Fig.1b). It was observed from histological examination that the Ge-132 treated group (Fig.1c) shows mild ductular proliferation with focal epithelial hyperplasia. Epithelial cells were uniform in size and anisonucleosis and mitosis were absent. Ge-132 group shows no observable distinct change from the control group (Fig.1a).

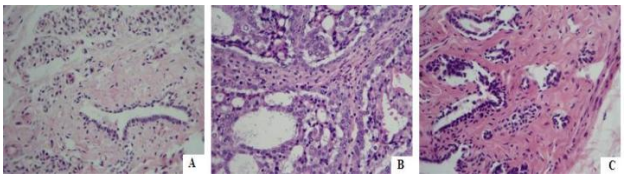

Figure 1: A. Mammary tissue showing normal architecture ; B. Mammary tissue of carcinogen control group at 34 wk post-NMU injection; C. Section of the NMU- treated mammary tissue showing more or less common architecture after continuous treatment of Ge-132 for 34wk (Hematoxylin and Eosin stain, 40 X magnification).

Table 1. Effect of Ge-132 on tumor size in NMU induced rat mammary carcinomas

Tumor size*	< 5mm				>5mm to <10mm				> 10mm			
Group I	-	-	-	-	-	-	-	-	-	-	-	-
Group II	1	1	2	2	20	27	23	18	5	5	10	18
Group III	-	3	3	6	-	11	10	9	-	2	3	3
Group IV	-	-	-	-	-	-	-	-	-	-	-	-

*Tumor sizes were recorded 19, 24, 29 and 34 weeks after NMU injection; the numbers in the table represent the total number of tumors per group

Table 2: Effect of Ge-132 on mean tumor volume in NMU induced rat mammary carcinomas

Group	Mean tumor volume		Reduction (%)
	25 wk	34 wk	
I	-	-	-
II	0.69	1.044	-
III	0.23	0.18	21.74
IV	-	-	-

Values represents mean; all measurements are in cubic millimeters

DISCUSSION

Animal models are particularly useful for the study of human mammary carcinogenesis. Since the rat mammary gland shows a high susceptibility to developing neoplasms which closely mimic human breast cancer[19]. In the present study, Ge-132 treatment exhibited potential anticancer activity on MNU-induced mammary tumors in rats. Histology and morphometry were taken as end-point biomarkers of preneoplasia. Histological observations clearly showed that MNU alone obviously damaged the normal architecture of rat mammary tissues. A typical epithelial hyperplasia with slight proliferation of mammary lobules, a preneoplastic condition, was observed. With Ge-132 treatment, the tissue architecture was almost normal with mild preneoplastic conditions proving that Ge-132 obtainable protected against MNU induced damage. Vanadium found to restore histological characteristics of mild ductular proliferation with focal epithelial hyperplasia in carcinogen induced rat mammary tumor model[20]. Present study showed a significant inhibition of mammary tumour size and volume in female Albino Wistar rats by Ge-132 treatment. Similarly selenium treated rat model reported a reduction in average tumour size and volume[21]. The efficiency of various drugs or compounds on inhibition or promotion of experimental cancer was evaluated in terms of number of observed tumours per animal and the rate of tumour development, which have their own limitations[22, 23].

This study may contribute to the regression of rat mammary tumors after Ge-132 treatment. It is anticipated that the comprehension of the mechanisms governing the programmed cell death will have a profound impact on the design of anticancer therapies.

ACKNOWLEDGEMENT

The authors are thankful to the authorities of Karpagam University, Coimbatore, and Tamilnadu for granting permission to use lab facility and for their encouragements.

REFERENCES

1. Cameron D, Bell R, Optimizing treatment for patients with breast cancer. Seminar in Oncol 2004; 31: 4-5.
2. Parkin DM, Pisani P, Ferlay J, Estimates of the worldwide incidence of 25 major cancers in 1990. Int J Cancer 1999; 80: 827-841.
3. Schultz LB, Weber BL. Recent advances in breast cancer biology. Curr Opin Oncol 1999; 11: 429-434.
4. Ali SM Harvey HA, Lipton A. Metastatic breast cancer: overview of treatment. Clin Orthop Relat Res 2003; 132–137.
5. Alexandrov VA, Bespalov VG. Preclinical and clinical study of cancer chemopreventive agents. St. Petersburg: Esculap 1997; 32 (in Russian).
6. Kelloff GJ. Perspectives on cancer chemoprevention research and drug development. Adv Cancer Res 2000; 78: 199-334.
7. Bradlow HL, Telang NT, Sepkovic DW, Osborne MP. Phytochemicals as modulators of cancer risk. Adv Exp Med Biol 1999; 472: 207-21.
8. Yun TK. Update from Asia. Asian studies on cancer chemopreven Chemoprevention. Bradlow HL, Fishman J, Osborne MP, editors, Cancer prevention. Novel nutrient and pharmaceutical developments. Ann NY Acad Sci 1999; 889: 157-92.
9. Rosenberg B, Van Camp L, Grimley EB, Thomson AJ. The inhibition of growth or cell division in Escherichia coli by different ionic species of platinum(IV) complexes. J Biol Chem 1967; 242: 1347–1352.
10. Boulikas T, Vougiouka M, Cisplatin and platinum drugs at the molecular level. Oncol Rep 2003; 10: 1663–1682.
11. Gottesman MM, Hall MD, Liang XJ, Shen DW (2009) Resistance to cisplatin results from multiple mechanisms in cancer cells. In A Bonetti, R Leone, FM Muggia, SB Howell, eds, Platinum and Other Heavy Metal Compounds in Cancer Chemotherapy—Molecular Mechanisms and Clinical Applications. Totowa: Humana Press Inc., pp 83–88.
12. Lu MS. Anti-cancer effect of tonic traditional Chinese medicine and organic germanium. Trace Elements Science 1998; 5: 57–59.
13. Hirayama C, Suzuki H, Ito M, Okumura M, Oda T. Propagermanium: a nonspecific immune modulator for chronic hepatitis B. J Gastroenterol 2003; 38: 525-532.
14. Mainwaring MG, Poor C, Zander DS, Harman E, Complete remission of pulmonary spindle cell carcinoma after treatment with oral germanium sesquioxide. Chest 2000; 117(2): 591–593.
15. Gusterson BA, Williams JC, N-nitrosomethylurea-induced rat mammary tumours as models of human breast cancer. J R Soc Med 1981; 74: 56-59.
16. Costa I, Solanas M, Escrich K, Histopathologic characterization of mammary neoplastic lesions induced with 7,12-dimethylbenz(a)anthracene in the rat. A comparative analysis with human breast tumor, Arch Pathol Lab Med 2002; 126: 915-927.
17. Thompson, H.J., 2000. Methods for the induction of mammary carcinogenesis in the rat using either 7,12-dimethylbenz[a]antracene or 1-methyl-1-nitrosourea. In: Ip, M., Asch, B.B. (Eds.), Methods in Mammary Gland Biology and Breast Cancer Research, eighth ed. Kluwer Academic/Plenum Publishers, New York, 19-29.
18. Wagner, Carston R, Ballato, George, Akanni, Abraham O, McIntee, Edward J, Larson, Richard S, Chang, Shu-ling, Abul-Hajj, Yusuf J, Potent Growth Inhibitory Activity of Zidovudine (AZT) on Cultured Human Breast Cancer Cells and Rat Mammary Tumors. Cancer Research 1997; 57: 2341-2345.
19. Flohe L, Otting F, Superoxide dismutase assays, Meth. Enzymol 1984; 105: 93.
20. Rajarshi Sankar Ray, Mitali Basu, Balaram Ghosh, Kartick Samanta, Malay Chatterjee, Vanadium, a Versatile Biochemical Effector in Chemical Rat Mammary Carcinogenesis. Nutrition and Cancer 2005; 51(2): 184–196.
21. Ghadi FE, Ghara AR, Bhattacharyya S, Dhawan DK, Selenium as a chemopreventive agent in experimentally induced colon carcinogenesis. World J Gastrointest Oncol 2009; 1(1): 74-81.
22. Kokoska, SM, Hardin JM, Grubbs CJ, Hsu C, The statistical analysis of cancer inhibition/promotion experiments. Anticancer Res 1993; 13: 1357-1363.
23. Dunson, BD and Dinse GE, Distinguishing effects on tumor multiplicity and growth rate in chemoprevention experiments. Biometrics 2000; 56: 1068-1075.