

*Critical Review***Cardioprotection by Vanadium Compounds Targeting Akt-Mediated Signaling**Md. Shenuarin Bhuiyan¹ and Kohji Fukunaga^{1,2,*}¹Department of Pharmacology, Graduate School of Pharmaceutical Sciences, Tohoku University, Aramaki-Aoba, Aoba-ku, Sendai 980-8578, Japan²Tohoku University 21st Century COE Program "CRESCENDO", Sendai 980-8578, Japan

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Abstract. Treatment with inorganic and organic compounds of vanadium has been shown to exert a wide range of cardioprotective effects in myocardial ischemia/reperfusion-induced injury, myocardial hypertrophy, hypertension, and vascular diseases. Furthermore, administration of vanadium compounds improves cardiac performance and smooth muscle cell contractility and modulates blood pressure in various models of hypertension. Like other vanadium compounds, we documented bis(1-oxy-2-pyridinethiolato) oxovanadium (IV) [VO(OPT)] as a potent cardioprotective agent to elicit cardiac functional recovery in myocardial infarction and pressure overload-induced hypertrophy. Vanadium compounds activate Akt signaling through inhibition of protein tyrosine phosphatases, thereby eliciting cardioprotection in myocardial ischemia/reperfusion-induced injury and myocardial hypertrophy. Vanadium compounds also promote cardiac functional recovery by stimulation of glucose transport in diabetic heart. We here discuss the current understanding of mechanisms underlying vanadium compound-induced cardioprotection and propose a novel therapeutic strategy targeting for Akt signaling to rescue cardiomyocytes from heart failure.

Keywords: myocardial hypertrophy, myocardial ischemia/reperfusion, bis(1-oxy-2-pyridinethiolato) oxovanadium (IV) [VO(OPT)], protein kinase B/Akt

1. Introduction

Vanadium has become subject of interest among nutritionists since the discovery that various marine species have this metal as an essential element (1, 2). Foods are the major source of exposure to vanadium for the general population because most of foods contain a low amount of vanadium (<1 ng/g) (3). Foods rich in vanadium include mushrooms, shellfish, dill seed, parsley, black pepper, and so on (3, 4). It occurs at extremely low concentrations in mammalian tissues (0.014 – 7.2 μ M) with an estimated amount of 100 – 200 μ g in humans (5 – 7). While its necessity for some lower organisms has been demonstrated, convincing evidence

to support an essential role for this element in humans is still lacking (8).

The estimated daily intake of the U.S. population from foods ranges from 10 – 60 μ g vanadium. Vanadyl sulfate is a common supplement used to enhance body weight during training in athletes at doses up to 60 mg/day (3). Studies of vanadyl sulfate for weight loss and exercise performance have been variable, with most showing only modest effects on body composition. Although vanadium has become a popular dietary supplement among bodybuilders, there are only limited data to support claims of increased muscle mass and strength (9, 10). One study of oral vanadyl sulfate (0.5 mg/kg per day) treatment on anthropometry, body composition, and performance investigated in a 12-week, double-blind, placebo-controlled trial involving weight-training volunteers showed that oral vanadyl sulfate was ineffective on body composition in weight-training athletes (11). In vitro and animal studies indicate that vanadate and other vanadium compounds

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increase glucose transport activity and improve glucose metabolism (3).

Over the last several years, a diverse range of biological actions of various vanadium compounds has been documented (6, 12–14). Among these, the cardioprotective effects of vanadium has been studied in great detail, and several studies have demonstrated that the vanadyl (IV) form of vanadium possesses cardioprotective properties (15) in diabetic rats (16, 17), myocardial ischemia/reperfusion-induced injury in rats (18, 19), spontaneously hypertensive rats (SHR) (20, 21), and fructose-fed rats (22), representing a genetic and diet-induced model of hypertension (23). Among the several oxidation states of vanadium II to V, vanadium ion in living cells exists exclusively as vanadyl (IV) cation and a small amount of vanadate (V) anion (24). The vanadyl ion is less toxic than the vanadate ion, as judged by the LD₅₀ values in several animals (25), and the vanadyl state is likely the pharmacologically active form of vanadium in cells (26). In addition, vanadate derivatives were shown to have prohypertensive properties in chronically exposed rats (27), whereas vanadyl derivatives were shown to be antihypertensive in spontaneously hypertensive rats and in fructose-induced hypertensive rats (20–23).

In the cardiovascular system, protein kinase B (PKB)/Akt plays an important role in the regulation of cardiac hypertrophy, angiogenesis, and apoptosis (18, 19, 28–31). The Akt subfamily comprises three mammalian isoforms, PKB α /Akt1, PKB β /Akt2, and PKB γ /Akt3, which are products of distinct genes and share a conserved structure that includes three functional domains: an N-terminal pleckstrin homology domain, a central kinase domain, and a C-terminal regulatory domain containing the hydrophobic motif phosphorylation site [FxxF(S/T)Y] (32). Among the three Akt genes, only Akt1 and Akt2 are highly expressed in the heart. Consistent with the general trophic function of Akt, the Akt1 whole-genome-knockout mice weigh approximately 20% less than their wild-type littermates and have a proportional reduction in size of all somatic tissues, including the heart (33, 34). In contrast, Akt2-knockout mice have only a modest reduction in organ size (35). Thus, data from the available Akt-knockout models support a critical role specifically for Akt1 in normal growth of the heart (36). The observations that acute activation of Akt in cardiomyocytes *in vivo* and *in vitro* protects against cardiomyopathies provide the possibility that agents targeting Akt activation may become a novel therapeutic strategy for limiting myocardial injury (37). To develop a novel therapeutic drug to protect cardiomyocytes from heart diseases, we selected a novel vanadyl (IV) compound having the VO²⁺

chelate: bis(1-oxy-2-pyridinethiolato) oxovanadium (IV) [VO(OPT)], which is a potent activator of the Akt signaling pathway both *in vivo* and *in vitro* (38–41). We previously documented that VO(OPT)-mediated Akt activation elicited cardioprotection in ischemia/reperfusion-induced injury in rats (18, 19) and myocardial pressure overload-induced hypertrophy in ovariectomized female rats along with cardiac functional recovery (28). Vanadium compounds inhibit protein tyrosine phosphatases (38, 39) and promote an increase in protein tyrosine phosphorylation, leading to the upregulation of Akt (38).

2. Akt activator vanadium compounds for treatment of cardiac diseases

Until now, four vanadium compounds, namely, sodium orthovanadate (OV) (42, 43); vanadyl sulfate (VS) (20, 22, 30); bis(maltolato)oxovanadium (IV) [BMOV] (21, 44, 45); and VO(OPT) (18, 19, 28), have been found to have cardioprotective effects in different heart injury models (Fig. 1). Organo-vanadium compounds have been shown to be more potent activators of the Akt signaling pathway and potent tyrosine phosphorylation compared to inorganic vanadium compounds in Chinese hamster ovary cells overexpressing human insulin receptor (CHO-HIR cells) (40). Moreover, the relative ordering of intensities of tyrosine phosphorylated proteins parallels the chemical stability of the VO²⁺ chelates and their ability to remain intact in the midst of proteins. Ou et al. (38) using 3T3-L1 adipocytes showed that in the presence of both albumin and transferrin, the relative intensities to tyrosine phosphorylation of proteins stimulated by the vanadium compounds followed the order VO(OPT) > BMOV > VS. Moreover, Mehdi and Srivastava (40) tested the potentials of protein tyrosine phosphatase inhibition and Akt activation by different vanadium compounds in CHO-HIR cells, and the relative intensities of the vanadium compounds followed the order BMOV > VS > OV (40). Moreover, comparative studies of the stability of VO²⁺ organic chelates demonstrate the enhanced stability of VO(OPT) in solution as an intact complex compared to that of the BMOV compound (38).

3. Targeting Akt by vanadium to inhibit cardiomyocyte apoptosis

In the cardiovascular system, Akt plays critical roles in the regulation of cardiac hypertrophy, angiogenesis, and apoptosis (31). In cardiomyocytes, activation of the phosphatidylinositol 3-kinase (PI3-K)/Akt pathway by insulin-like growth factor 1 (IGF-I) treatment appears to

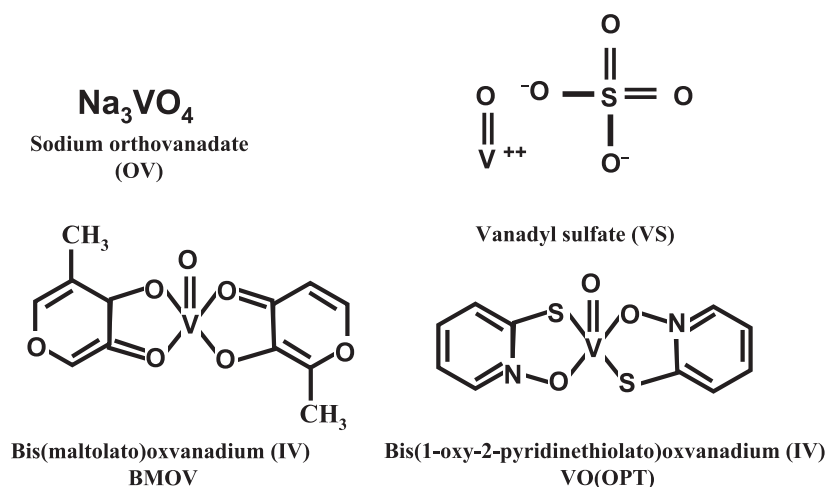


Fig. 1. Chemical structures of cardioprotective vanadium compounds. Sodium orthovanadate (OV); vanadyl sulfate (VS); bis(maltolato)oxovanadium (IV) [BMOV]; bis(1-oxy-2-pyridinethiolato)oxovanadium (IV) [VO(OPT)].

be necessary for the anti-apoptotic effects of IGF-I (46). Adenoviral expression of constitutively active mutants of either PI3-K or Akt reduces hypoxia-induced apoptosis in cardiomyocytes in vitro. Adenoviral gene transfer of constitutively active Akt (myr-Akt) transiently to the heart in vivo results in a dramatic reduction in cardiomyocyte apoptosis and infarct size (37). Along with the anti-apoptotic effect, Akt activation preserved cardiomyocyte function both in vitro and in vivo, while inhibition of Akt activity with a dominant negative construct greatly accelerated hypoxia-induced cardiomyocyte dysfunction (37). Taken together, acute Akt activation by genetic manipulation can be beneficial in the heart through combined effects of promoting both cardiomyocyte survival and its functional recovery.

We recently reported that decreased Akt activity is involved in ischemia/reperfusion-induced myocardial injury and that increased Akt activity by treatment with vanadium compounds accounts for cardioprotection in rat heart (18, 19, 42). Like other cardioprotective vanadium compounds (30, 42, 43), VO(OPT) is a potent Akt activator both in vitro (38) and in vivo (18, 19, 28, 47, 48). In vitro studies using 3T3-L1 mouse adipocytes demonstrate that treatment with VO(OPT) increased the tyrosine phosphorylation of proteins including the β -subunit of the insulin receptor and insulin receptor substrate-1 (38, 48), suggesting that VO(OPT) can inhibit PTPase. Recently, investigators studying PTPase inhibition by vanadate have proposed that the vanadium molecule interacts with the thiol of the catalytic cysteine residue of PTPase, forming a thiol–vanadyl ester linkage, thereby leading to a competitive inhibition of PTPase (49). It is speculated that the same mode of action might be involved in PTPase inhibition by VO(OPT), but

further studies using a variety of structurally diverse vanadyl complexes are necessary in order to determine its mode of inhibitory action. Thus, the vanadyl compound VO(OPT) inhibits protein tyrosine phosphatases (50, 51) and produces hydrogen peroxide (52), thereby promoting PI3-K/Akt activities through receptor tyrosine kinase activation. Moreover, vanadium compound-mediated cardioprotection accounts for the post-ischemic cardiac functional recovery (18, 19, 30, 42, 43). Indeed, Akt activation increases myocardial contractility with a combined mechanism of increased intracellular free calcium and sensitization of the myofilaments to calcium [Ca^{2+}], with marginal increased myocardial oxygen consumption (53). The reduced oxygen consumption required for Ca^{2+} transport in turn limits the threat of arrhythmias and results in inhibition of Ca^{2+} -dependent transcriptional and translational mechanisms leading to hypertrophy and heart failure. Moreover, Akt activation in mesenchymal stem cells may enhance cardiac function in heart failure models to a greater extent than that observed in nontransduced stem cells (54, 55).

Akt activation is believed to suppress apoptosis through phosphorylation of several substrates, including the Bcl-2 family member Bad (56, 57). Akt is known to phosphorylate Bad at Ser-136, which is critical for sequestration by the 14-3-3 protein, thereby inactivating Bad (58–60). We documented that Bad dephosphorylation in the myocardium is associated with decreased Akt activity following myocardial ischemia/reperfusion injury, and VO(OPT)-induced rescue of Akt activity promoted Bad phosphorylation (18). Akt phosphorylates forkhead transcription factors (FOXOs) such as FKHR and FKHL1 as downstream targets in cell survival signaling (61, 62). Phosphorylation of FOXOs reduces

their DNA-binding activities, interferes with interactions with other transcription factors, and promotes nuclear export and tethering in the cytoplasm by binding to 14-3-3 proteins (63). The decreased Akt activity following myocardial ischemia/reperfusion injury is closely correlated with dephosphorylation of FOXOs such as FKHR (Ser 256) and FKHL1 (Ser 253) in ventricular cardiomyocytes (18, 19, 30). Treatment with vanadium compounds significantly inhibited FKHR and FKHL1 dephosphorylation after myocardial ischemic injury, with concomitant Akt activation (18, 19, 30). These results suggest that FOXOs are Akt downstream targets and their phosphorylation mediates cardioprotective action by inhibiting their transcriptional activities following treatment with vanadium compounds.

Fas and Fas ligand trigger activation of caspase 8 through the adaptor molecule FADD (Fas-associated death domain) and in turn caspase 8 either directly activates caspase 3 or induces the translocation of Bid, another apoptotic Bcl-2 family member, to the mitochondria. The FOXOs such as FKHR and FKHL1 are known to induce the expression of cell death-related genes Fas ligand and Bim (64–66). Indeed, both Fas ligand and Bim are induced in the ventricular cardiomyocyte after myocardial ischemia/reperfusion-induced injury (18, 30). Moreover, we previously documented the FOXOs-mediated increased expression of Fas ligand and Bim in ischemic brain injury in a mouse bilateral common carotid artery occlusion model (61, 62, 66, 67). VO(OPT) treatments prevented ischemia/reperfusion injury-induced expression of Fas ligand and Bim in rat heart (18, 19) and brain (51). However, to fully explore the therapeutic potential of this pathway in the heart, we must understand the effects of Akt signaling on cardiac growth and hypertrophy.

4. Targeting Akt by vanadium to protect against myocardial hypertrophy

Signaling through the PI3-K/Akt pathway is important for the physiological growth and inhibition of pathological hypertrophy (37, 68, 69). Moreover, physiological hypertrophy induced by exercise training also requires the activation of myocardial Akt. By contrast, pathological hypertrophies induced by pressure overload causes an inactivation of the Akt signal pathway (70). We recently introduced a novel heart injury model using ovariectomized (OVX) pressure overloaded (PO) female rats, which is attractive for testing cardioprotective drugs in hypertension-induced cardiac injury in postmenopausal women (29). The most relevant phenomenon in the heart of OVX-PO female rats was impairment of Akt and endothelial nitric oxidase synthase (eNOS) signaling

(28, 29). Lack of estrogen by ovariectomy and pressure overload led to reduction of Akt activity (29). The decreased Akt activity is accompanied by reduced eNOS phosphorylation.

The Akt activation by VO(OPT) suppressed the OVX-PO-induced hypertrophy in female rats. Like the single treatment (18), the repeated oral administration of VO(OPT) for 14 days dose-dependently increased Akt activity in cardiomyocytes (Fig. 2A) (28). Under these conditions, Akt activation by VO(OPT) promotes cardiac remodeling to recover the physiological contraction and relaxation in the cardiomyocyte. The inhibition of cardiac hypertrophy by VO(OPT) was closely associated with recovery of heart rate, mean artery blood pressure (MABP), and contractile heart functions (28).

Akt can directly phosphorylate recombinant eNOS or eNOS in situ, at serine 1177 (human) / 1179 (bovine) (71, 72), thereby enhancing the eNOS activity. Interestingly, the repeated VO(OPT) treatment for 14 days not only increased Akt-mediated eNOS phosphorylation on Ser-1179 but also enhanced eNOS protein expression at the high dose (2.5 mgV/kg) (Fig. 2B). This observation is consistent with a previous observation in which inorganic vanadate induces nitric oxide (NO) release via eNOS phosphorylation and increases binding of HSP 90 to eNOS in bovine lung microvascular cells (73). NO in vascular smooth muscle activates soluble guanylyl cyclase (sGC) to increase cGMP formation, thereby leading to a decrease in $[Ca^{2+}]_i$ with subsequent inhibition of myosin light chain phosphorylation and contraction (74–76). This was also confirmed by our study showing decreased MLC phosphorylation by VO(OPT) treatment (28). VO(OPT) promotes cardiac remodeling to recover the physiological contraction and relaxation in cardiomyocytes partly by increasing NO levels and decreasing MLC phosphorylation (Fig. 3). The recovery of the heart from OVX-PO-induced cardiac injury is likely mediated by the increased eNOS expression and/or phosphorylation. Our results are consistent with inhibition of cardiac hypertrophy by 17 β -estradiol in OVX-PO female rats (77), in which upregulation of PI3-K/Akt and suppression of calcineurin/NF-AT3 mediated the 17 β -estradiol effects. In this context, we should examine the calcineurin/NF-AT3 signaling in the VO(OPT)-treated heart in the future.

Localization and activity of eNOS are regulated by making a complex in the caveolae with a chaperone protein, heat shock protein 90 (HSP 90), and caveolin 3 in cardiomyocytes (72). We did not find any significant changes in HSP 90 in the OVX-PO group; thus HSP 90 level is not the primary target for VO(OPT)-induced cardioprotection (28). Likewise, caveolin-3 level was not affected by VO(OPT) treatment (28). Thus, eNOS

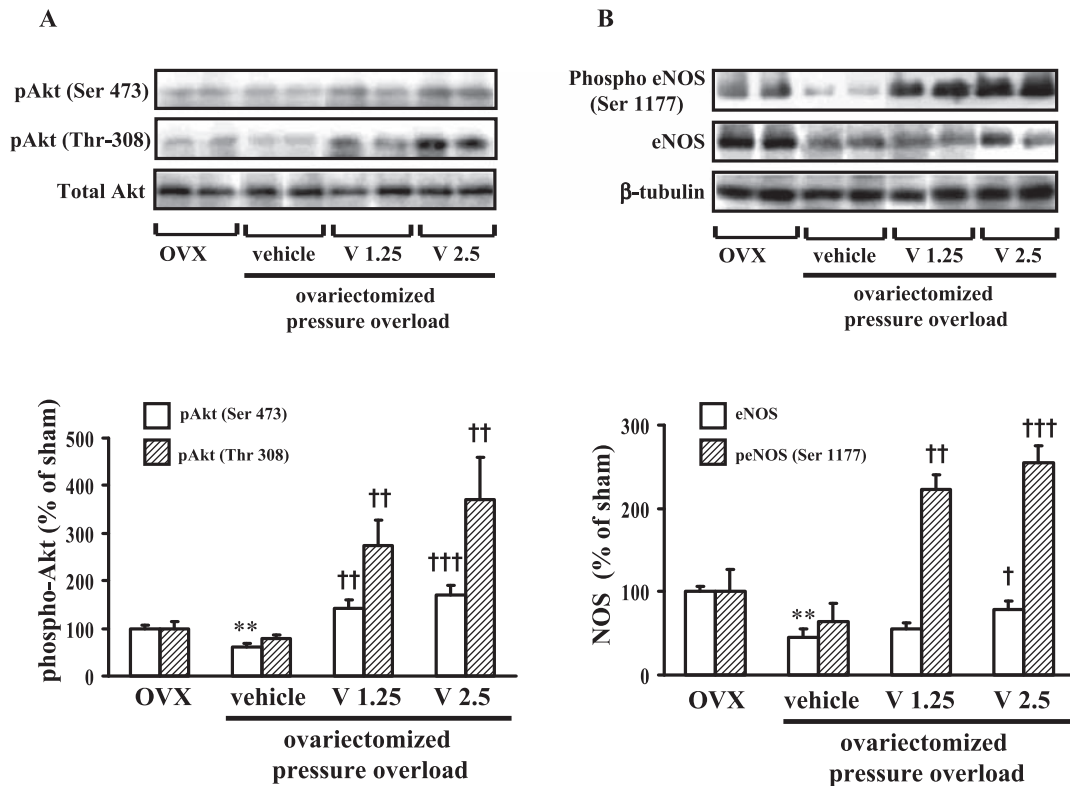


Fig. 2. Effects of VO(OPT) on Akt phosphorylation (A) and eNOS expression and phosphorylation (B) in the left ventricle. Representative Western blot analysis using cell extracts from OVX (n = 8), OVX-PO-vehicle (n = 8), OVX-PO-VO(OPT) containing 1.25 mg/kg vanadium (V 1.25) (n = 8), and OVX-PO-VO(OPT) containing 2.5 mg/kg vanadium (V 2.5) (n = 8) treated hearts probed with phospho-Akt (Ser-473), phospho-Akt (Thr-308), total Akt, eNOS, and phospho-eNOS (Ser 1177) antibody. Quantitative measurements of protein levels were determined by densitometric analyses of the Western blots. Data are expressed as percentage of the value of OVX rats. Each column represents the mean \pm S.E.M. ** $P < 0.01$ vs. the OVX group; † $P < 0.05$, †† $P < 0.01$, and ††† $P < 0.001$ vs. the OVX-PO-vehicle treated group. (modified from Ref. 28 with permission)

phosphorylation is the predominant mechanism in VO(OPT)-induced cardioprotection. Since HSP-90 level tended to decrease following OVX-PO and increase by VO(OPT) treatment, more extensive studies are required to determine temporal changes and immunohistochemical localization of HSP 90 and eNOS following OVX-PO. We also previously documented increased dystrophin breakdown after OVX-PO in female rats (29) and after prolonged stimulation with endothelin-1 in cultured cardiomyocytes (78). The marked breakdown of dystrophin suggests involvement of calpain in OVX-PO-induced cardiac injury. We also found a significant decrease in calpastatin levels following OVX-PO. Only treatment with a high dose of VO(OPT) (2.5 mgV/kg) significantly inhibited both calpastatin and dystrophin breakdown, suggesting that this was a consequence of the cardioprotection by VO(OPT) (28). The β -adrenoceptor (β -AR) agonists differently affect heart rate during the estrous cycle in female rats and in ovariectomized rats with or without estrogen replacement (79). Importantly, Kam et al. (80) demonstrated

that β -AR stimulation with isoproterenol led to a significantly greater increase in electrical stimulation-induced Ca^{2+} elevation, Ca^{2+} -uptake through cardiac L-type Ca^{2+} channels, heart rate, and contractility in hearts of ovariectomized rats compared to sham rats. These responses were rescued by estrogen replacement. Interestingly, Kaplan-Meier survival data in our study clearly indicates that significantly increased mortality occurs only in the OVX-PO group following chronic β -adrenergic stimulation by isoproterenol (5 mg/kg) (Fig. 4) (28, 29). No death was observed in sham and PO groups treated with low doses (5 mg/kg) of isoproterenol. VO(OPT) treatment dose-dependently increased survival following acute cardiac stress caused by chronic β -adrenergic stimulation, suggesting that cardiac remodeling and recovered cardiac functions by VO(OPT) treatment contributes to the reduced mortality (28). This is supportive evidence for the cardioprotective effects of VO(OPT) in the treatment of cardiac injury in postmenopausal women.

Thus, simultaneous severe reduction of eNOS and

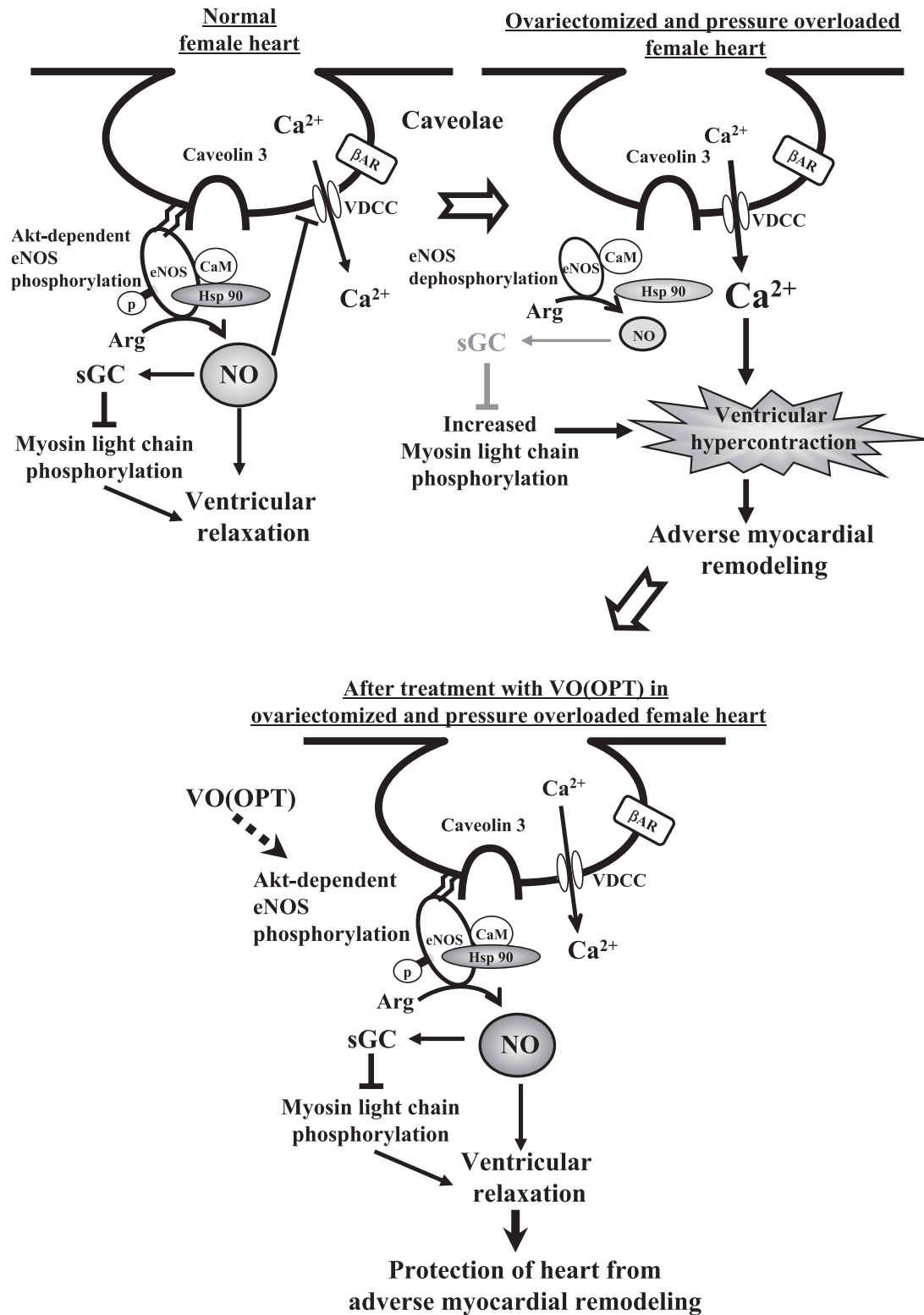


Fig. 3. Putative mechanism of VO(OPT)-mediated cardioprotection. In normal female heart, eNOS is localized to the caveolae through its interaction with caveolin 3 and compartmentalized with L-type Ca^{2+} channel and beta-adrenergic receptor. Activation of Akt signaling ultimately leads to eNOS phosphorylation, which activates eNOS, and thereby activates nitric oxide signaling pathways. The resulting combination of these effects subsequently confers ventricular dilation and cardioprotection. Pressure overload-induced hypertrophy severely impairs eNOS and Akt signaling pathways, thereby resulting in an imbalance of the NO-mediated cardioprotective action. Treatment with VO(OPT) activates the Akt activity and enhances Akt-mediated eNOS activity and subsequently confers cardioprotection against myocardial hypertrophy.

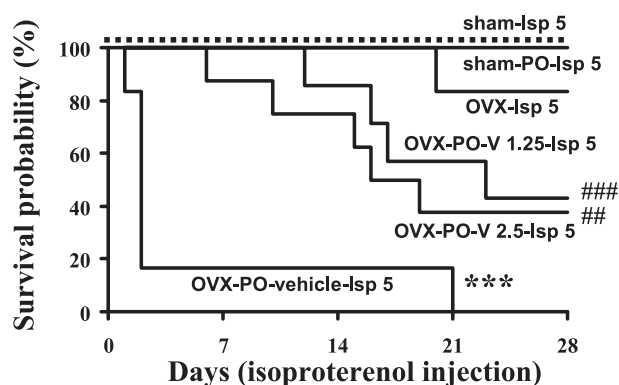


Fig. 4. Kaplan-Meier survival analysis following chronic isoproterenol treatment. Isoproterenol (5 mg/kg, intraperitoneally) (Isp 5) was administered once daily for 4 weeks to sham, sham-pressure overloaded (sham-PO), ovariectomy (OVX), ovariectomy-pressure overloaded plus vehicle (OVX-PO-vehicle), OVX-PO-VO(OPT) containing 1.25 mg/kg vanadium (V 1.25), and OVX-PO-VO(OPT) containing 2.5 mg/kg vanadium (V 2.5) treated groups. Survival over 28 days was monitored. *** $P < 0.001$ vs. the OVX group; ## $P < 0.01$ and ### $P < 0.001$ vs. the OVX-PO-vehicle-treated group. (modified from Ref. 28 with permission)

Akt activities in OVX-PO female rats likely triggers compensatory hypertrophy with increased heart contractility. Potentiation of the Akt and eNOS signaling pathways, along with inhibition of dystrophin breakdown, by treatment with VO(OPT) after OVX-PO likely contributes to increased survival following acute cardiac stress caused by chronic β -adrenergic stimulation (Fig. 4). VO(OPT) likely accounts for the anti-hypertrophic effect and cardiac remodeling to rescue cardiomyocytes from heart failure through activation of Akt and eNOS signaling pathways.

5. Effect of vanadium compounds on the diabetic heart

Vanadate can also scavenge free radicals (81), and vanadate administration has been associated with a decrease in oxidative damage in the diabetic heart (82). The beneficial effect of vanadate on diabetic heart dysfunction may be related to its ability to serve as a scavenger of free radicals (44). Vanadate also mimics the stimulatory effect of insulin on cardiac K^+ currents (83). Further studies demonstrate that vanadate augmented glucose uptake and improved ischemic tolerance in hypertrophied heart (84). Improvement in cardiac function following vanadate treatment may result from restoration of aberrant myocardial ATP and phosphocreatine production as well as creatine kinase activities in diabetic rats (45, 85, 86).

Extensive studies have been done to evaluate the effect of vanadium on glucose transport, in particular

glucose transporter 4 (GLUT-4) translocation and expression both in isolated cardiomyocytes (87) and in the myocardium (88, 89) due to the importance of glucose in cardiac physiology. GLUT-4 is the primary glucose transporter expressed in the heart and a decreased GLUT-4 expression was observed in streptozotocin-induced diabetes (90). Prolonged treatment with VS normalized GLUT-4-mRNA expression and restored both sarcolemmal and total cardiac GLUT-4 protein levels in the diabetic rat hearts (88). In contrast, BMOV treatment of streptozotocin-induced diabetes in rats normalized GLUT-4 levels in skeletal muscle (91) and enhanced GLUT-4 translocation in response to insulin without change in cardiac GLUT-4 levels (89). The reversal of aberrant GLUT-4 function by vanadium may serve as part of a mechanism responsible for the observed vanadium-mediated improved cardiac performance.

Vanadium-mediated stimulation of the glucose transport in isolated adult cardiomyocyte demonstrates a potential role of the PI3-K/Akt pathway in the mediation of glucose transport (88, 92, 93). Studies demonstrate that vanadate-induced glucose transport was accompanied by Akt activation, an effect that was completely abolished by wortmannin (88, 92). Studies reveal that in cardiomyocytes from obese Zucker rats, neither insulin nor vanadate could induce glucose uptake or Akt activation to the same extent as in lean control animals, but in combination they elicited a stronger response (92, 94). Moreover, chronic exposure to a low concentration of vanadate stimulated glucose uptake, an action mediated via activation of both PI3K and p38 MAPK signaling pathways (93, 95). Thus, the ability of vanadium compounds to stimulate glucose transport and glucose oxidation in the myocardium could contribute to the cardioprotective effects observed in various diabetic models following vanadium therapy.

6. Effect of vanadium compounds on vascular diseases

Since 1980 it has been known that vanadium compounds are able to induce contraction of various types of smooth muscle cells, including vascular smooth muscle cells (96). The contraction might be due to the ability of vanadate to inhibit Na^+/K^+ ATPase activity and to the resulting increase in Na^+/Ca^{2+} exchange (97). However, the observation that vanadate was still able to induce muscle contraction even when Na^+/K^+ ATPase activity had been inhibited with ouabain ruled out the contribution of Na^+/K^+ in this process (98). On the other hand, vanadate was found to augment free intracellular Ca^{2+} in smooth muscle cells, thereby promoting increased vascular tone and contractility (99). The increased level

of tyrosine phosphorylation, typically described in the mechanism of the insulinomimetic effects of vanadium derivatives (13, 100), was also found to be associated with the smooth muscle contractile activities by vanadium compounds (101, 102).

Carr et al. (103) demonstrated that protein tyrosine phosphatase (PTPase) inhibition by BMOV enhances the endothelial receptor tyrosine kinase (RTK) activity and improves collateral blood flow. In cultured endothelial cells, pretreatment with BMOV augmented vascular endothelial growth factor receptor-2 (VEGFR-2) and Tie-2 tyrosine phosphorylation and enhanced VEGF- and angiopoietin-1-mediated cell survival. In rat aortic ring explants, BMOV enhanced vessel sprouting, a process that is influenced by both VEGFR-2 and Tie-2 activation. Moreover, 2-week treatment of BMOV in a rat model of peripheral vascular disease enhanced the collateral blood flow similarly to VEGF. Four weeks later, the BMOV effect was superior to that by VEGF (103). Moreover, BMOV has protective effects against vascular endothelial dysfunction induced by diabetic mellitus and hyperhomocysteinemia. The BMOV-induced inhibition of PTPase may close ATP-sensitive K^+ channels and consequently activate eNOS to reduce oxidative stress and subsequently improve the vascular endothelial dysfunction (104). In addition, the BMOV-induced inhibition of PTPase also improved vascular endothelial dysfunction in high fat diet-induced hypercholesterolemia (105) and deoxycorticosterone acetate-induced hypertension in rats (105).

Demonstrating the therapeutic potential of activating endothelial RTKs for the treatment of atherosclerotic cardiovascular disease, delivery of polypeptide growth factors, including fibroblast growth factor (FGF) (106), VEGF (107), and angiopoietin-1 (108), results in improved collateral blood flow in atherosclerotic cardiovascular disease models. However, because polypeptide growth factors are expensive to produce and difficult to absorb, their usage as therapeutic agents is currently limited. In addition, like the development of the embryonic vasculature, optimal collateral development in vivo may require the coordinated activation of multiple RTKs and necessitate the delivery of multiple growth factors (109, 110). Thus, there has been growing interest in developing alternative approaches to activate endothelial RTKs by vanadium compounds for treatment of occlusive atherosclerotic cardiovascular disease.

7. Vanadium toxicity

In general, the toxicity of vanadium compounds is low. The most common toxic effects reported for inorganic vanadium compounds are diarrhea, decreased

fluid and food uptake, dehydration, and reduced body weight gain (111, 112), which can, however, be corrected by adding sodium chloride to drinking water, adjusting the pH of the solution to neutrality, and by gradually increasing the dose of vanadium (111, 113, 114). More recently, it was shown that organic vanadium compounds were much safer than inorganic vanadium salts and did not cause any gastrointestinal discomfort and showed no hepatic or renal toxicity (41, 111). Along with the previous studies, we also did not find any gastrointestinal, hepatic, or renal toxicity in the VO(OPT)-treated rats (47, 48). Oral administration of VO(OPT) (10 mg V/kg) for 21 days had no effect on blood urea nitrogen and body weight in streptozotocin-induced diabetic rats (47). In our present series of experiments, none of the rats died in any of the studies conducted; and no gastrointestinal, hepatic, or renal toxicity was observed after 14 days of VO(OPT) treatment as reported previously (28, 47, 48). Moreover, VO(OPT)-treated rats continued to gain weight throughout the experimental period as reported previously (47, 48). This suggests that the reduced weight gain caused by VO(OPT) administration is due to reduced food and fluid intake (28, 47, 48).

In addition to gastrointestinal discomfort, other toxic effects of vanadium salts include hepatotoxicity, nephrotoxicity, and teratogenicity as well as developmental/reproductive toxicity (115, 116). In contrast, recent work has demonstrated that vanadium compounds inhibited serum- and growth factor-stimulated mitogenesis (117, 118) and possess anti-tumor activity (119, 120). Many other studies have, however, failed to detect any change in the levels of urea, creatinine, glutamic oxaloacetic transaminase, and glutamic pyruvic transaminase (111, 121–123), indices of kidney and liver functions. Moreover, no significant changes in the histopathology of several tissues, including the liver, spleen, stomach, heart, and lung, have been observed among control and VS-treated animals (124). Electron microscopic examination of *ob/ob* mice treated with OV for 47 days revealed no sign of hepatotoxicity (114).

In patients treated with vanadium salts, gastrointestinal discomfort was the most common side effect, which could be corrected by decreasing the dose level (125, 126). Moreover, clinical studies have been of short duration (up to 6 weeks) and utilized lower doses than those administered in animal experiments; thus, the long-term toxicity of vanadium in humans remains to be explored. Clearly, at present, there is no consensus on the toxic effects of vanadium compounds, and detailed and systematic investigations are needed to evaluate the toxicity of various vanadium compounds before undertaking long-term clinical trials in humans. It

should be noted that use of chelating agents (115) and organo-vanadium compounds, such as VO(OPT), have shown significantly reduced vanadium toxicity and may serve as more potent cardioprotective agents than inorganic vanadium salts.

8. Future directions

Overexpression of IGF-1 (127) or nuclear-targeted Akt (128) in the post natal heart by cardiac-specific transgenesis results in hyperplastic growth without myocyte hypertrophy, suggesting that Akt is responsible for the promotion of cellular proliferation in the postnatal heart. Furthermore, both physiological growth and hemodynamic adaptability are blunted when Akt1 is knocked out (129) or when the dominant negative mutant of the PI3-K subunit P110 γ is overexpressed (130). Mitotic replication of mature myocytes within the adult myocardium remains controversial, but the recent demonstration of progenitor cells within the myocardium (131, 132) raises the possibility that potentiation of this resident cardiac committed stem cell pool could contribute to myocardial cellular proliferation driven by Akt. We recently documented that vanadium compound-mediated Akt activation induces neurogenesis in the adult rat subventricular zone (133) and mouse hippocampal subgranular zone after focal cerebral ischemia (134). We hypothesize that the potent Akt activator organovanadium VO(OPT) may also induce cardiogenesis to rescue myocardial infarction and should be subjected to further extensive study.

Coupled with the well-established cardioprotective properties displayed by Akt-activator vanadium compounds against myocardial ischemia/reperfusion-induced injury and myocardial hypertrophy, the observed remarkable cardiac functional recovery opens new scenarios regarding clinical heart disease (18, 19, 28, 30, 42, 43). Indeed, changes in the activation status of Akt have been recently reported in experimental myocardial ischemia/reperfusion injury (18, 19, 30), pressure overload-induced myocardial hypertrophy (28, 29), diabetic cardiomyopathy (135), and human heart failure (136), thus painting Akt as a target for novel therapeutic strategies. To support this view, studies have demonstrated beneficial effects following elevated myocardial Akt signaling in experimental heart failure (137). Thus, treatment with vanadium compounds targeting Akt activation provides a unique therapeutic strategy to rescue cardiomyocytes, and at the same time, modulation of the Akt pathway may provide novel therapeutic targets for which a new class of cardioprotective drugs can be designed.

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