in vivo *21*: 973-978 (2007)

Four Cardiac Hormones Eliminate up to Two-Thirds of Human Breast Cancers in Athymic Mice

DAVID L. VESELY[1,3], BRIAN A. VESELY[1], EHRENTRAUD J. EICHELBAUM[1], YING SUN[1], ABDEL A. ALLI[2] and WILLIAM R. GOWER JR.[2,3]

[1]Departments of Internal Medicine, [2]Molecular Medicine and [3]Molecular Pharmacology and Physiology University of South Florida Cardiac Hormone Center, and James A. Haley Veterans Medical Center, Tampa, Florida U.S.A.

Abstract. *Background: Four cardiac hormones i.e. atrial natriuretic peptide (ANP), vessel dilator, long acting natriuretic peptide (LANP) and kaliuretic peptide have anticancer effects in vitro. Materials and Methods: These four cardiac hormones were infused subcutaneously for 28 days with weekly fresh hormones at 3 nM min^{-1} kg^{-1} body weight in athymic mice bearing human breast adenocarcinomas. Results: Vessel dilator, LANP, kaliuretic peptide and ANP eliminated 67%, 50%, 67% and 33% of the HTB-132 human breast adenocarcinomas. LANP eliminated 100% and vessel dilator 1/3 of CRL-2327 breast adenocarcinomas. There was no recurrence of the breast cancers in the primary site and no metastasis except in the ANP-treated group in one year post-treatment. The natriuretic peptide receptors-A and -C were decreased 50% and 31%, respectively, in metastatic versus primary ANP-treated breast adenocarcinomas. Conclusion: Four cardiac hormones eliminate up to two-thirds of human breast adenocarcinomas in athymic mice.*

It is estimated that in 2007 there will be 180,510 new cases of breast cancer and 40,910 deaths from breast cancer in the United States (1). Breast cancer is the second leading cause of death from cancer in women and the leading cause of death in women aged 40 to 55 in the United States (2). Breast cancer is the leading cause of cancer death in women worldwide (2). The number of new cases of breast cancer worldwide was estimated to be 1.05 million with 370,000 deaths in 2000 (2). Obviously there is a need for new therapies for breast cancer.

Correspondence to: David L. Vesely, MD, Ph.D., Professor of Medicine, Molecular Pharmacology and Physiology, Director, USF Cardiac Hormone Center, 13000 Bruce B. Downs Blvd. Tampa, FL 33612, U.S.A. Tel: +813 972 7624, Fax: +813 972 7623, e-mail: david.vesely@med.va.gov

Key Words: Breast cancer, natriuretic peptides, new therapeutics, receptors, metastasis.

The rational for the present study is that four cardiac hormones, *i.e.* atrial natriuretic peptide (ANP), vessel dilator, long acting natriuretic peptide (LANP) and kaliuretic peptide (each at 1 μM) decrease the number of human breast adenocarcinomas cells in cell culture and their DNA synthesis up to 85% (3). These four cardiac hormones are synthesized within the heart and stored in the atrial myocyte as a prohormone for rapid release in response to stimuli (4-6). One gene in the heart synthesizes a 126 amino acid (a.a.) prohormone which contains these four cardiac hormones (7). The present *in vivo* investigation was designed to determine whether these peptide hormones can eliminate human breast adenocarcinomas in athymic mice when infused subcutaneously for 28 days with the peptide hormone being refreshed weekly.

Materials and Methods

Human breast adenocarcinoma cells. Several cell lines of human breast adenocarcinoma cells were purchased from the American Type Culture Association (ATCC), Manassas, VA, USA. One breast adenocarcinoma cell line (ATCC number CRL-2327) was derived in 1995 from a 49-year-old Caucasian female who harboured a homozygous deletion in exon four of the fragile histidine triad (FHIT) gene (8). This is a slow growing breast cancer cell model. A second *in vivo* human breast cancer cell model incorporated a faster growing human breast adenocarcinoma, *i.e.* ATCC-HTB 132 (also called MDA-MB-468) from a 51-year-old lady that develops tumors in approximately 21 days in athymic mice (9).

Culture of breast adenocarcinoma cells for tumor formation in vivo. Propagation of CRL-2327 human breast adenocarcinoma cells was in Roswell Park Memorial Institute (RPMI) 1640 medium with 2 mM L^{-1}-glutamine adjusted with addition of 1·5 g L^{-1} sodium bicarbonate, 4·5 g L^{-1} glucose, 10 mM HEPES, 1 mM of 90% sodium pyruvate and heat-inactivated 10% fetal bovine serum (Sigma Chemical Company, St. Louis, MO, USA) at a temperature of 37°C as recommended by the ATCC. The HTB-132 human breast adenocarcinoma cells were propagated in Leibovitz's L-15 medium with 2 mM L-glutamine supplemented with 10% fetal bovine serum as suggested by the ATCC. The breast cancer cells were dispensed

into new flasks with subculturing every 6-8 days. The growth medium was changed every three days. A single cell suspension with a viability of >90% were used for the injections. Cell pellets were resuspended in saline prior to injections.

Animals. Human breast adenocarcinomas in athymic mice. Male and female homozygous (nu/nu) athymic mice 4 to 6 weeks old were purchased from National Cancer Institute (NCI). Animals were cared for under the "Guiding Principles for Research Involving Animals and Human Beings".

Twenty-gram mice were given subcutaneous injections of one million human breast adenocarcinoma cells in 250 μl of phosphate buffered saline, pH 7.4, on the left side of the back of the mice with ketamine 80 mg/kg and xylazine 10 mg/kg body weight intraperitonealy as anesthesia. Tumor growth was followed by electronic digital Vernier caliper measurements every day with tumor volume recorded daily (10). Tumor volume was calculated by the formula $V=(a \times b^2)/2$, where a=the largest superficial diameter and b=the smallest superficial diameter (10).

Research protocol. The injected HTB-132 breast adenocarcinoma cells coalesced into well-defined tumors of at least 1 mm x 1 mm (volume = 0.5 mm^3) in approximately 21 days. The CRL-2327 was a much slower growing human breast cancer *in vivo* and only five of 63 athymic mice (25 male and 38 female mice) developed breast cancers in 84 days of observation. This was a distinctly different model than the HTB-132 breast cancer model as a single breast cancer lesion formed in the HTB-132 animals while in the CRL-2327 animal model many of the animals developed 5 to 9 lesions simultaneously. Osmotic pumps (Alzet Model 1007D, Duret Corporation, Cupertino, CA, USA) containing either 0.9% saline (control infusion) or one of the respective hormones in 0.9% saline were implanted subcutaneously under anesthesia in the upper back of the athymic mice after three independent investigators confirmed that a tumor was present. All of the peptide hormones for these experiments were synthesized by Phoenix Pharmaceuticals Inc., Belmont, CA, USA. The Alzet Model 1007D osmotic pump for mice delivers all of its contents (100 μl) over 7 days at a rate of 0.5 μl/h and then stops pumping. A 1 mM concentration of these cardiac hormones *in vitro* eliminates up to 97% of cancer cells *in vitro* within 24 hours (11, 12). When a 1 mM concentration in the osmotic pump is infused over seven days the concentration per kg body weight/minute is 3 nM/kg/body weight/min or 99 nM/30 gram mouse/minute, which is the concentration utilized in the present investigation. This research protocol was approved by the Institutional Animal Care and Use Committee of the University of South Florida and the James A. Haley Veterans Medical Center and followed the "Guiding Principles for Research Involving Animals and Human Beings".

Natriuretic peptide receptors (NPR)-A and -C in primary versus metastatic breast adenocarcinomas. When two of the human breast adenocarcinomas in the ANP-treated group become metastatic in the present investigation, we hypothesized that the metastatic lesions may have mutated losing their NPR-A and/or -C receptors in order that they could no longer respond to ANP similar to breast cancers which lose their estrogen and/or progesterone receptors being more prone to metastasize. NPR-A and -C receptors were evaluated by Western blotting with the following modifications for evaluation of solid tumors *versus* evaluation of individual cancer cells.

Western blot analysis of primary and malignant human breast adenocarcinomas. 50-100 mg pieces of human breast adenocarcinomas were homogenized and then 50 μg of protein from the pellet and supernatant fractions were loaded onto separate lanes of a Criterion Precast 7.5% Tris-HCl gel (Bio-Rad; Hercules, CA, USA). The proteins were separated by SDS-PAGE (25 volts for 8 hours) and then electrically transferred onto a nitrocellulose membrane (Hybond-C Extra, Amersham Biosciences Corporation, Piscataway, NJ, USA) for 75 minutes at 100 volts in Towbin buffer followed by Ponceau S staining to verify transfer, as previously described from our laboratory (11). The membranes were incubated overnight at 4˚C with gentle rocking and with a 1:4000 dilution of R1214 polyclonal antibody directed against the COOH terminus of the NPR-A receptor (generously provided by Dr. David L. Garbers, University of Texas Southwestern, Dallas, TX, USA) or with a 1:1000 dilution of Omori polyclonal antibody to the NPR-C receptor (kindly provided by Dr. Kenji Omori, Osaka, Japan). The blot was washed four times with 1X TBS and then incubated for 1 hour at room temperature with moderate shaking in a solution of 5% nonfat dry milk goat anti-rabbit horseradish peroxidase (HRP)-conjugated secondary antibody (Bio-Rad, Hercules, CA, USA) at a dilution of 1:7000 for NPR-A or 1:3000 for NPR-C. The immunoreactive bands were then detected by using the Super Signal West Pico Chemiluminescent Substrate (Pierce; Rockford, IL, USA) according to instructions by the manufacture. Precision plus protein dual color standards were used to identify bands corresponding to the NPR-A and NPR-C receptors.

Densitometric analysis of immunogenic bands. Densitometry of immunogenic bands was performed using the ImageQuant software version 5.2 (Molecular Dynamics, Sunnyvale, CA, USA). The abundance of NPR-A and NPR-C protein in the metastatic tumor was determined relative to that of primary tumor in a ANP-treated breast carcinoma which had a metastatic lesion large enough to evaluate. The metastatic lesion in the second ANP-treated animal was so small that there was not enough tissue to do receptor analysis.

Statistical analysis. Data are expressed as means±SEM and evaluated using analysis of variance (ANOVA) with repeated measures design for within-group comparisons. A $p<0.05$ was considered the criteria to be statistically significant.

Results

The four cardiac hormones as a group eliminated the human HTB-132 breast adenocarcinomas growing in 54% of the athymic mice and in 60% of the CRL-2327 breast adenocarcinomas bearing mice. Thus, each of the four cardiac peptide hormones had the ability to completely eliminate some of the human breast adenocarcinomas growing *in vivo*. In the present investigation, vessel dilator and kaliuretic peptide had the most consistent anticancer effects in female mice harboring HTB-132 human breast adenocarcinomas, each eliminating the primary human breast adenocarcinomas in 67% (*i.e.* two out of three breast cancers) (Figure 1). LANP caused 50% of HTB-132 human breast adenocarcinomas to disappear while ANP caused 1/3 of the human HTB-132 breast adenocarcinomas to be eliminated (Figure 1). There

was not a single incidence of a breast adenocarcinoma recurring in the primary site when followed for one year post-treatment when the HTB-132 breast cancer was eliminated by any one of these cardiac hormones. In the control animals that were not treated with any of the cardiac hormones (n=8) there was an approximate doubling in the size of the human breast carcinoma every two weeks. There was, thus, a dramatic difference in the control groups and the treated groups where up to two-thirds of the human breast cancers were completely eliminated by two of the cardiac hormones. Two of the ANP-treated (*e.g.* numbers 3 and 4 of Table I) HTB-132 breast cancers each developed a metastatic lesion. None of the three other treated groups developed a metastatic lesion in the one year of post-treatment follow-up.

The CRL-2327 human breast cancer was found to be a much slower growing human breast cancer model with all 38 female athymic mice not developing a single human breast cancer when followed for 84 days as opposed to development within 21 days of HTB-132 human breast cancers in athymic mice. In male mice, 5 of the 25 mice developed CRL-2327 human breast cancers which occurred at 51 to 62 days. The CRL-2327 breast cancer, thus, is much slower growing with the majority of HTB-132 human breast cancers developing in 21 days. The CRL-2327 is, thus, a more difficult cell line of human breast cancer to establish *in vivo* as only 5 of 63 athymic mice (male and female) developed human breast cancers after getting an identical one million breast cancer cells as the HTB-132 breast cancer mice. To our knowledge this is the first time that CRL-2327 human breast cancers have been shown to develop in an *in vivo* model. The CRL-2327 breast cancer model is further differentiated by forming multiple breast cancer lesions as opposed to only one lesion in the HTB-132 breast cancer model.

Of the five male athymic mice with CRL-2327 human breast cancers, two were treated with LANP and after three weeks their breast cancer lesions disappeared (Table II). These two animals had five and nine breast cancer lesions, respectively, before being treated with LANP (Table II). The other three male mice which developed human breast cancers were treated with vessel dilator. After three weeks, one animal (#1 of Table II), which had five breast cancer lesions, had all five disappear and none of these lesions ever recurred. A second vessel dilator-treated male animal (#3 of Table II) had the CRL-2327 breast cancer decrease in size during the first weeks of treatment, but after the infusion was completed, increased in size and never regressed in follow-up. The third vessel dilator-treated male animal with CRL-2327 breast cancer had a large tumor (165 mm^3) before treatment that decreased 37% in volume during the first week of treatment (#2 of Table II) and was less than 1/3 of its original volume one week after the four-week vessel dilator treatment. This 2/3 decrease in tumor volume slowly increased but in another month its volume was still 45% less than its original tumor volume at which time the animal was euthanized. Autopsy at this time revealed no metastasis.

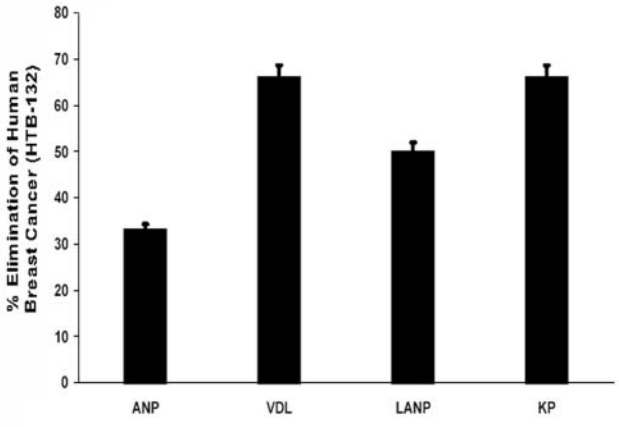

Figure 1. *Elimination of human breast adenocarcinomas with cardiac hormones. The graph for each peptide hormones indicates the percent of complete elimination of the HTB-132 human breast adenocarcinomas in female athymic mice. Atrial natriuretic peptide = ANP, long acting natriuretic peptide = LANP; vessel dilator = VDL; and kaliuretic peptide = KP. The elimination of human breast cancer with vessel dilator, kaliuretic peptide and LANP were significant at $p<0.0001$ while ANP was significant at $p<0.01$ when evaluated by analysis of variance (ANOVA) with repeated measures design for within group comparisons.*

NPR-A and -C receptors in primary and metastatic breast cancers. The reasoning for the hypothesis to study the NPR receptors in the primary and metastatic cancer tissues is based upon the knowledge that the more aggressive breast cancers are the ones that have lost their estrogen/progesterone receptors and with respect to the human breast cancers, our hypothesis is that the more aggressive human breast adenocarcinomas may have also lost part of their receptors for ANP in the metastatic lesions to allow them not to respond to ANP. When the primary and metastatic lesions were examined by Western blots, the NPR-A and -C receptors were demonstrated to be present in the primary lesion but the NPR-A or active receptor was markedly reduced (50% less) and the NPR-C receptor was 31% less in the metastatic breast cancer compared to primary lesion (Figure 2).

Side effects. There was no evidence of any side effects with the peptide hormones *in vivo*. The animals were monitored daily for any evidence of side effects and none of the animals had seizures, hypotension, nausea, vomiting or any signs of pain or distress which in mice may be evidenced by immobility and silence, withdrawal, reduced grooming, hunched up posture, or reduced food and water intake. None of these occurred in the treated animals, which were lively and social. Physiological variables such as reduced depth of respiration, increased heart rate or reduced hydration status did not occur with the cardiac hormone treatments.

Table I. *Cardiac hormones slow the growth and/or eliminate human HTB-132 breast adenocarcinomas in vivo.*

Volume (mm^3) of tumor	Weeks												
	Treatment												
	0	1	2	3	4	5	6	7	8	9	10	11	12
Vessel dilator													
1.	6.7	30.1	16.8	6.8	0	0	0	0	0	0	0	0	0
2.	11.9	9.7	23.6	21.3	0	0	0	0	0	0	0	0	0
3.	11.9	15.3	14.2	13.8	0	0	0	0	0	0	0	0	0
4.	11.1	8.8	16.5	0	0	0	0	0	0	0	0	0	0
5.	42.6	131.9	109.3	209.3	192.9	179.7	225.6	210.1	180.7	328.8	371.8	485.7	928.7*
6.	7.4	75.2	37.6	33.5	40.3	75.3	108.5	157.2	159.3	186.6	266	269	400.7*+
Kaliuretic peptide													
1.	10.6	5.6	0	0	0	0	0	0	0	0	0	0	0
2.	8.8	6.6	0	0	0	0	0	0	0	0	0	0	0
3.	4.7	10.9	18.6	0	0	0	0	0	0	0	0	0	0
4.	8.8	14.9	0	0	0	0	0	0	0	0	0	0	0
5.	14.0	10.4	20.9	41.2	38.4	62.8	70.3	72.4	114.7	108	290	245	592.7*
6.	7.8	4.4	29.7	28.8	32.5	30.6	20.7	27.4	43.5	53.6	55.9	55.9	70.7++
LANP													
1.	64	8.1	13.5	27.9	13.2	0	0	0	0	0	0	0	0
2.	23.5	8.1	0	0	0	0	0	0	0	0	0	0	0
3.	9.3	9.8	8.4	9.9	40.9	25.9	28.1	61	38.7	20.2	0	0	0
4.	10.0	13.5	8.9	11.1	11.5	11.8	7.7	6.9	10.3	5.5	10.5	0	0
5.	20	18.3	18	22.4	26.6	59.5	61.8	108.5	115.2	181.2	230.0	237*	
6.	6.2	12.3	6.7	11	17.5	26.4	27.2	35.7	50.6	116.3	171.5	487.1*+++	
ANP													
1.	7.7	3.4	0	0	6.5	0	0	0	0	0	0	0	0
2.	15.3	10.4	13.4	27.4	15.5	60.2	47.1	57.3	49.6	85.0	62.5	36.7	0+++
3.	22.2	3	43.7	23.1	36.8	42	74.4	137.3	137.3	237.2	267.7	291.9*Δ	–
4.	10.4	66	9.1	16.5	18.2	22.6	20.2	42.0	158.6	117.4	103.4	102.7*Δ	–
5.	7.4	13.1	34.7	45.5	73.6	119.7	216	196.9	287.9	235.5	334	363.8*	–
6.	4.9	12.2	30.8	55.2	91.6	122.5	161.7	219.6*	–	–	–	–	

ANP = Atrial natriuretic peptide. LANP = long acting natriuretic peptide. Weeks: Beginning with start of infusion for four weeks followed by eight weeks post-infusion. "0" weeks represents the baseline size of the tumor prior to infusion of the respective peptide hormones. *Mouse euthanized and autopsy performed. +=15 weeks, euthanized. ++=16 weeks, tumor growth stabilized. +++=15 weeks euthanized. ++++=18 weeks post-treatment breast cancer completely disappeared and never returned; Δ=metastatic lesion. 0 volume=tumor no longer present. –=no data, mouse euthanized.

Discussion

This is the first investigation demonstrating that cardiac hormones can completely eliminate (*i.e.* cure) human breast cancers growing *in vivo*. Human breast adenocarcinomas were chosen for this investigation since there are 370,000 deaths/year globally secondary to breast cancer even with current surgery and current chemotherapy (2). In this regard it is important to note that the cures of the human breast cancers in the present investigation were without surgery to remove the breast cancer before treatment with the four different cardiac hormones. The cardiac hormones themselves, *i.e.* without any other treatment, caused the HTB-132 human breast cancers to disappear and never recur in over 50% of the female mice carrying these breast cancers. With vessel dilator and with kaliuretic peptide two out of every three human breast cancers were completely eliminated.

In none of the animals where the breast cancer was eliminated did the cancer ever recur in the primary site or have a metastasis in the vessel dilator, long acting natriuretic peptide or kaliuretic peptide-treated groups. With LANP 1/2 and with ANP 1/3 of female mice with human breast cancer had complete cures. None of the primary human adenocarcinomas that were eliminated recurred in the primary site in any of the four peptide hormone treatment groups. In the male animals with CRL-2327 human breast carcinomas, it was the larger lesions that did not respond as well in this small group that developed breast cancers but 60% of the breast cancers were eliminated in the male animals as well. This knowledge that the larger breast cancers may not respond as well to these peptide hormones would suggest that once a breast cancer is

Table II. *Cardiac hormones elimination of CRL-2327 human breast adenocarcinomas in vivo in athymic mice.*

Volume (mm^3) of tumor	Weeks												
	Treatment												
	0	1	2	3	4	5	6	7	8	9	10	11	12
LANP													
1.	17.7	7.7	3.9	0	0	0	0	0	0	0	0	0	0
2.	15.9	3.9	2	0	0	0	0	0	0	0	0	0	0
Vesssel dilator													
1.	3.1	3.6	0.6	0	0	0	0	0	0	0	0	0	0
2.	165.4	4.4	94.1	117	108	72.5	97.5	100.9	90.9	–	–	–	–
3.	9.1	2.6	0.8	2.8	29.3	131.7	156.6	–	–	–	–	–	–

LANP = Long acting natriuretic peptide. Weeks: Beginning with start of infusion for four weeks followed by eight weeks post infusion. "O" weeks represents the baseline size of the tumor prior to infusion of the respective peptide hormones. – = mouse euthanized. "0" volume in table=tumor no longer present.

removed surgically and/or debulked for very large breast cancers, these peptide hormones may have benefit as adjunct agents if given when the tumor load becomes small. It is further important to note that in the male mice the majority developed five to nine lesions simultaneously and all of these lesions disappeared in the animals which responded to cardiac hormones, which is encouraging.

To put this one year cancer-free post-treatment in perspective, the lifespan of athymic mice is approximately one year (source: Harlan, Indianapolis, IN, USA). The mice in the present investigation were evaluated for the normal lifespan of healthy cancer-free athymic mice. Thus, they were one month old when they arrived from the NCI, took one month to develop the human breast adenocarcinomas, were treated for one month and had a one year post-treatment follow up. The mice did not die of breast cancer but rather died of old age with no cancer on autopsy in the animals that had the human breast carcinomas eliminated by these cardiac hormones.

Two of the ANP-treated animals which did not have the primary lesion disappear, developed a metastatic lesion (one lesion in each animal). Western blots of the NPR-A and -C receptor in the present investigation revealed that the metastatic lesion had less NPR-A receptor (50% less) and NPR-C (31% less) as a possible cause as how metastatic lesions grow, *i.e.*, they have lost a significant amount of the receptor(s) that allows the cancer to respond to ANP. Only the ANP-treated breast cancers had metastatic lesions, *i.e.* in none of the other three cardiac hormone treated groups had any metastatic lesions develop. This information that metastatic breast cancer lesions have less NPR-A receptors than the primary lesion suggests that measuring NPR-A receptor on human breast cancers surgically removed might help as a further guide in addition to estrogen/progesterone receptors as to which tumors will grow more aggressively

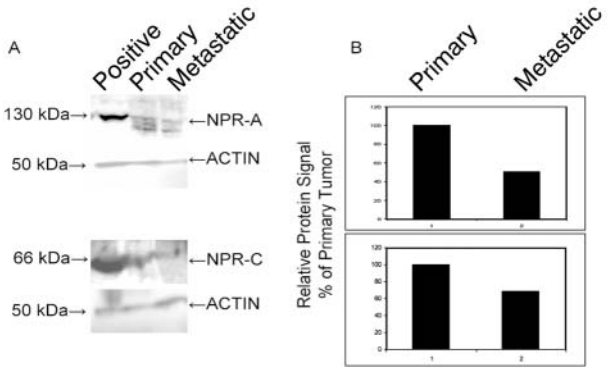

Figure 2. *Western blot analysis of NPR-A (top panel) and NPR-C (lower panel) of human breast adenocarcinoma primary tumor and metastatic lesion in ANP-treated athymic mouse which developed a metastatic lesion. Western blot analysis (50 μg protein/lane) revealed that relative to the primary tumor, the total amount of NPR-A receptor was 50% less ($p<0.01$) in the metastatic lesion and the amount of NPR-C receptor was 31% less in the metastatic lesion compared to the primary breast adenocarcinoma when analyzed by repeated measures of analysis of variance (ANOVA). Band intensities were assessed by densitometry and the results were expressed as percentages of primary tumor values. The positive control for NPR-A receptor was human lung and for NPR-C receptor was rat gastric mucosa.*

and which ones will respond to ANP as an adjunct therapy. The loss of the NPR-A (or active) receptor helps to explain why the metastatic lesions treated with ANP were not eliminated. Loss and/or a marked decrease of the NPR-A receptor by the metastatic lesion would cause metastatic lesions not to respond to and/or have a decreased response to ANP. ANP works via the NPR-A receptor while vessel dilator and long acting natriuretic peptide have receptors distinct from the NPR-A receptor (13-15). Loss of the NPR-A receptor by the metastatic lesion would cause metastatic

lesions not to respond to ANP but loss of this receptor would still allow the metastatic lesion to respond to vessel dilator and long acting natriuretic peptide which as above, have their own distinct receptors (13-15).

Cardiac hormone receptors on human breast adenocarcinomas, as shown in the present investigation, are involved in the elimination of breast cancer. This is the first demonstration that breast adenocarcinomas growing *in vivo* have receptors which mediate ANP's effects. After binding to their specific receptors, the anticancer mechanism(s) of action of these four cardiac hormones involves inhibition (up to 98%) of the activation (*i.e.* phosphorylation) of extracellular-signal regulated kinases (ERK) 1/2, important for the growth of cancers (16, 17). They also inhibit up to 97% of MEK 1/2 kinase, the upstream kinases in the RAS/RAF-MEK 1/2,-ERK 1/2 kinase cascade of causing cancer (18, 19). The final step in the RAS/RAF-MEK 1/2-ERK 1/2's cancer promoting effects is stimulation of DNA synthesis within the nucleus of the cancer cell and these four cardiac hormones strongly inhibit DNA synthesis within the human CRL-2327 breast cancer cells of the present investigation (3). All four of these peptide hormones localize to nucleus of cancer cells where they can directly inhibit DNA synthesis as well as localize to the cytoplasm of cancer cells where they cause a strong inhibition of MEK 1/2-ERK 1/2 kinases (20).

Acknowledgements

This investigation dedicated to Clo Vesely, wife of the first author and mother of the second author, who died of breast cancer. We thank Charlene Pennington for excellent secretarial assistance, and Liesl Wiesen, Shelby Peel and John Soto for excellent care of the mice. This work has been supported in part by Merit Awards from the United States Department of Veterans Affairs (DLV, WRG).

References

1 Jemel A, Siegel R, Murray T, Xu J and Thun MJ: Cancer Statistics, 2007. CA Cancer J Clin *55*: 43-66, 2007.

2 Parkin DM: Global cancer statistics in the year 2000. Lancet Oncol *2*: 533-543, 2001.

3 Vesely BA, Song S, Sanchez-Ramos J, Fitz SR, Solivan SR, Gower WR Jr and Vesely DL: Four peptide hormones decrease the number of human breast adenocarcinoma cells. Eur J Clin Invest *35*: 60-69, 2005.

4 Brenner BM, Ballermann BJ, Gunning ME and Zeidel ML: Diverse biological actions of atrial natriuretic peptide. Physiol Rrev *70*: 665-699, 1990.

5 Vesely DL: Natriuretic peptides and acute renal failure. Am J Physiol *285*: F167-F177, 2003.

6 Vesely DL: Natriuretic hormones. *In*: The Kidney: Physiology and Pathophysiology, 4th edition. Alpern RJ and Herbert SC (eds.). Elsevier Inc, pp. 947-977, 2007.

7 Vesely DL: Atrial natriuretic peptide prohormone gene expression: Hormones and diseases that upregulate its expression. IUBMB Life *53*: 153-159, 2002.

8 Ahmadian M, Wistuba II, Fong KM, Behrens C, Kodagoda DR, Saboorian MH, Shay J, Tomlinson GE, Blum J, Minna JD and Gazdar AF: Analysis of the FHIT gene and FRA3B region in sporadic breast cancer, preneoplastic lesions, and familial breast cancer probands. Cancer Res *57*: 3664-3668, 1977.

9 Pathak S, Siciliano MJ, Cailleau R, Wiseman CL and Hsu TC: A human breast adenocarcinoma with chromosome and isoenzyme markers similar to those of the HeLa line. J Natl Cancer Inst *62*: 263-271, 1979.

10 Duguay D, Mercier F, Stagg J, Martineau D, Bramson J, Servant M, Lin R, Galipeau J and Hiscott J: *In vivo* interferon regulatory factor 3-tumor suppressor activity in B16 melanoma tumors. Cancer Res *62*: 5148-5152, 2002.

11 Vesely BA, Alli AA, Song S, Gower WR Jr, Sanchez-Ramos J and Vesely DL: Four peptide hormones specific decrease (up to 97%) of human prostate carcinoma cells. Eur J Clin Invest *35*: 700-710, 2005.

12 Gower WR Jr, Vesely BA, Alli AA and Vesely DL: Four peptides decrease human colon adenocarcinoma cell number and DNA synthesis via guanosine 3'5'-cyclic monophosphate. Int J Gastrointest Cancer *36*: 77-88, 2005.

13 Vesely DL, Cornett LE, McCleod SL, Nash AA and Norris JS: Specific binding sites for prohormone atrial natriuretic peptides 1-30, 31-67, and 99-126. Peptides *11*: 193-197, 1990.

14 Vesely DL, Arnold WC, Winters CJ, Sallman AL and Rico DM: Increased circulating concentration of the N-terminus of the atrial natriuretic prohormone in persons with pheochromocytomas. J Clin Endocrinol Metab *71*: 1138-1146, 1990.

15 Vesely DL, Sallman AL and Bayliss JM: Specific binding sites for pro atrial natriuretic factors 1-30, 31-67, and 99-126 on distal nephrons, proximal tubules, renal cortical and medullary membranes. Renal Physiol Biochem *15*: 23-32, 1992.

16 Sun Y, Eichelbaum EJ, Wang H and Vesely DL: Vessel dilator and kaliuretic peptide inhibit activation of ERK 1/2 in human prostate cancer cells. Anticancer Res *26*: 3217-3222, 2006.

17 Sun Y, Eichelbaum EJ, Wang H and Vesely DL: Atrial natriuretic peptide and long acting natriuretic peptide inhibit ERK 1/2 in prostate cancer cells. Anticancer Res *26*: 4143-4418, 2006.

18 Sun Y, Eichelbaum EJ, Wang H and Vesely DL: Vessel dilator and kaliuretic peptide inhibit MEK 1/2 activation in human prostate cancer cells. Anticancer Res *27*: 1387-1392, 2007.

19 Sun Y, Eichelbaum DJ, Wang H and Vesely DL: Atrial natriuretic peptide and long acting natriuretic peptide inhibit MEK 1/2 activation in human prostate cancer cells. Anticancer Res *27*: 3813-3818, 2007.

20 Saba SR, Garces AH, Clark LC, Soto J, Gower WR Jr and Vesely DL: Immunocytochemical localization of atrial natriuretic peptide, vessel dilator, long acting natriuretic peptide, and kaliuretic peptide in human pancreatic adenocarcinomas. J Histochem Cytochem *53*: 989-995, 2005.

Received August 23, 2007
Accepted September 20, 2007