

Review

Heart Peptide Hormones: Adjunct and Primary Treatments of Cancer

DAVID L. VESELY

*Departments of Medicine, Molecular Pharmacology and Physiology,
University of South Florida Morsani School of Medicine, Tampa, FL, U.S.A.*

Abstract. Four heart hormones, namely atrial natriuretic peptide (ANP), long-acting natriuretic peptide (LANP), vessel dilator and kaliuretic peptide reduce up to 97% of cancer cells *in vitro*. These four cardiac hormones eliminate up to 80% of human pancreatic adenocarcinomas, two-thirds of human breast carcinomas and up to 86% of human small-cell lung carcinomas growing in athymic mice. ANP given intravenously for 3 hours after 'curative' lung surgery as an adjunct to surgery results in a 2-year relapse-free survival of 91% compared to 75% for those treated with surgery alone. The anticancer mechanisms of action of these peptides involve binding to receptors on the cancer cells, followed by 95% inhibition of the conversion of inactive to active rat sarcoma-bound guanosine triphosphate (RAS)-mitogen-activated protein kinase (MAPK) kinases 1/2 (MEK 1/2) (98% inhibition)-extracellular signal-related kinases 1/2 (ERK1/2) (96% inhibition) cascade in cancer cells. They are dual inhibitors of vascular endothelial growth factor (VEGF) and its VEGF2 receptor (up to 89%). They also inhibit MAPK9, i.e. c-JUN-N-terminal kinase 2. One of the downstream targets of VEGF is β -catenin, which these peptides inhibit by up to 88%. These four peptide hormones inhibit the Wingless-related integration site (WNT) pathway 68% and WNT secreted-Frizzled protein is reduced by up to 84%. Signal transducer and activator of transcription 3 (STAT3), a final 'switch' that activates gene expression that leads to malignancy, is specifically reduced up

to 88% by these peptides but they do not affect STAT1. There is crosstalk between the RAS-MEK 1/2-ERK 1/2 kinase cascade, VEGF, β -catenin, JNK, WNT, and STAT pathways and each of these pathways and their crosstalk is inhibited by these peptide hormones. They enter the nucleus of cancer cells where they inhibit the proto-oncogenes c-FOS (by up to 82%) and c-JUN (by up to 61%). Conclusion: These multiple kinase inhibitors have both adjunct and primary anticancer effects.

The amino acid sequences of atrial natriuretic peptide (ANP) and the ANP prohormone from which it is derived were determined in 1984 (1-8). ANP has anticancer effects both *in vitro* (9-20) and *in vivo* (21, 22). ANP eliminates 80% of human pancreatic carcinomas (21) and 43% of human small cell lung carcinomas growing in mice (22). Recently ANP has been used for 3 hours intravenously as an adjunct after 'curative' lung cancer surgery, which resulted in a 2-year relapse-free survival of 91% (77 patients) *versus* 75% ($p=0.018$) in 390 patients treated with surgery alone (23). When peer-matched patients (77 each) were analyzed by propensity score matching, the 2-year relapse-free survival was 91% for those treated with ANP *versus* 67% for those treated with surgery alone ($p=0.0013$) (23). This study would suggest that adding ANP after surgery as an adjunct may be helpful in preventing recurrence after surgery (23). The present review focuses on the mechanism of how ANP and other cardiac peptide hormones eliminate primary carcinomas *in vivo* and metastatic lesions (21, 22).

This article is freely accessible online.

Correspondence to: David L. Vesely, Departments of Medicine, Molecular Pharmacology and Physiology, University of South Florida Morsani School of Medicine, Tampa, FL, U.S.A. E-mail: david.tampabay@gmail.com.

Key Words: Adjunct therapy, primary therapy, new therapeutics, peptides, mechanisms of action, metastasis, method of treatment, review.

ANP

ANP prohormone can produce four hormones, one of which is ANP. The ANP prohormone gene encoding the synthesis of the 126 amino acid ANP prohormone consists of three exons sequences, separated by two introns, which encode for a mature mRNA transcript approximately 900 bases long (3, 24-27). Translation of the human ANP prohormone mRNA results

in a 151 amino acid pre-prohormone (3, 24-26). A signal peptide on the *N*-terminus of the pre-prohormone is cleaved from the pre-prohormone in the endoplasmic reticulum resulting in a 126 amino acid prohormone, which is the storage form of four hormones from this prohormone and they are the major constituents of the atrial granules found in the heart (24-26). The ANP prohormone is cleaved into four peptide hormones upon release from the heart (28, 29). These four peptide hormones consist of the first 30 amino acids of the prohormone's *N*-terminal end (*i.e.* amino acids 1-30) named long-acting natriuretic peptide (LANP), amino acids 31-67 of the ANP prohormone produce vessel dilator, amino acids 79-98 produce kaliuretic peptide and amino acids 99-126 produce ANP (27, 28). These peptides were named for their most potent known biological effects at the time of naming (28). Each of these four peptide hormones has anticancer effects *in vitro* (9-20) and *in vivo*, where they eliminate up to 86% of human small lung carcinomas in mice (21, 22).

Metastatic Lesions

Nojiri *et al.* reported that mice pretreated with ANP exhibited dramatic reduction in lipopolysaccharide (which mimics surgical stress)-induced pulmonary metastasis of introduced cancer cells, suggesting that ANP can prevent tumor metastasis in mice (23). This notion is supported by the authors' finding that mice which overexpress the receptor for ANP in vascular endothelium have reduced metastasis (23). This is consistent with the fact that ANP (and the three other peptide hormones derived from the ANP prohormone) inhibit vascular endothelial growth factor (VEGF) and the VEGFR2 receptor (by up to 87%) (30) that cause vascular endothelium to grow into cancer (31-33). Inhibiting VEGF and the VEGFR2 receptor would reduce the number of metastases as they would outgrow the blood supply bringing oxygen and nutrients to the metastasis (30). In addition to helping to prevent metastatic lesions from forming, metastatic lesions that are already present can be dramatically reduced and even eliminated by ANP and the other cardiac peptide hormones when used either alone or in combination in a sequential manner (21, 22).

Other Anticancer Peptides

In addition to the four anticancer peptides synthesized from the ANP prohormone within the heart (27-29), there are other peptides with anticancer effects (27-29). Thus, C-natriuretic peptide (CNP) also synthesized in the heart has anticancer effects but only at concentrations 100-fold higher than the four peptide hormones derived from the ANP prohormone (13, 19). On exposure to 100 μ M CNP for 24 hours, there was a 10% ($p=0.04$) decrease in human renal carcinoma cells (13). Brain natriuretic peptide (BNP) originally found in porcine brain, but misnamed as 50-fold more BNP is

found in the heart, has no significant anticancer effects at any studied concentration (11-13, 16, 19).

In the kidney, as opposed to all other tissues, differential processing of ANP pro hormone occurs, where instead of cleaving the prohormone between amino acids 98 and 99 to form ANP and kaliuretic peptide, it is cleaved between amino acids 95 and 96 (29, 34-36). This results in four amino acids from the *C*-terminal of kaliuretic peptide (*i.e.* threonine-alanine-proline-arginine) being attached to the *N*-terminal of ANP, with the resulting peptide being called urodilatin (29, 34-36). It is important to note that the amino acids in urodilatin are identical to the four *C*-terminal amino acids of kaliuretic peptide and identical to all of the amino acids in ANP (29, 34-36). Urodilatin reduces the number of renal carcinoma cells by 66% at 100 μ M, while ANP and kaliuretic peptide at the same concentration eliminate 70% and 74% of renal carcinoma cells in 24 hours (13). Similar findings have been found for the effects of urodilatin on small cell-lung cancer cells (22).

Dendroaspis augusticeps peptide (DNP) found in the venom of the green mamba snake, *Dendroaspis augusticeps*, has similar amino acids to ANP (37) and also has anticancer effects (18). DNP has anticancer effects on human glioblastoma cells but the four peptide hormones derived from the ANP prohormone eliminate 4-fold more glioblastoma cells (18).

Mechanism of Action of Cardiac Hormones in Cancer Cells

The mechanism of action of cardiac peptide hormones anticancer effects have been reviewed in detail previously (38) so a brief summary of their effects will be presented in this review.

Receptors. Human cancer cells have natriuretic peptide receptors (NPR) A and C to mediate the effects ANP in cancer cells (Figure 1) (10-12, 19). Western blot analysis has confirmed that both the NPR A and C receptors are present on human cancer cells (19). Metastatic pancreatic adenocarcinomas are adapted to treatment with ANP by reducing the NPR A receptor by 33% in abdominal metastases and 55% in liver metastases compared with the number of receptors in the primary pancreatic adenocarcinoma; thereby reducing the ability of ANP to eliminate metastatic lesions (21). Thus, metastatic lesions lose a significant amount of the receptor that allows ANP to have anticancer effects (21). Such metastatic lesions, however, can be eliminated by utilizing one of the other cardiac peptide hormones (vessel dilator, LANP or kaliuretic peptide) whose effects are not mediated *via* the NPR A receptor, which binds only ring structured peptides such as ANP; the linear peptide hormones (without a ring structure) have their own receptors to mediate their anticancer effects (39-41).

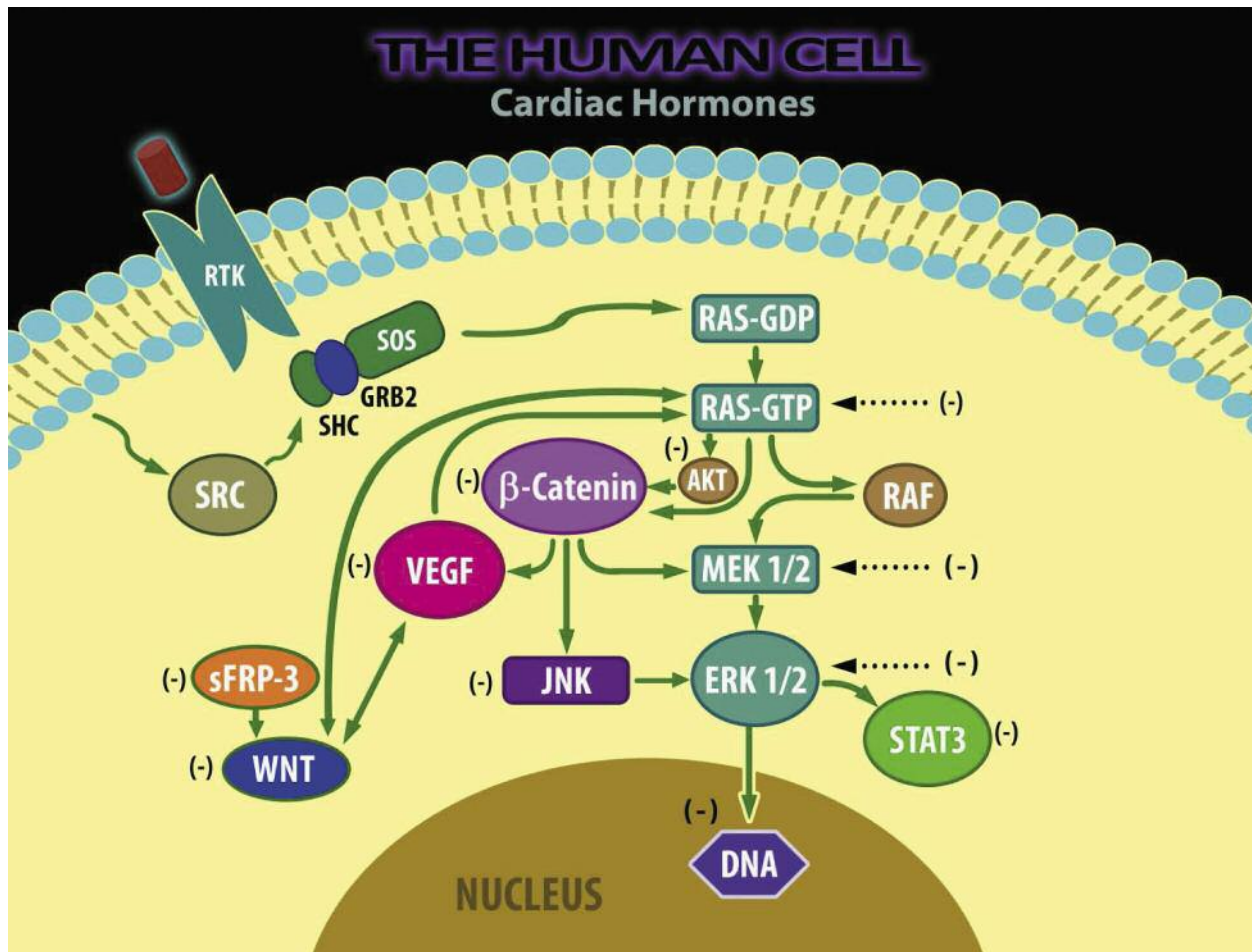


Figure 1. Heart hormones inhibit cellular oncogenes *c-FOS* (up to 82%) and *c-JUN* (up to 65%) and inhibit 95% of the conversion of rat sarcoma-bound guanosine diphosphate (RAS-GDP) to rat sarcoma-bound guanosine triphosphate (RAS-GTP). They inhibit each step of the RAS-mitogen protein kinase kinase (MEK1/2; 98% inhibition of phosphorylation) and extracellular signal-regulated kinases 1/2 (ERK1/2; 96% inhibition of phosphorylation) pathways. Other targets which the cardiac hormones inhibit in cancer cells are β -catenin, *c-JUN*-N-terminal kinase 2 (JNK), secreted frizzled-related protein 3 (sFRP3), vascular endothelial growth factor (VEGF) and the VEGFR2 receptor, Wingless-related integration site (WNT) pathway and the signal transducer and activator of transcription 3 (STAT3). As indicated by (-), the cardiac hormones inhibit several steps in the feedback loop that stimulate each other (kinases) and the nuclear oncogenes *c-FOS* and *c-JUN*, interrupting the vicious cycle of cancer growth. RKT: Tyrosine kinase receptor; SRC: rous sarcoma viral proto-oncogene tyrosine kinase; SHC: rous sarcoma SH2 C-terminal-binding domain adapter protein; GRB2: growth factor receptor-bound protein 2; SOS: son of Sevenless gene; RAF: rapidly accelerated fibrosarcoma serine/threonine protein kinase; AKT: AK mouse strain with "T" for thymoma. Modified with permission from (45).

Rat sarcoma (RAS)-mitogen-activated protein kinase kinase (MEK)1/2-extracellular signal-regulated kinases (ERK)1/2 kinase cascade. Inside cancer cells, the cardiac peptide hormones have multiple targets (Figure 1). Vessel dilator, LANP, kaliuretic peptide, and ANP are multiple kinase inhibitors that inhibit the conversion of inactive rat sarcoma-bound guanosine diphosphate (RAS-GDP) to active rat sarcoma bound guanosine triphosphate (RAS-GTP) by 95%, 90%, 90%, and 83%, respectively (42, 43). This inhibition appears to be mediated by cyclic guanosine monophosphate

(cyclic GMP) which inhibits this conversion itself by 89% (42, 43). In addition to directly inhibiting the conversion to RAS-GTP, these four cardiac hormones inhibit the stimulation of RAS by mitogens such as epidermal growth factor (EGF) and insulin (44, 45).

The next step (Figure 1) in the RAS-MEK1/2-ERK1/2 kinase cascade involves two kinases, MEK1 and MEK2 (46, 47). Vessel dilator, LANP, kaliuretic peptide and ANP inhibit the phosphorylation of MEK1/2 kinases by 98%, 97%, 81% and 88%, respectively (48, 49).

ERK1/2 are important targets for inhibiting the growth of cancer (50, 51). Growth factors such as EGF and VEGF mediate their cancer effects *via* ERK kinase activity (50). Vessel dilator, LANP, kaliuretic peptide and ANP inhibit the phosphorylation of ERK kinases by 96%, 88%, 70% and 94%, respectively (52, 53). ERK1/2 kinases can directly translocate to the nucleus to stimulate the production of several nuclear oncoproteins such as c-FOS (50, 51). These four peptide hormones have been demonstrated by immunocytochemical techniques to enter the nucleus of cancer cells (54, 55) where they reduce expression of *c-FOS* and *c-JUN* proto-oncogenes (56).

c-JUN N-terminal kinases. c-JUN N-terminal-kinase-2 is associated with cancer development (57,58) and the invasion of cancer cells (59). As part of the crosstalk among the kinases in cancer cells, JNK is activated by the MEK kinases (60). Further related to this crosstalk, the activation of JNK by EGF is dependent upon H-Ras activation (61, 62). The loss of JNK activation coupled with the loss of ERK activation promotes cell death (63). Vessel dilator, LANP, kaliuretic peptide, and ANP maximally reduce the expression of JNK in human small cell-lung cancer cells by 89%, 88%, 77% and 89% (64). Thus, they inhibit another important target is cancer cells (Figure 1).

β -Catenin. One of the downstream targets of VEGF is β -catenin (65). β -Catenin is a multifunctional protein located on the intracellular side of the cytoplasmic membrane and causes growth of a variety of different cancer types (68-80). Vessel dilator, LANP, kaliuretic peptide and ANP reduce β -catenin expression by up to 88%, 83%, and 73% in human pancreatic, colorectal adenocarcinoma and renal adenocarcinoma cells, respectively (81). ANP is associated with a re-distribution of β -catenin from nuclear and cytoplasmic compartments to cell to cell junction sites and a decrease in the proliferation of colonic adenocarcinomas (82). β -Catenin activates JNK and VEGF as illustrated in Figure 1 (65, 83).

WNT signaling pathway. The WNT signaling pathway is a signal transduction pathway that is enhanced in a number of cancer types (68, 84). WNT signaling is stimulated by RAS and VEGF (85) and both contribute to the pathobiology of colonic cancer, in part through the WNT pathway (86) (Figure 1). The four peptide hormones from the heart maximally reduce WNT3a by up to 68% in human pancreatic cancer cells (87). The complex interplay of WNT and RAS in causing cancer and VEGF in maintaining its growth (84-89) are interrupted by the four cardiac peptide hormones, which helps to explain their anticancer effects (22).

Secreted frizzled-related protein 3. Secreted frizzled-related protein-3 (sFRP3) is a 300 amino acid glycoprotein (57-91) that promotes renal cancer growth when injected into

athymic mice (92). sFRP3 also causes growth of other types of cancer (93). ANP affects activation of the frizzled receptor (94, 95). ANP and the frizzled receptor co-localize on the cell membrane within 30 minutes after ANP addition to cell culture medium (82). Vessel dilator, LANP, kaliuretic peptide and ANP reduce the level of sFRP by 77-78% in human pancreatic cancer cells, 82-83% in human colorectal cancer cells, and 66-68% in human renal cancer cells (96).

Signal transducers and activators of transcription (STATs). STATs are cytoplasmic transcription factors (Figure 1) (97, 98) which are the final 'switches' activating gene expression leading to cancer (97-103). Vessel dilator, LANP, kaliuretic peptide and ANP reduce STAT3 by 88%, 54%, 55% and 65% respectively in human small-cell lung cancer cells and by 66%, 57%, 70% and 77% in pancreatic cancer cells (104). These heart peptide hormones do not reduce STAT1 in pancreatic adenocarcinoma nor in small-cell lung cancer cells (104). Thus, these heart peptide hormones are significant inhibitors of STAT3 but do not affect STAT1 which suggests a specificity of their anticancer mechanism(s) of action.

The Best Way to Administer Cardiac Hormones

The treatment of human pancreatic adenocarcinomas (21) and human small-cell lung carcinomas (22) in mice with cardiac hormones was *via* osmotic pumps for 28 days. On the other hand, the treatment of congestive heart failure with vessel dilator in humans was *via* intravenous infusion (105). To determine which of these methods might be best for treating humans with cancer, pharmacokinetics were compared between intravenous bolus (IvB), subcutaneous bolus (ScB) and subcutaneous infusion for 3 hours in male Fischer 344 rats (106). The half lives of vessel dilator after ScI, IvB and ScB were 54, 43, and 30 minutes (106). The time to reach peak concentrations in plasma (t_{max}) after IvB, ScB and ScI of vessel dilator was 1.5, 23, and 156 minutes (106). The peak plasma concentrations (C_{max}) with ScI, IvB and ScB were 3749, 887, and 471 ng/l (normalized against the dose used for ScB and IvB) (106). The area under the curve (AUC) for vessel dilator was 1,166, 880, and 1,652 ng h/ml (normalized) following IvB, ScB and ScI administration (106). The volume of distribution for vessel dilator was 2.38, 0.92 and 1.08 l following IvB, ScB and ScI administration, with its corresponding clearance being 1.69, 1.50 and 0.78 l/h, respectively (106). Plasma concentrations of vessel dilator after each of these three methods of administration mirrored their predicted concentration profiles (106). Vessel dilator administered *via* ScI led to a significantly greater AUC and half-life and slowed clearance compared to IvB and SCB ($p<0.001$), suggesting that based upon pharmacokinetics, subcutaneous infusion is the preferred method of administration to treat cancer.

Acknowledgements

Studies reported herein were funded by grants from the United States Department of Veterans Affairs, grants from the American Heart Association, the Mama Mare Foundation, the Darren Manelski Foundation, the James and Ester King Florida Biomedical Research Program and numerous individuals who contributed to the University of South Florida Research Foundation. The research involving human beings adhered to the principle of the Declaration of Helsinki and Title 45, U.S. Code of Federal Regulations, Part 46. Protection of Human Subjects, effective December 13, 2001. Research with the rats or mice adhered to the Guiding Principles in the Care and Use of Animals approved by the Council of the American Physiological Society.

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Received February 20, 2016

Revised May 13, 2016

Accepted May 24, 2016