The ANP Family of Peptides and Cancer Treatment

Michael R Kozlowski*

Arizona College of Optometry, Midwestern University, Glendale, Arizona, USA

*Corresponding author: Michael R Kozlowski, Midwestern University, 19555 North 59th Avenue, Glendale, AZ 85308, USA.

Abstract

Atrial natriuretic peptide (ANP) was discovered over 40 years ago, and the other members of the ANP family of peptides nearly 30 years ago. One of the effects of these peptides was found to be inhibition of the proliferation of both normal and cancer cells *in vitro* as well as *in vivo*. For the past 20 years, numerous studies have characterized the antiproliferative effects of ANP family peptides on cancer cells and proposed a role for them in the treatment of cancer. Yet, as of 2021, no anti-cancer drugs have been developed from ANP. Recently, however, events have propelled two ANP-related agents into clinical development for treating cancer. The present mini review outlines the history of ANP, from its discovery to the recent entry of ANP-related peptides into clinical trials for cancer.

Keywords: Atrial natriuretic peptide; ANP; Small peptide; Cancer treatment; Pancreatic cancer

Abbreviations: ANP: Atrial natriuretic peptide; MAPK: Mitogen activated protein kinase; KP: Kaliuretic peptide; LANP: Long-acting atrial natriuretic peptide; VDL: Vessel dilator protein

Introduction

Atrial natriuretic peptide (ANP) was first identified in the 1950's as the component in cardiac atrium extracts that lowered blood pressure by stimulating sodium and water excretion [1]. The peptide was subsequently isolated, purified and characterized in the 1980's. It was determined that ANP was generated by the cleavage of a larger precursor protein, proANP. In the early 1990's, cleavage of this protein also was found to produce three other peptides that also modulated blood pressure and fluid regulation, although in divergent ways. These peptides were kaliuretic peptide (KP), long-acting atrial natriuretic peptide (LANP), and vessel dilator protein (VDL) [2,3]. In this mini review, this group of peptides, together with ANP, will be called the ANP family of peptides, and each individual peptide referred to as an ANP family peptide. The major functions of ANP were characterized in the 1980's and 90's, and found to include diuresis, natriuresis, vasorelaxation, increased vascular permeability, and antagonism of the renin-angiotensin system. In addition to these hemodynamic effects, however, ANP was also found to inhibit cardiac hypertrophy and remodeling in response to pressure overload. This suggested that ANP may play a role in regulating cell growth and physiology. The effects of ANP on cell functioning were further investigated in several studies performed beginning in the 1990's. One of the effects identified was inhibition of cell proliferation [1]. Since unregulated cell proliferation is a major component of the pathology of cancer, such an activity points to a potential role of ANP in the treatment of cancer.

Inhibition of the Proliferation of Normal Cells by ANP

In a number of studies, beginning in the early 1990's, ANP was shown to inhibit the proliferation of a variety of types of normal cells, including rat astrocytes, mouse and rat mesangial cells, rat thymocytes, human airway smooth muscle cells, bovine aortic endothelial cells, rat gastric epithelial cells, and rat ventricular

fibroblasts [4-15]. The concentration range for the inhibitory effects varied from nanomolar [4,10,14] to micromolar [8,9]. The reduction in cell proliferation was not always accompanied by reduced viability, suggesting a primary effect of ANP on the generation of new cells [10]. The effectiveness of ANP against some cell types was only explored under conditions of mitogen or growth factor-stimulated proliferation [5,8,10,12]. In one study, inhibition of growth by ANP was only seen in the presence of a growth factor with no effect noted on basal growth [5]. This finding, along with later work, suggested that ANP may have a minimal effect on the basal growth of normal cells [16]. Certain ANP analogs (e.g., cANP4-23) and other peptides related to ANP (e.g., C-type natriuretic peptide) were also found to have similar effects [7,17,18]. Several mechanisms were proposed for the reduction of cell proliferation by ANP, including inhibition of signaling through the MAPK or Akt pathways [12,13,19, 20]. This also pointed to a potential role of ANP in cancer treatment since deregulation of signaling in each of these pathways has been proposed as contributing to the development of cancer [21,22].

Inhibition of the Proliferation of Cancer Cells by ANP

A more direct indication that ANP might be useful in cancer treatment came from examining its effects on cancer cells themselves. These studies, which began in the early 2000's, showed that ANP could reduce the proliferation of a number of types of cancer cells in vitro, including those derived from human breast cancer, human cervical cancer, human and rat colon cancer, human and mouse gastric cancer, human glioblastoma, human heart cancers, human kidney cancer, human small cell and squamous lung cancers, human and mouse melanoma, human and mouse neuroblastoma, human prostate cancer, and human thyroid cancer [3,18-20,23,24]. The concentration range for the antiproliferative effects extended from nanomolar to micromolar, as it did in normal cells, but in some of these studies, ANP had a non-monotonic concentration-response curve for inhibition. In one case, higher concentrations were inhibitory whereas lower concentrations stimulated cell proliferation while, in another case, lower concentrations were inhibitory whereas higher concentrations had no effect [19,23]. This is instructive when considering the use of ANP in the treatment of cancer, suggesting that using the correct dosage could be important for effective therapy. The effects that ANP family peptides produce on cancer cells were not seen in a number of types of normal cells that were unstimulated by growth factors or mitogens [16]. The potential use of ANP as an anticancer agent was addressed in many of these studies [3,19,20,23].

Effects of ANP Family Peptides on Cancer Cells in Vitro and in Vivo

Based on the inhibitory effects of ANP on cancer cell proliferation, Vesely and his colleagues conducted a series of studies to determine whether the other ANP family peptides had similar effects, beginning in the early 2000's. They demonstrated that KP, LANP, and VDL were also able to inhibit the proliferation of cells derived from human pancreatic, colon, prostate, breast, kidney and thyroid carcinomas; heart angiosarcoma; small cell and squamous lung cancers; and glioblastoma [3]. These effects occurred at nanomolar to micromolar concentrations, as in the studies discussed above, but the degree of inhibition of proliferation was greater than that reported those studies. The enhanced effect may have been due to a higher sensitivity to ANP family peptides in the particular types of cells used by Vesely and his colleagues since ANP was also tested in these studies and likewise produced a greater effect than it had in the earlier studies [3].

Based on these compelling results, Vesely and his colleagues also examined the effect of ANP family peptides on the growth of tumors produced by human pancreatic carcinoma, human breast carcinoma, and human small cell lung cancer in a mouse xenograft model system [25-27]. In these experiments the peptides were given either as a continuous subcutaneous infusion or as bi-weekly intravenous injections. Both types of administration appeared equally effective [28]. They found that these peptides were not only able to decrease tumor growth but, in many cases, to entirely eliminate the tumors. The reason for the magnitude of these effects may lie in the xenograft protocol used in the experiments. In their protocol, a relatively small number of cells was ingrafted [25-27] and Matrigel™ was not given with the cells to provide a matrix for tumor formation. These conditions are not the most favorable for tumor development [29]. Thus, Vesely and his colleagues may have been measuring not only the inhibition of tumor growth, but also the failure of tumor establishment. If this is the case, it does not diminish the significant of these peptides as potential treatments for cancer but suggests roles in reducing both tumor growth as well as new tumor establishment (e.g., the development of metastases). More recently Nojiri and colleagues found that ANP given during lung cancer surgery reduced subsequent development of cancer metastases [30]. This was a serendipitous finding as Nojiri and his colleague were actually attempting to reduce cardiopulmonary complications arising from lung cancer surgery (e.g. chronic obstructive pulmonary disease and atrial fibrillation) by administering ANP. They found that giving patients ANP during surgery did indeed reduce these post-surgical complications [31-33]. In following up the patients, however, they also noted a reduction in the risk of developing metastases following the surgery in the patients given ANP [30].

Optimization of the Antiproliferative Effects of the ANP Family Peptides

The effectiveness of the ANP family peptides in inhibiting the proliferation of a variety of human cancer cell types both *in vitro* and *in vivo* was, at first, difficult to understand because these peptides

share no apparent sequence homology [34]. This lack of homology made their common ability to inhibit cell proliferation hard to explain [3]. A closer examination of their sequences ultimately revealed that they all contain the same 8-amino acid length motif [34]. Subsequent work further revealed that a peptide consisting of only an optimized version of this motif (KTH-222) was more effective than an ANP family peptide containing the native motif (i.e., VDL) in decreasing the growth of human pancreatic tumors in a mouse xenograft model [34]. In this study, the percentage of inhibition of tumor growth by VDL was less than that originally reported by Vesely and colleagues [25]. This may be because the xenograft system employed in this study used a larger number of cells to seed the tumors and added Matrigel™ to the cells to provide a matrix for tumor development, thereby producing more robust tumor growth than the system used by Vesely and his colleagues [29]. Of particular significance, KTH-222 was found to be more effective in reducing the growth of human pancreatic cell tumors than the drug, gemcitabine, which is routinely used to treat pancreatic cancer in human patients [34].

Conclusion

At the time of the writing of this mini review, ANP family peptides have finally been brought to the threshold of entering clinical use in cancer by two very different paths. The first is through the discovery of a small motif found in each of the ANP family peptides that appears to underly their antiproliferative activity. KTH-222, an optimized version of this motif [34], is in the early stages of clinical development for treating pancreatic cancer. The second way is through the serendipitous finding by Nojiri and colleagues that ANP given during the perioperative period of curative lung cancer surgery reduced the recurrence of the cancer [30]. A clinical trial is now underway in lung cancer comparing surgery alone with surgery plus ANP on the development of metastases [35]. The verdict concerning the utility of ANP family peptides in the treatment of cancer will ultimately depend upon the outcome of these, and subsequent, clinical trials.

Acknowledgement

Conflict of Interest

M.R. Kozlowski is a consultant for Kalos Therapeutics Inc., which is developing KTH-222.

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