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Cardiac Hormones for the Treatment of Prostate Cancer

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Abstract

The heart is a sophisticated endocrine gland synthesizing the atrial natriuretic peptide prohormone which contains four peptide hormones, i.e., atrial natriuretic peptide, vessel dilator, kaliuretic peptide and long-acting natriuretic peptide, which decrease up to 97% of human prostate adenocarcinoma cells in cell culture. Their signaling in human prostate cancer cells after binding to specific receptors includes inhibition of up to 95% of the basal activity of Ras, 98% inhibition of the phosphorylation of the MEK 1/2 kinases and 96% inhibition of the activation of basal activity of the ERK 1/2 kinases mediated via the intracellular messenger cyclic GMP. They also completely block the activity of mitogens such as epidermal growth factor's ability to stimulate ERK and Ras. The final step in their anticancer mechanism of action is that they enter the nucleus as demonstrated by immunocytochemical studies to inhibit DNA synthesis within cancer cells, which is also mediated by cyclic GMP. These cardiac hormones cause cell death of prostate cancer cells but not of prostate cells from healthy individuals.

The present review provides up-to-date information on 4 new potential treatments (cardiac hormones) of prostate cancer and their molecular targets (Ras-MEK 1/2-ERK 1/2 kinase cascade).

Keywords: Cardiac hormones; Prostate cancer; Ras kinase; MEK 1/2 kinases; ERK 1/2 kinases

Introduction

Prostate cancer is the leading cause of death in men in developed countries and the second most frequently diagnosed cancer in developing countries [1]. World-wide there were 903, 500 new cases of prostate cancer and 258,400 estimated deaths in the most recent year (2008) for which world-wide statistics are available [2]. In developed countries there were 648,400 estimated new cases and 136,500 estimated deaths secondary to prostate cancer in 2008 [1,2]. In developing countries there were an estimated 255,000 new cases of prostate cancer and an estimated 121,900 cases of death secondary to prostate cancer in 2008 [2]. With prostate cancer being the leading cause of death in men in developed countries and the second leading cause of death in men in all countries world-wide [1,2], there is definitely a need for new therapies which can prolong survival and decrease mortality. This review will concentrate on four hormones synthesized within the heart which decrease up to 97% of human prostate cancer cells in culture [3] and their mechanism(s) of doing so.

The heart synthesizes a family of peptide hormones which help moderate blood pressure and blood volume in healthy individuals [4,5]. When these peptide hormones are given in higher concentrations than normally made by the heart they have anticancer effects on human tumors (breast, pancreatic, and small-cell lung cancers) growing in athymic mice [6-8]. A 126 amino acid (a.a.) prohormone which has been designated the atrial natriuretic peptide prohormone since the 4 peptides contained in the prohormone are synthesized mainly in the atrium of the heart during adult life, have salt excreting properties, i.e. natriuresis, and are peptides [9,10]. Within the ANP prohormone are four peptide hormones, i.e. long-acting natriuretic peptide (LANP), vessel dilator, kaliuretic peptide and atrial natriuretic peptide (ANP), whose main known biological properties are blood pressure regulation and maintenance of plasma volume in animals [4,11-15] and humans [5,16,17].

Four cardiac hormones decrease the number of prostate adenocarcinoma cells in culture

The addition of 1 μM of long-acting natriuretic peptide (LANP) for 24 hours decreases the number of human prostate cancer cells 32% (p<0.05; Figure 1). Vessel dilator at 1 μM for 24 hours decreased the number of human prostate cancer cells the most of the cardiac hormones, i.e. 62% (p<0.001) (Figure 1). Kaliuretic peptide at 1 μM for 24 hours decreased the number of human prostate adenocarcinoma cells by 30% (p<0.05) (Figure 1).

ANP (1 μM) decreased the number of human prostate adenocarcinoma cells in culture by 37% (p<0.05). Brain natriuretic peptide (BNP) and C-natriuretic peptide (CNP), peptides with similar ring structures to ANP but with different amino acids, each at 1 μM, only decreased the number of human prostate adenocarcinoma cells by 0.8% and 1%, respectively, after 24 hours of incubation (not significant). Thus, with respect to their ability to inhibit the growth of human prostate cancer cells, when these cells were exposed to identical concentrations of these peptide hormones for 24 hours, vessel dilator > ANP > LANP > kaliuretic peptide [3]. In the wells with the decreased number of cells secondary to the cardiac hormones there was evidence of cellular debris suggesting that necrosis was occurring [3].

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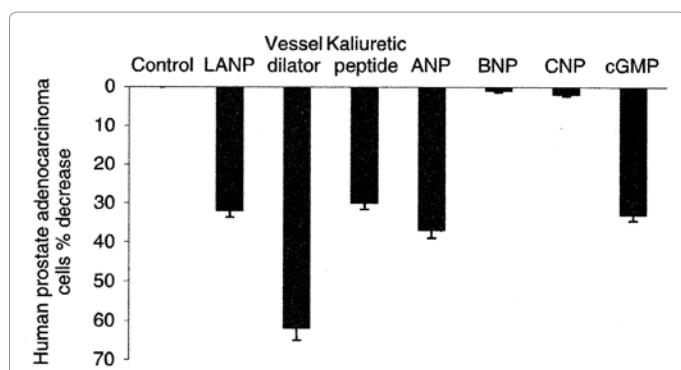
Specificity of the ability of the cardiac hormones to decrease the number of human prostate adenocarcinoma cells

To determine their specificity, each of the cardiac hormones (at 1 µM) was incubated with their specific antibodies (Ab, 5 µM). The decrease in cancer cell number secondary to vessel dilator alone of 63% was reduced to only 1% (89 ± 2 cells in control vs. 87 ± 2 cancer cells in Ab plus vessel dilator) [3]. There was no decrease in cell number with LANP plus its antibody and kaliuretic peptide plus its antibody resulted in only a 0.4% decrease in prostate cancer cell number [3]. These antibodies studies also indicated that ANP's effects were specific with only a 2% decrease when its antibody was added. Thus, the addition of specific antibody blocked each of these cardiac hormones' ability to decrease cancer cells by 98 to 100% at p<0.0001, suggesting that the anticancer effects were specifically due to the respective cardiac hormones.

When these specificity experiments were extended to 48, 72, and 96 hours of incubation of cardiac hormone plus antibody, the decrease in number of cancer cells was 1% or 0% at 48, 72, and 96 hours for vessel dilator, LANP, kaliuretic peptide, and ANP (p<0.0001) [3].

Decreased cellular proliferation after initial decrease in human prostate adenocarcinoma cell number

With the prostate adenocarcinoma cells exposed for 48, 72, and 96 hours, to vessel dilator, LANP, kaliuretic peptide, and ANP, each at 1 µM, there was a 31 to 38% inhibition of proliferation of the prostate cancer cells after the initial decrease in the number of these cancer cells at 24 hours by these cardiac hormones [3]. Thus, proliferation was inhibited by these peptide hormones for 3 days after the initial decrease in cell number in the first 24 hours. There was no significant decrease (1%) in human prostate adenocarcinoma cancer cell number secondary to BNP or CNP (each at 1 µM) at 48, 72, or 96 hours.

Dose-response evaluations of the cardiac hormones on human prostate cancer cells
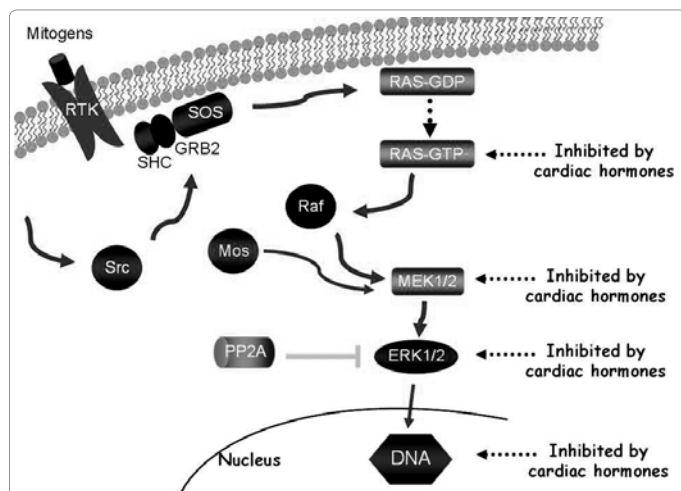
Dose-response studies utilizing 10-, 100- and 1000-fold higher concentrations for 24 hours revealed that with each increase in the concentrations of the four peptide hormones synthesized by the ANP prohormone gene there was a further decrease (p<0.05) in the number of prostate cancer cells [3].

Vessel dilator decreased the number of human prostate adenocarcinoma cells 60% at 1µM, 72% at 10 µM, 91% at 100 µM, and 97.4% at 1 mM when incubated for 24 hours [3]. Thus, vessel dilator at 1 mM eliminated almost all of the human prostate adenocarcinoma cells within 24 hours (i.e. there were only 3 ± 2.24 cells remaining, with several of the fields that were examined having no cancer cells whatsoever still alive) [3]. Long-acting natriuretic peptide (LANP), kaliuretic peptide and ANP decreased the number of cancer cells 87 to 89% at their 1 mM concentration [3].

Mechanism(s) of action of cardiac hormones in human prostate adenocarcinoma cells

Natriuretic Peptide Receptors (NPR) A and C are present in human prostate cancer cells: The first step in most hormones action(s) is binding to their specific receptors [9,10,18]. ANP works via several receptors [9,10,18]. ANP binding to the NPR-A or active receptor begins its effects within a cell and it also binds to NPR-C or clearance receptor which helps to remove ANP from the circulation [9,10,18]. When human prostate adenocarcinoma cells were evaluated by Western blots, the NPR-A and –C receptors were found to be present in prostate cancer cells [3].

Cardiac hormones inhibit Ras in human prostate cancer cells: The Ras mitogen-activated protein kinase (MAPK)/extracellular signal-related kinase (ERK) kinase (MEK)-ERK kinase cascade (Figure 2), hereafter referred to as the Ras- MAPK pathway, is the prototypical signal transduction pathway in cancer [19,20]. This pathway is aberrantly activated in many types of neoplasms, including prostate, with this activation being associated with a poor prognosis [19,20]. Structural alteration in the upstream GTPase Ras occurs in 25 to 30% of human cancers [21,22]. This is usually due to point mutation in


Figure 1: Decrease in human prostate adenocarcinoma cells after 24 hour exposure to 1 µM of long-acting natriuretic peptide (LANP), vessel dilator, kaliuretic peptide, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP). The decrease in prostate cancer cells was significant at p<0.001 with vessel dilator, p<0.01 with ANP, and p<0.05 with kaliuretic peptide and LANP when evaluated by repeated analysis of variance (ANOVA). There was no significant decrease in prostate cancer cell number secondary to BNP or CNP when evaluated by ANOVA. The decrease in number of human prostate cancer cells secondary to vessel dilator was significantly greater (p<0.05) than the decrease secondary to any of the other natriuretic peptides when evaluated by ANOVA. Cyclic GMP's (cGMP) (1 µM) decrease in prostate adenocarcinoma cells was significant at p<0.05 when evaluated by ANOVA (n=60 for each group). Reprinted with permission from Eur. J. Clin. Invest. 35:700-710, 2005.

Figure 2: Cardiac hormones inhibit five metabolic targets, i.e. Ras-GTP, MEK 1/2, and ERK 1/2 kinases of the Ras-MEK 1/2-ERK 1/2 kinase cascade by 95-98%. They are also strong inhibitors (i.e. 91%) of DNA synthesis within cancer cells. Reprinted with permission from Eur. J. Clin. Invest. 40:408-413, 2010.

one of three Ras genes, i.e. H-Ras, K-Ras or N-Ras, which encode for highly similar proteins with molecular weight of 21,000 [23]. This point mutation abolishes the intrinsic GTPase activity of Ras protein [23]. This pathway contributes to enhanced survival of tumor cells while also facilitating their metastatic spread to distant organs [24,25]. Ras is a small GTPase that cycles between an inactive GDP-bound and an active GTP-bound form [26]. A large variety of ligands that stimulate cell surface receptors induce the activation of Ras [26]. In dose-response and time sequence studies vessel dilator inhibited the activation of Ras by a maximum of 95% (p<0.00001) which occurred at 45 minutes at its 1 μM concentration (Figure 3) [27]. It was found that vessel dilator could inhibit the activation of Ras for 24 hours (p<0.0001) and then the effects began to wane at 48 and 72 hours [27].

Kaliuretic peptide (1 μM) caused a significant (64%; p=0.003) decrease in Ras activation in 30 minutes with a maximal 90% (p<0.0001) decrease in Ras in human adenocarcinoma cells at 60 minutes [27]. ANP at a concentration of 0.1 μM inhibited the activation of Ras by a maximum of 90% (p<0.00001) at 15 minutes and inhibited Ras 88% (p<0.00001) at 30 minutes [28]. LANP at 1 μM caused a maximal (71%; p=0.009) decrease in Ras at 30 minutes with a still significant (p<0.03) 63% decrease in the activation of Ras in human prostate cancer cells at 45 minutes [28]. These investigations on Ras kinase indicate that all 4 cardiac endogenous hormones derived from ANP prohormone can significant (p<0.0001) inhibit the activation (i.e. activity) of Ras.

Cardiac hormones inhibit the phosphorylation of MEK 1/2 kinases in human prostate cancer cells: The next step in the Ras-Raf-MEK 1/2-ERK 1/2 kinase cascade is two kinases termed MEK 1 and 2. With respect to these two kinases, the prototype member, designated MAP kinase kinase (MKK-1) or MEK-1, specifically phosphorylates the MAP kinase regulatory threonine and tyrosine residues present in the Thr-Glu-Tyr motif of ERK 1/2 [29,30]. A second MEK family member, namely MEK-2, resembles MEK-1 in its substrate specificity
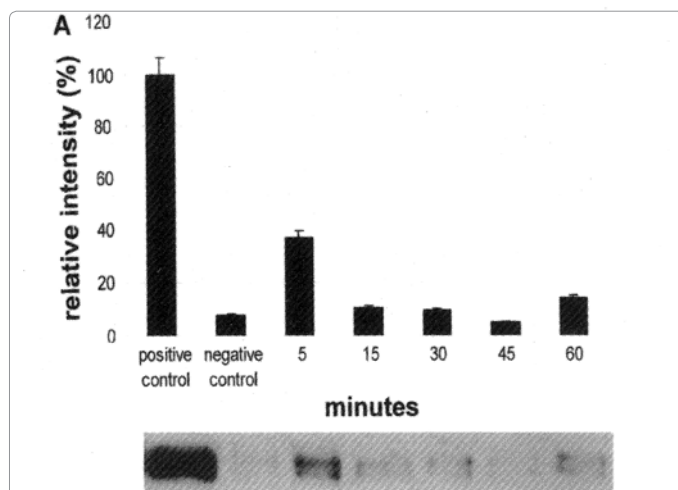
Figure 3: Dose response of vessel dilator on the activation of Ras in human prostate cancer cells at 1 μmol/L in time-sequenced experiments at 5, 15, 30, 45 and 60 minutes. These was a significant (p<0.0001) inhibition of the activation of Ras at each time point when evaluated by analysis of variance (ANOVA). Ras-GPT (i.e. active Ras) is at 21 kD. The relative intensity in this bar graph is a comparison of Western blots against the positive control (untreated Ras-GPT) with one typical Western blot illustrated. The illustrated negative control in this graph is Ras- GDP at 21 kDs. Reprinted with permission from Anticancer Res. 29:971-975, 2009.
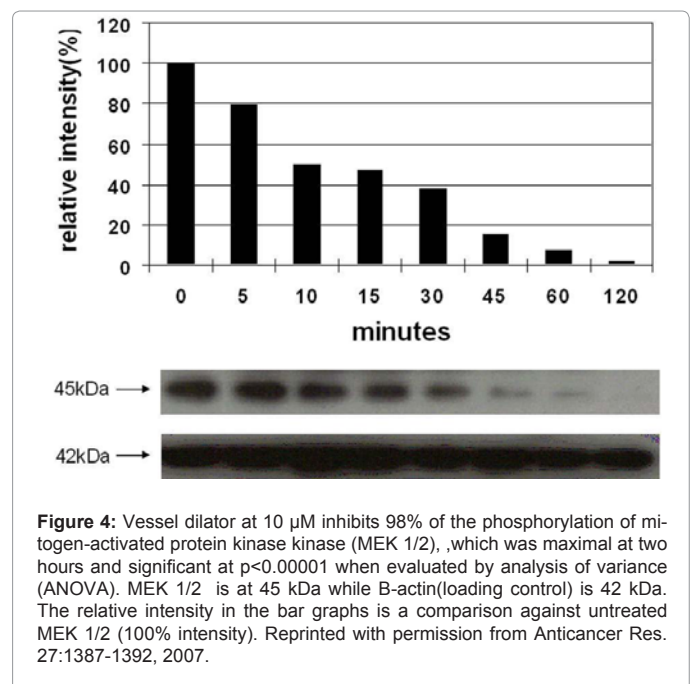
Figure 4: Vessel dilator at 10 μM inhibits 98% of the phosphorylation of mitogen-activated protein kinase kinase (MEK 1/2), ,which was maximal at two hours and significant at p<0.00001 when evaluated by analysis of variance (ANOVA). MEK 1/2 is at 45 kDa while B-actin(loading control) is 42 kDa. The relative intensity in the bar graphs is a comparison against untreated MEK 1/2 (100% intensity). Reprinted with permission from Anticancer Res. 27:1387-1392, 2007.

but is seven residues longer than MEK-1 with its amino acid sequence being 81% identical to MEK-1 [30].

Vessel dilator and kaliuretic peptide (each 10 μM) inhibited the phosphorylation of MEK 1/2 kinase by 98% (p<0.0001) (Figure 4) and 91% (p<0.001) respectively [31]. The inhibition of MEK 1/2 lasted for at least two hours, where it was maximal secondary to both peptides [31]. ANP and LANP, like vessel dilator and kaliuretic peptide, decreased the activation of MEK 1/2 over a concentration range of 0.01 μM to 10 μM [32]. LANP and ANP (each 10 μM) inhibited the phosphorylation of MEK 1/2 kinase by 97% (p<0.00001) and 88% (p<0.00001) respectively [32].

Cardiac hormones inhibit ERK 1/2 kinases in prostate cancer cells: Extracellular-signal regulated kinase (ERK 1/2) is a mitogen activated protein kinase (MAP kinase) important for the growth of cancer(s) [33,34]. Growth factors such as epidermal growth factor (EGF), fibroblast growth factor, platelet-derived growth factor and vascular endothelial growth factor (VEGF), after binding to their specific receptor tyrosine kinases, work via ERK 1/2 kinase to cause proliferation [33]. EGF, for example, when it binds to its EGF receptor, causes this receptor to autophosphorylate on tyrosine residues and recruits the Grb2-Sos complex to turn on membrane-associated Ras, which then activates the Ras-Raf-MEK 1/2 – ERK 1/2 kinase cascade [33]. Of the mitogen- activated protein kinases, ERK 1 and 2, 42 and 44 kDa proteins, can directly translocate to the nucleus and stimulate DNA synthesis and the production of several intermediate early genes such as c-fos and c-myc, which are implicated causing cells to divide and grow [33,34].

Vessel dilator and kaliuretic peptide decrease the activation (i.e. phosphorylation) of ERK 1/2 kinases over a concentration range of 0.01 μM to 1 μM [35]. Vessel dilator and kaliuretic peptide (each 1 μM) inhibit the phosphorylation of ERK 1/2 kinases by 96% (p<0.0001) and 70% (p<0.001), respectively [35]. The inhibition of ERK 1/2 lasts for at least two hours secondary to both [35].

ANP and LANP, likewise, decrease the activation of ERK 1/2 kinases over a concentration range of 0.01 μM to 10 μM [36]. ANP and LANP's maximal inhibition the phosphorylation of ERK 1/2 kinases were 94% and 88% (p<0.0001), respectively [36]. The inhibition of ERK 1/2 kinases lasted for at least two hours, where it was maximal, secondary to ANP and LANP [36].

Mitogens such as epidermal growth factor's stimulation of Ras and ERK 1/2 kinases are also blocked by the cardiac hormones: Epidermal growth factor (EGF) has been shown to directly activate Ras [37-40]. Vessel dilator, LANP, ANP and kaliuretic peptide, each at 1 μM, inhibit 73%, 79%, 33% and 45%, respectively, of 5 ng/mL EGF stimulation of Ras [41]. Another mitogen, i.e. insulin's, ability to contribute to cancer formation and proliferation is thought to be mediated in part by its ability to convert inactive GDP-Ras to active GTP-Ras [42]. Vessel dilator, LANP, ANP and kaliuretic peptide, each at 1 μM, inhibit 88%, 94%, 56% and 47%, respectively, of insulin's (1 μM) activation of Ras [43].

Growth promoting hormones such as insulin and epidermal growth factor (EGF) also work by stimulating ERK 1/2 kinases to cause growth [33,34]. Insulin (1 μM) and EGF (10 ng/mL) each enhance the phosphorylation of ERK 1/2 by 66% [44]. This enhanced phosphorylation of ERK 1/2 by EGF and insulin is decreased to 10%, 8%, 27% and 13% above non-stimulated ERK 1/2 by vessel dilator, kaliuretic peptide, LANP and ANP, respectively [44].

Cardiac hormones' ability to decrease prostate cancer cell number and inhibit Ras, MEK 1/2 and ERK 1/2 kinases is mediated by the intracellular messenger cyclic GMP: Cyclic GMP, the intracellular messenger of the cardiac hormones [45,46], decreases the number of human   prostate cancer cells is culture itself by 33% [3]. Further, the use of cyclic GMP antibody incubated with the respective cardiac hormones blocks their ability to decrease prostate cancer cells numbers, strongly suggesting that cyclic GMP mediates their effects on prostate adenocarcinoma cells [3].

Cyclic GMP antibody also inhibits the ability of the cardiac hormones to block the basal activity of Ras [27,28], MEK 1/2 [31,32] and ERK 1/2 kinases [35,36]. Cyclic GMP itself inhibits the activation of Ras by 89% [27], the phorylation of MEK 1/2 by 93% [32], and the activation of ERK 1/2 kinases by 83% [36]. Cyclic GMP, thus, appears very important for mediating the offset on prostate cancer cells, in general, and on each step of the kinase cascade in Figure 2.

Cardiac hormones inhibit DNA synthesis within the nucleus of prostate cancer cells: Vessel dilator, LANP, kaliuretic peptide and ANP, each at 1 μM concentration, inhibit DNA synthesis when incubated with human prostate adenocarcinoma cells for 24 hours by 89%, 68%, 76% and 79%, respectively (p<0.001 for each) [3]. Immunohistochemical studies have revealed that each of these cardiac hormones enter the nucleus of cancer cells [47,48] where they can inhibit DNA synthesis. 8-bromo cyclic GMP, the cell permeable antibody of cyclic GMP, inhibits DNA synthesis in adenocarcinoma cells by 56% [3]. Since cyclic GMP mimics the effects of the cardiac hormones on DNA synthesis in human prostate cancer cells, this suggests that cyclic GMP is one of the mediators of these cardiac hormones' effects to inhibit DNA synthesis in cancer cells.

Four cardiac hormones cause cell death of human prostate cancer cells but not of prostate cells from healthy individuals: Exposure to the cardiac hormones for 2 hours causes cell death in up to 28% (p<0.001) of prostate cancer cells over a concentration range of

100 pM to 10 μM [49]. Cell death of the human prostate cancer cells was quantified by measurement of nuclear matrix protein 41/7 which is a function of the number of dead or dying cells [50]. There was no cell death of prostate cells from healthy individuals [49].

Thus, cardiac hormones do not cause cell death of healthy prostate cells at the same concentrations that cause cell death of prostate cancer cells. These findings suggest that healthy prostate cancer cells are spared cell death at concentrations which have anticancer effects in mice [49].

Conclusions

Vessel dilator, long-acting natriuretic peptide, atrial natriuretic peptide and kaliuretic peptide each significantly decrease up to 97% of human prostate cancer cells in culture. After binding to specific receptors, their signaling in prostate cancer cells involves at 95% inhibition of the activation of Ras, 98% inhibition of the activity of MEK 1/2 kinases, and 96% inhibition of the phosphorylation of ERK 1/2 kinases mediated by cyclic GMP. They also inhibit DNA synthesis within prostate cancer cells mediated by cyclic GMP.

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