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Pituitary adenylate cyclase-activating polypeptide (PACAP) protects against mitoxantrone-induced cardiac injury in mice

Venkat Subramaniam[a], Gin Chuang[a], Huijing Xia[a,b], Brendan Burn[a], Jessica Bradley[a,b], Jerome L. Maderdrut[c], David H. Coy[c], Kurt J. Varner[a,b,*]

[a] Department of Pharmacology and Experimental Therapeutics, Louisiana State University, Health Sciences Center, New Orleans, LA 70112-1393, United States
[b] Cardiovascular Center of Excellence, Louisiana State University, Health Sciences Center, New Orleans, LA 70112-1393, United States
[c] Peptide Research Laboratory, Department of Medicine, Tulane University School of Medicine, New Orleans, LA 70112-2699, United States

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ABSTRACT

Mitoxantrone (MXT) is an androstenedione that is used to treat cancers and progressive forms of multiple sclerosis; however, its use is limited by its cardiotoxicity. Pituitary adenylate cyclase activating polypeptide (PACAP) is a member of the secretin/growth hormone-releasing hormone/vasoactive intestinal peptide family and has many functions, including cytoprotection and immunosuppression. We tested the hypothesis that PACAP can protect against MXT-induced cardiotoxicity in mice. Female BALB/c mice were treated once weekly for 4 weeks with saline (n = 14) or MXT (3 mg/kg, i.p.; n = 14). Half of the mice in each group received PACAP (10 μg, i.p.) 1 h before and 24 and 48 h after MXT, while the remaining mice received injections of saline on the same schedule. Echocardiography was used to assess cardiac structure and function. In mice treated with MXT and saline, body weight was significantly reduced after the third dose of MXT. PACAP significantly attenuated the reduction in body weight; however, the weights did not return to control level. Compared to controls, MXT-treated mice had significantly increased left ventricular (LV) diameter and LV volume and decreased LV posterior wall thickness. Fractional shortening (FS) and ejection fraction (EF) were also significantly decreased. Treatment with PACAP prevented MXT-induced LV dilation and significantly attenuated the reductions in FS and EF, although FS and EF did not return to control level. PACAP38 did not prevent MXT-induced decreases in LV posterior wall thickness. MXT dose-dependently decreased the viability of cultured U937 (human leukemia) cells; PACAP did not protect cultured U937 cells from MXT-mediated cell death. In conclusion, PACAP can attenuate MXT-mediated LV dilation and dysfunction in mice.

1. Introduction

Mitoxantrone (MXT) is an androstenedione chemotherapeutic agent that is used to treat acute nonlymphocytic leukemia, acute lymphoblastic leukemia, prostate cancer, metastatic breast cancer, and non-Hodgkin's lymphoma [13,14]. It is also approved by the Food and Drug Administration (FDA) for progressive/relapsing (also called worsening relapsing-remitting) and secondary progressive multiple sclerosis (MS) [7,47]. Its chemical structure is similar to that of doxorubicin, a widely used anthracycline chemotherapeutic agent. MXT causes apoptosis in proliferating and non-proliferating cells by inhibiting DNA replication, DNA-dependent RNA synthesis and DNA repair by topoisomerase II [13]. The drug also has numerous effects on immune function, resulting in decreased lymphocyte proliferation, decreased cytokine release, suppression of T-cell and B-cell function, including antibody production [9,13]. However, the therapeutic efficacy of MXT is limited by its irreversible cumulative dose-related cardiotoxicity [33,60] and its potential to cause secondary leukemias [5,13].

Pituitary adenylate cyclase-activating polypeptide (PACAP) was first isolated from the hypothalamus during a search for novel hypophysiotropic factors [48], but it rapidly became clear that it is a multifunctional peptide with potent anti-inflammatory and potent cytoprotective properties [69]. PACAP is a member of the secretin/growth hormone-releasing hormone/vasoactive intestinal peptide (VIP) family.

Abbreviations: d, diastole; EF, ejection fraction; FDA, Food and Drug Administration; FS, fractional shortening; i.p., intraperitoneal; IVS, intraventricular septum; LV, left ventricle; LVID, internal diameter of the left ventricle; LVPW, thickness of the posterior wall of the left ventricle; MS, multiple sclerosis; MXT, mitoxantrone; PACAP, pituitary adenylate cyclase-activity polypeptide; s, systole; TBST, tris-buffered saline with 0.05% Tween 20 containing 3% bovine serum albumin; VIP, vasoactive intestinal peptide; Vol, volume

* Corresponding author at: Department of Pharmacology and Experimental Therapeutics, Louisiana State University, Health Sciences Center, 1901 Perdido Street, New Orleans, LA 70112-1393, United States.
E-mail address: kvarne@lsuhsc.edu (K.J. Varner).

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It exists as two α-amidated peptides with 38 (PACAP38) or 27 (PACAP27) amino acids, and PACAP27 has 68% sequence identity with VIP. PACAP binds to three subtypes of Class II (secretin-type) G protein-coupled receptors that are called the PAC1, VPAC1 and VPAC2 receptors [23]. PACAP binds not only to the PAC1 receptor with high affinity, but it also binds to the VPAC1 (VIP1) and VPAC2 (VIP2) receptors with affinities comparable to or greater than VIP. On the other hand, VIP binds to the PAC1 receptor with an affinity 100–1000 times lower than PACAP [24]. PACAP is most abundant in the brain, but there are high levels in other organs, including the thymus, spleen, lymph nodes, and duodenal mucosa [69]. The cytoprotective effects of PACAP have been most extensively studied in the brain and kidney [40,69,72]. PACAP has both direct and indirect cytoprotective effects in the brain and kidney [32,72]. The direct protective effects of PACAP in the brain and kidney are mediated mainly via the cyclic AMP/protein kinase A signal transduction pathway, while the indirect protective effects of PACAP are mediated by multiple signal transduction pathways [32,69,72]. PACAP protects the brain, kidney and liver against ischemia/reperfusion injury [28,31,59,68] and protects the brain and kidney from injury caused by a wide range of therapeutic agents [2,32,40,41,69]. Despite extensive library screens by major pharmaceutical companies over several decades, small molecule orthosteric agonists have not been found for the cognate receptors for any member of the secretin/growth hormone-releasing hormone/VIP family [24,27].

PACAP/VIP receptors are found in the myocardium: the PAC1 receptor is the predominant receptor in cardiac myocytes and the VPAC2 receptor is the predominant receptor in cardiac fibroblasts [63]. The VPAC2 receptor is also the predominant PACAP/VIP receptor in vascular smooth muscle cells [63,69]. PACAP has positive inotropic and chronotropic effects on the heart [61] and increases blood flow to most major organs [69]. PACAP has only a slight transient effect on systemic blood pressure [4,39]. PACAP directly protects cardiomyocytes in vitro against oxidative stress and doxorubicin [18,19,56,57]. In addition, the cardiotoxicity elicited by doxorubicin is exacerbated in PACAP-deficient mice and significantly reduced in PACAP-deficient mice by treatment with PACAP38 [49]. Mori and colleagues [49] did not determine whether PACAP can protect wild-type mice against doxorubicin. Whether PACAP can protect against MXT-induced cardiotoxicity has not been determined in vitro or in vivo. Therefore, the purpose of this study was to test the hypothesis that PACAP can block MXT-induced cardiotoxicity in mice. We show for the first time that PACAP significantly attenuates MXT-induced left ventricular dysfunction and remodeling in wild-type mice in vivo. However, the clinical utility of these observations would be limited if PACAP also blocked the therapeutic actions of MXT. Therefore, we also demonstrated that PACAP does not protect cultured human leukemia cells against MXT-induced cell death. PACAP has already been shown by several laboratories to be efficacious in preclinical in vivo models for MS [15,20,30,66,67].

2. Materials and methods

2.1. Animals

All studies were approved by the Louisiana State University Health Sciences Center Animal Care and Use Committee and performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Experimental Animals. The animal experiments used 12-week-old female BALB/c mice (Harlan, Indianapolis, IN). The mice were group-housed in a pathogen-free, climate-controlled room with a 12-h light/dark cycle. The standard rodent chow and tap water were available ad libitum. After their arrival in the animal care facility, the mice were allowed 1 week to acclimate before beginning the experiments.

2.2. Peptide synthesis and purification

PACAP38 was prepared at the Peptide Research Laboratory (Tulane University, New Orleans, LA) by solid-phase synthesis using Fmoc chemistries on a CEM microwave-assisted automatic peptide synthesizer (Matthews, NC). Mass spectroscopic analysis of intermediate sequences revealed a tendency for incomplete couplings to occur near the center of the *alpha*-helical region of PACAP38 (residues 16–27) and peptide synthesizers were programmed to make many repeat couplings in this region. The crude PACAP38 was cleavage from the Rink amide resin with trifluoroacetic acid/triisopropylsilane/water (95:1:4) followed by precipitation with ether and filtration. The white powder was dissolved in dilute acetic acid and applied directly to a preparative chromatography column (5 × 50 cm) containing Vydac C-l8 silica (300-Å pore size) and eluted with a linear gradient (20–60%) of acetonitrile/0.1% trifluoroacetic acid. The fractions containing PACAP38 but no contaminating deletion sequences were identified using analytical high-performance liquid chromatography and matrix-assisted laser desorption/ionization mass spectrometry. These fractions were pooled and lyophilized several times from deionized water. The overall yield of PACAP38 with > 95% purity was ~10%. PACAP was reconstituted in sterile 0.9% NaCl (saline) prior to injection, or in distilled water for cell culture studies.

2.3. Design of animal experiments

Four experimental groups were used: saline-treated mice (saline), mice treated with PACAP, mice treated with MXT, and mice treated with MXT plus PACAP (MXT + PACAP). All solutions were injected intraperitoneally in a volume of 100 µl. On day 0, the saline (n = 7) and MXT (n = 7) groups were injected with saline, while the PACAP (n = 7) and PACAP + MXT (n = 7) groups were injected with PACAP (10 µg/mouse). One hour later, echocardiograms were recorded for each mouse under light isoflurane anesthesia (~1%). Immediately after completing the echocardiogram, the mice in the saline and PACAP groups were given injections of saline, while the mice in the MXT and PACAP + MXT groups were injected with MXT (3 mg/kg; Tocris Bioscience, Ellisville, MO) dissolved in saline. This dose was chosen because it produced cardiac toxicity in female mice [3]. Twenty-four and 48 h later, the saline and MXT groups received saline injections, while the PACAP and the PACAP + MXT groups received injections of PACAP (10 µg/mouse). This regimen of PACAP injections was chosen because a similar regimen prevented cisplatin-induced renal failure in mice [41]. The 3-day dosing protocol was repeated on experimental days 7, 14 and 21 (Fig. 1). The echocardiograms were repeated on days 14 and 21, with a final echocardiogram performed 1 week after the last saline or MXT treatment (day 28). After the last echocardiogram, the mice were deeply anesthetized and the heart removed, rinsed, patted dry, and weighed.

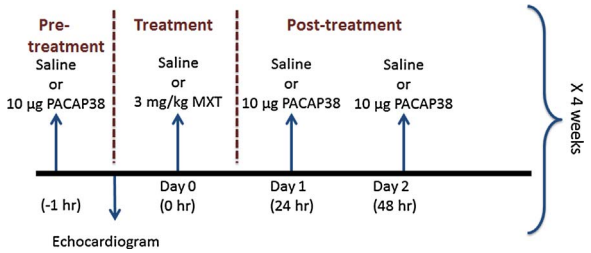

Fig. 1. Schematic drawing of the experimental protocol. The weekly dosing of MXT in mice was intended to mimic the monthly dosing of MXT in human cancer patients [13,14]. The order of the weekly injections for the Saline Group was saline, saline, saline, and saline; the order of the weekly injections for the PACAP Group was PACAP, saline, PACAP, and PACAP; the order of the weekly injections for the MXT Group was saline, MXT, saline, and saline; and the order of the weekly injections for the PACAP + MXT Group was PACAP, MXT, PACAP, and PACAP.