IN TRANSLATION

Emerging Role of the Bufadienolides in Cardiovascular and Kidney Diseases

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The bufadienolides are a group of steroid hormones that circulate in blood and are excreted in urine. They have the ability to inhibit the adenosine triphosphatase sodium-potassium pump (Na^+-K^+-ATPase), with predilection for its $\alpha 1$ isoform. This capability enables them to share with other cardiac glycosides the facility to cause an increase in sodium excretion, produce vasoconstriction resulting in hypertension, and act as cardiac inotropes. Bufadienolides have been implicated in instances of volume expansion–mediated hypertension, syndromes in which they are considered capable of causing a vascular leak, interfering with cellular proliferation, and inhibiting cellular maturation. An antagonist to the most well-studied bufadienolide, marinobufagenin, is resibufogenin, a compound that provides promise for the treatment of disorders in which excessive levels of marinobufagenin are present and are etiopathogenetic.

BACKGROUND

The bufadienolides are a group of steroid compounds that belong to a class of circulating substances collectively called cardiac glycosides[1-3] (Fig 1). Included in the latter category are the cardenolides, the prototype of which is ouabain and its derivative digoxin.[1-3] Although the bufadienolides and cardenolides show structural and physiologic differences, these 2 groups of compounds share certain characteristics. (1) They act primarily because of their ability to inhibit the ubiquitous transport enzyme, the adenosine triphosphatase sodium-potassium pump (Na^+-K^+-ATPase). This enzyme thus represents their signal transducer. (2) By virtue of their ability to inhibit the enzyme, they are natriuretic. (3) They have the capacity to cause hypertension because they are vasoconstrictive. (4) Perhaps their best known effect is that of cardiac inotropy. Thus, the beneficial effect of digoxin in heart failure is well established related to its ability to improve cardiac output.[4,5] Use of the ancient Chinese medication "Chan Su" for this purpose apparently is because this medicament, a natural product of plants and trees, contains a mixture of cardiac glycosides.[6] Differences in these endogenous cardiotonic steroids include structural differences: the cardenolides have an unsaturated 5-membered lactone ring, whereas the bufodienolides have a doubly unsaturated 6-membered lactone ring (Fig 1). Mammalian bufadienolides are synthesized in the adrenal cortex[7] and placenta.[8] Volume expansion with sodium chloride stimulates increased secretion of marinobufagenin in the brain and plasma of experimental rats.[9] The bufadienolides are cleared unchanged in urine.[10] The cardenolides have been determined to have a predilection for the $\alpha 2$ and $\alpha 3$ isoforms of Na^+-K^+-ATPase, whereas the bufodienolides act primarily on the $\alpha 1$ isoform,[3] the predominant form of the enzyme in the kidney.[1] Na^+-K^+-ATPase is complex and heterogeneous, with the potential for multiple $\alpha\beta$ combinations, each with distinct properties.[11] Currently, the 3 classic features of this membrane protein (the pump, enzyme, and receptor to cardiotonic steroids) have been described in more detail.[12] There is evidence for an alternative or "signaling" function for Na^+-K^+-ATPase. This model proposes that certain plasmalemmal Na^+-K^+-ATPases reside in the caveolae of cells that do not seem to actively "pump" sodium and are
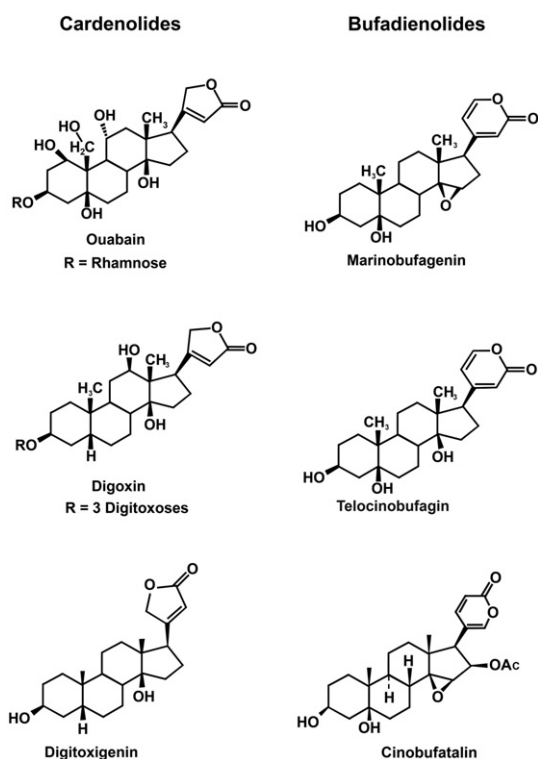
Figure 1. Chemical structures of the cardenolides (left) and bufadienolides (right).

closely associated with other key signaling proteins.[13,14]

Of the cardenolides, an ouabain-like substance,[15] endogenous digitalis-like factor,[16,17] and endogenous ouabain[18,19] have been isolated from mammals. The bufadienolides have been well described in both plants and animals.[20] Their appellation was derived from their identification in skin and venom of the common toad, *Bufo marinus*. Bufalin,[21] marinobufagenin,[22] and telocinobufagin[23] have been isolated from mammals. Currently, the most actively studied of these compounds is marinobufagenin (Fig 1). Finally, the bufadienolides appear to be more important in certain disease states than the cardenolides.[3] Pre-eclampsia may be an example of the latter circumstance.[10,12,24]

CASE VIGNETTE

A 19-year-old gravida 1 para 0 was admitted to the labor and delivery unit at 28 weeks of gestation because of new onset of hypertension. She also had noted significant edema of the lower extremities in the past 2 weeks. Before these events, she had undergone an uneventful pregnancy. Physi-

cal examination showed blood pressure of 145/95 mm Hg, pulse of 90 beats/min, and respiratory rate of 16 breaths/min. Examination of the lung fields and precordium showed no abnormalities. Abdominal examination showed fundal height of 26 weeks, and fetal heart tracing indicated no evidence of distress. There was significant pedal edema extending to the knees bilaterally. Metabolic panel showed normal electrolyte, serum urea nitrogen, creatinine, glucose, and liver enzyme levels. Apart from the physiologic anemia of pregnancy, complete blood cell count was normal. Random urine dipstick showed proteinuria (2+) on 2 separate occasions and 24-hour urine analysis showed 2.5 g of protein, whereas calculated creatinine clearance was 130 mL/min. The initial decision was made to start her on expectant management, including bed rest and steroid administration. Parameters being assessed frequently included blood pressure, proteinuria, serum chemistry, fetal heart rate, and biophysical profile. By week 29 of gestation, her condition had worsened. The patient's blood pressure reached 165/105 mm Hg and she was experiencing headaches and blurry vision. Additionally, a repeated biophysical profile showed oligohydramnios, and a nonstress test indicated fetal distress. The patient agreed to immediate delivery of the fetus. The pregnancy was terminated at 29 weeks, and the baby was transferred to the neonatal intensive care unit.

PATHOGENESIS

Essential Hypertension

The concept that circulating vasoactive substances might be involved in an etiologic and mechanistic manner in hypertension was raised first by Dahl et al[25] in parabiotic studies in the rat. This concept later was extended by de Wardner and Clarkson[26] based on exchange studies after volume expansion in the dog, and more recently by Blaustein and Hamlyn and their coinvestigators.[27-29] Their thesis is that Na^+-K^+-ATPase inhibition results in a consequent change in the sodium-calcium cotransporter in vascular smooth muscle, leading to increased intracellular calcium levels and hence increased vascular tone, resulting in hypertension.[27] Guyton[30] proposed that the relationship between vascular volume and resistance to blood flow determined blood pressure. Reflective of this physiologic relationship, Laragh et al[31] championed the idea that there are 2 forms of "essential" hypertension: (1) hypertension related to vasoconstriction, largely the result of action of the renin-angiotensin system, and (2) hypertension due to volume expansion (excess salt and water) in which renin activity is suppressed.[32] The interrelationships of these 2 mechanisms are suggested because angiotensin promotes sodium reabsorption.[33,34] Neverthe-

Table 1. Blood Levels of Marinobufagenin

Group	Marinobufagenin (nMol/L)
Healthy individuals (n = 38)	0.225 ± 0.045
Chronic renal failure (n = 24)	16.6[a] ± 5.3
Primary aldosteronism (n = 5)	13.5[a] ± 12.9
Congestive heart failure (n = 7)	1.69[a] ± 1.29
Essential hypertension (n = 27)	1.74[a] ± 0.67

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[a]$P < 0.05$ versus healthy controls.

less, it is clear that certain demographic groups behave as if the underlying pathogenesis of their hypertension relates primarily to volume expansion resulting from salt sensitivity.[35] These include African Americans, the elderly, the obese, a subset of patients with type 2 diabetes, and most likely Hispanics.

Whether the cardenolides and/or bufadienolides are important in volume expansion–mediated hypertension is still a matter of controversy. The latter debate most likely results from the following difficulties. (1) Ouabain, digoxin, and the bufadienolides are often "lumped together" as endogenous digitalis-like factors with respect to their involvement in volume expansion–mediated hypertension. (2) It is clear that these substances have differential responses and activities with respect to volume expansion and volume expansion–mediated hypertension. (3) Commercial methods for measurement of the bufadienolides are not yet available. (4) There is always the possibility that findings in experimental animals and in vitro studies are not transferable to the human condition.

With respect to the bufadienolides, the following evidence supports the premise that they have a role in the pathogenesis of those forms of hypertension related to excessive salt and water accumulation. (1) Increases in amounts of circulating and/or excreted bufadienolides in blood and/or urine of experimental animals are prominent in states of volume expansion and volume expansion–mediated hypertensive syndromes related to the accumulation of high levels of salt.[36-39] (2) Administered to experimental animals, the bufadienolides cause hypertension.[10,40,41] Additionally, Ahmad et al[42] reported that some hypertensive patients who were not using digitalis had a measurable digoxin-like immunoreactive substance in plasma. (3) In humans with hypertensive disorders associated with volume expansion (kidney failure, congestive heart failure, primary aldosteronism, and pre-eclampsia), bufadienolide levels show increases compared with controls[24,43-46] (Table 1). (4) Hypertension induced by administration of deoxycorticosterone acetate and salt to rats is resolved by the daily intraperitoneal injection of the antagonist of marinobufagenin, resibufogenin[10,47,48] (Fig 2). However, resibufogenin does not correct hypertension in a rat model in which blood pressure increase was caused by angiotensin infusion[49] (Fig 3). (5) Finally, antibodies to marinobufagenin decrease blood pressure in salt-loaded pregnant rats[10] and salt-sensitive hypertension in Dahl-S rats.[50]

Pre-eclampsia

Pre-eclampsia is a pregnancy-specific syndrome that affects 3%-10% of gestations.[51,52] It consists of new-onset hypertension and proteinuria after 20 weeks of pregnancy. Furthermore, intrauterine growth restriction (IUGR) may complicate the clinical presentation. In addition, ex-
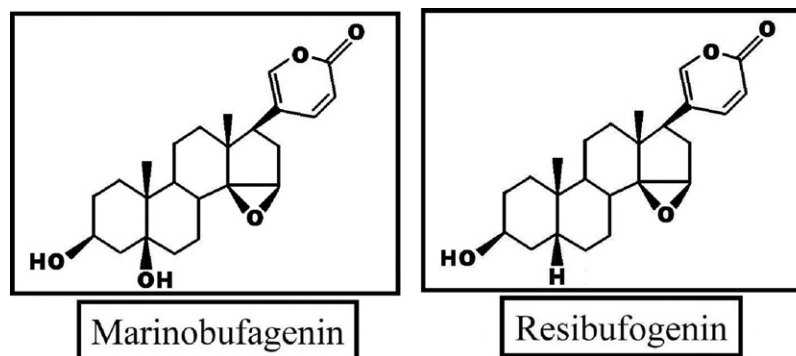

Figure 2. Structural formulas for marinobufagenin and resibufogenin. Resibufogenin differs from marinobufagenin only in the absence of a hydroxyl group in the β 5 position.
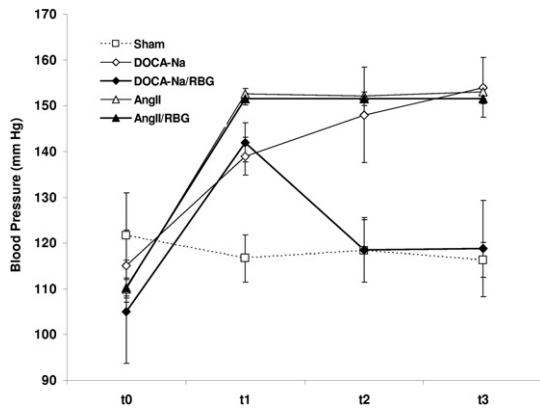
Figure 3. Resibufogenin (RBG) decreases blood pressure in an animal model of volume expansion–mediated hypertension, but is ineffective in vasoconstrictive hypertension. Abbreviations and definitions: AngII, animals infused with angiotensin II; AngII/RBG, animals infused with angiotensin II, then treated with RBG when hypertension had been established. DOCA-Na, uninephrectomized animals given deoxycorticosterone acetate (DOCA) and saline; DOCA-Na/RBG, uninephrectomized rats given DOCA and saline, then treated with RBG when hypertension was established; Sham, sham-operated animals. Time intervals: t0 = baseline; t1 = 10 days after saline and DOCA administration in DOCA-Na and DOCA-Na/RBG rats or 5 days after angiotensin II infusion in AngII and AngII/RBG groups; t2 = 3-5 days after institution of RBG in the DOCA-Na/RBG and AngII/RBG groups; t3 = 12-14 days after treatment with RBG in the DOCA-Na/RBG group and 7-10 days in the AngII/RBG group. RBG was effective in decreasing blood pressure to normal in the DOCA-Na/RBG group of rats ($P < 0.05$ vs DOCA-Na), but had no effect on blood pressure in AngII/RBG rats ($P > 0.05$ vs AngII). Reprinted from Danchuk et al[49] with permission from S. Karger AG, Basel.

cessive edema often is present. Hypertensive disorders of pregnancy are the second leading cause of maternal and fetal morbidity and mortality. The precise cause or causes of this syndrome are unknown. However, significant, reliable, and reproducible data have accumulated that favor the view that pre-eclampsia is a disorder of endothelial dysfunction[53] involving oxidative stress. Furthermore, the method for early diagnosis and treatment has not become available and prevention currently is not a possibility. Therefore, this illness represents an important example of medical unmet need. It seems clear that pre-eclampsia is not a single disorder, but a syndrome with multiple pathophysiologic factors and mechanisms.[51,52] Genetic and immunologic factors no doubt have importance in the pathophysiologic process of this disorder. In addition, there are 3 approaches to various pathogenetic

mechanisms of pre-eclampsia that presently are under intensive study: (1) the possibility that a significant segment of the pre-eclampsia population is responding to the elaboration and secretion of an excessive amount of the bufadienolides, and in particular, marinobufagenin;[54] (2) the likelihood that antiangiogenic factors are involved; imbalance of pro- and antiangiogenic factors has been suggested as contributory in the pathogenesis of pre-eclampsia;[55] and (3) the possibility that at least a proportion of pre-eclamptic patients develop agonistic autoantibodies to the angiotensin II type 1 (AT_1) receptor. Results of studies by Wallukat et al[56] indicate that pre-eclamptic patients develop stimulatory autoantibodies against the second extracellular AT_1 receptor loop. These novel autoantibodies may participate in angiotensin II–induced vascular lesions in these patients. Detailed discussion of the roles of angiogenic imbalance and autoantibodies is outside the scope of this review. Emphasis is placed on the first of these theses.

As mentioned, increased marinobufagenin levels have been reported in hypertensive disorders characterized by volume expansion and in pre-eclampsia.[24,44,45] Pregnancy is a state of expansion of extracellular fluid volume. During pregnancy, patients experience an increase in blood volume of 40%-50% by the end of the gestation period.[57] The authors and their colleagues have postulated that in patients with pre-eclampsia, excessive volume expansion very early in pregnancy has led to an initial increase in secretion and elaboration of marinobufagenin.[10] In a rat model of the syndrome, which resulted from replacement of their drinking water by saline and administration of the powerful mineralocorticoid deoxycorticosterone acetate,[58] the animals developed hypertension, proteinuria, excessive weight gain, and IUGR. The latter is a dreaded complication of the human syndrome. Interestingly, urinary excretion of marinobufagenin in these animals increased early in the course of the disorder, before the development of hypertension and proteinuria[10] (Fig 4). An important role in the pathogenesis of pre-eclampsia is believed to be inadequate placentation related to failure of cytotrophoblast cells to adequately remodel the vasculature of the uterus.[59] This results in hypoperfusion of the maternal-fetal unit, with the consequence of oxidative stress and endothelial
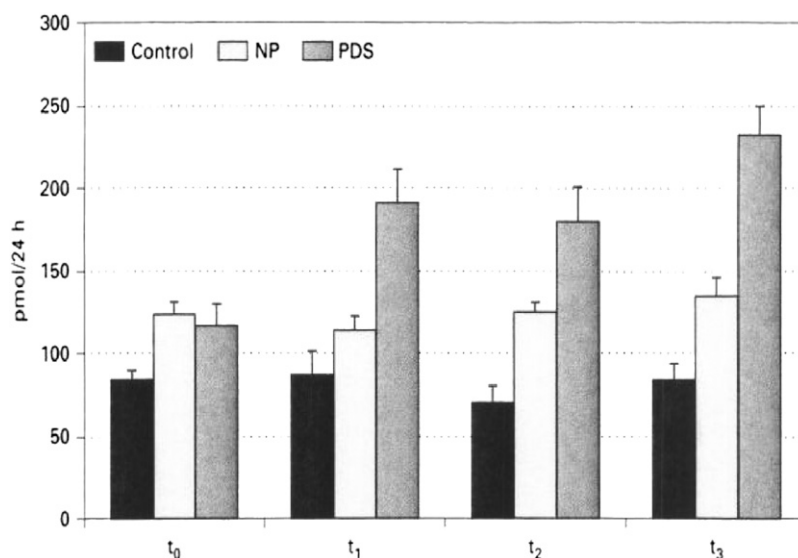
Figure 4. Urinary marinobufagenin excretion in nonpregnant (control), normal pregnant (NP), and pre-eclamptic rats (PDS; pregnant animals given 0.9% saline as drinking water and weekly injections of desoxycorticosterone acetate (DOCA) in depot form). It was found that even before hypertension is induced (t_1), a significant difference in marinobufagenin excretion exists between the control group and PDS group (86.4 ± 14.4 vs 189.7 ± 19.6 pmol/24 h; $P < 0.001$). This trend continued throughout the gestation period. Experimental periods are: t_o = Initial non-pregnant state; t_1 = 3-5 days of pregnancy; t_2 = 7-10 days of pregnancy; t_3 = 16-19 days of pregnancy. Reproduced from Vu et al[10] with permission from S. Karger AG, Basel.

dysfunction, which are responsible for the development of the syndrome.[52] Interestingly, marinobufagenin has been determined to interfere with proliferation, migration, and the invasive capacity of cytotrophoblast cells.[60,61] These are specialized cells derived from the placenta that invade the uterine decidua.[62] These cells remodel the vasculature of the uterus, converting arterioles from high-resistance small-diameter channels to wide-bore low-resistance vessels.[63] Administration of marinobufagenin to pregnant rats reproduces the syndrome, which includes hypertension, proteinuria, and IUGR.[47,64] We have recently reported the discovery of an antagonist to marinobufagenin, resibufogenin (Fig 2). Resibufogenin differs from marinobufagenin only in the absence of a hydroxyl group in the β 5 position. Resibufogenin corrects hypertension in the rat model of pre-eclampsia described,[47,58] and administered early in pregnancy, completely prevents all manifestations of pre-eclamptic syndrome.[48]

Because extracellular fluid volume expansion in pregnancy exceeds the increase in red blood cell mass, pregnant patients develop physiologic anemia.[57] Hematocrits routinely decrease from 40%-42%[65,66] to a mean of approximately 35%.[65] However, in pre-eclamptic patients, hematocrits are higher than those seen in normal pregnancy (approximately 36.5%),[65] but lower than those seen in control nonpregnant women. This finding of relative hemoconcentration in the setting of volume expansion has led obstetricians to speculate that pre-eclamptic patients show a vascular leak.[67,68] It also has been proposed for some time that this leak is related to effects of circulating vasoactive factor(s).[52] Recent studies have shown that pre-eclamptic animals provide evidence of a substantial leak in omental vessels[69] (Fig 5) and marinobufagenin is a cause of increased permeability in vivo[69] and in endothelial cell monolayers.[70] Thus, pre-eclampsia appears to be a syndrome in which both hemoconcentration and volume expansion occur simultaneously.[54,71] The concept of the pathogenesis of pre-eclampsia developed by the authors and their colleagues (Fig 6) includes excessive volume expansion, perhaps related to abnormalities in sodium disposal initially.[54,72] This leads to alterations in endothelial cell function in association perhaps with changes in pro- and antiangiogenic factors.[73] In addition, the development of agonistic
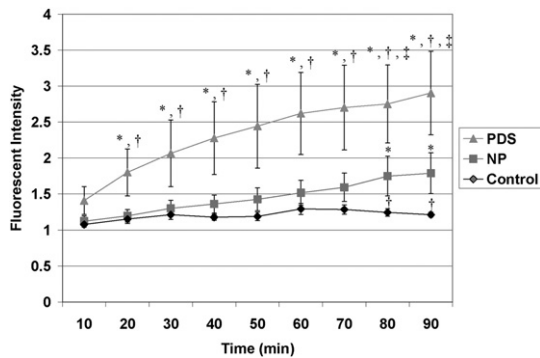
Figure 5. Comparison of vascular leakage in mesenteric postcapillary venules in 3 groups of female rats: nonpregnant female animals (control; n = 5); normal pregnant (NP) rats (n = 9); pregnant animals administered deoxycorticosterone acetate and saline (PDS; n = 9). NP rats showed leakage of dye at 80-90 minutes ($P < 0.05$ vs control). PDS rats showed significant leakage beginning at 20 minutes ($P < 0.05$ vs control and NP) compared with control and NP rats. *$P < 0.05$ vs control; †$P < 0.05$ vs NP; ‡$P < 0.006$ vs control. Reproduced from Uddin et al[69] with permission from S. Karger AG, Basel.

autoantibodies to the AT_1 receptor may have a role.[56] The upshot of all these abnormal pathophysiologic events is, as stated, hypoperfusion of the maternal-fetal unit, with the possibility that IUGR and/or other abnormalities will occur.[74] The abnormality in sodium excretion leading to excessive expansion of extracellular fluid volume is supported by evidence that pre-eclamptic patients are significantly more sodium retentive than their normal pregnancy counterparts.[72,75] This phenomenon could be related to the high deoxycorticosterone acetate levels seen in both normal and hypertensive pregnancy,[76] derived from progesterone and not the adrenal gland.[74] In addition, the authors have postulated that defective sodium transport is involved.[54]

RECENT ADVANCES

Pre-eclampsia

As mentioned, resibufogenin is a congener of marinobufagenin with a single difference in its chemical structure (Fig 2). We postulated that it might act as an antagonist of marinobufagenin in instances of volume expansion–mediated hypertensive disorders. We used as our model the competitive inhibition of another steroid hormone, aldosterone, by spironolactone. When

resibufogenin was given to animals rendered hypertensive and proteinuric in the manner previously described,[58] it decreased blood pressure to normal.[47] The same was seen in normal pregnant animals given marinobufagenin to induce hypertension.[10] When resibufogenin was given early in pregnancy, it completely prevented all manifestations of pre-eclampsia noted in this rat model, including IUGR.[48] These data suggest that resibufogenin or monoclonal antibody Fab fragments to marinobufagenin[50] may be important new therapeutic and/or preventative strategies in this syndrome. If so, these interventions would represent the first truly effective and rational pharmacologic agents in the treatment of pre-eclampsia.

As noted, we have determined that the MAPK (mitogen-activated protein kinase) system is involved in the deleterious effects of marinobufagenin on cytotrophoblast cells.[60,61] In vitro pretreatment of these cells with resibufogenin does not prevent this effect of marinobufagenin (J. Puschett, E. Agunanne, M.N. Uddin, unpublished observations, 2008). This action of marinobufagenin, which is mediated by p38 (encoded by the *MAPK1* gene) and involves activation of apoptosis, can be prevented in vitro by the addition of a p38 inhibitor[77] (Fig 7). These studies suggest that the use of p38 inhibition may be an important therapeutic strategy in pre-eclampsia in the future. However, little is known about the potential adverse effects of therapy using p38 inhibitors.

The potential involvement of cytokines is suggested because pre-eclampsia is associated with an increase in interleukin 6 release.[78] Thus, strategies to diminish the presence of this cytokine in the inflammatory cascade or combat the production of other cytokines (eg, with interleukin 10) or with anti-inflammatory agents[79] may represent another avenue in the approach to the treatment of this disorder. Interestingly, derivatives of resibufogenin have been reported to antagonize the effect of interleukin 6 and/or its receptor.[80-82] Finally, the bufadienolides have immunoregulatory properties related to their ability to inhibit T-cell activity.[83] Rostafuroxin, currently undergoing clinical trials,[84] is a novel experimental agent that has the ability to specifically interact with Na^+-K^+-ATPase.
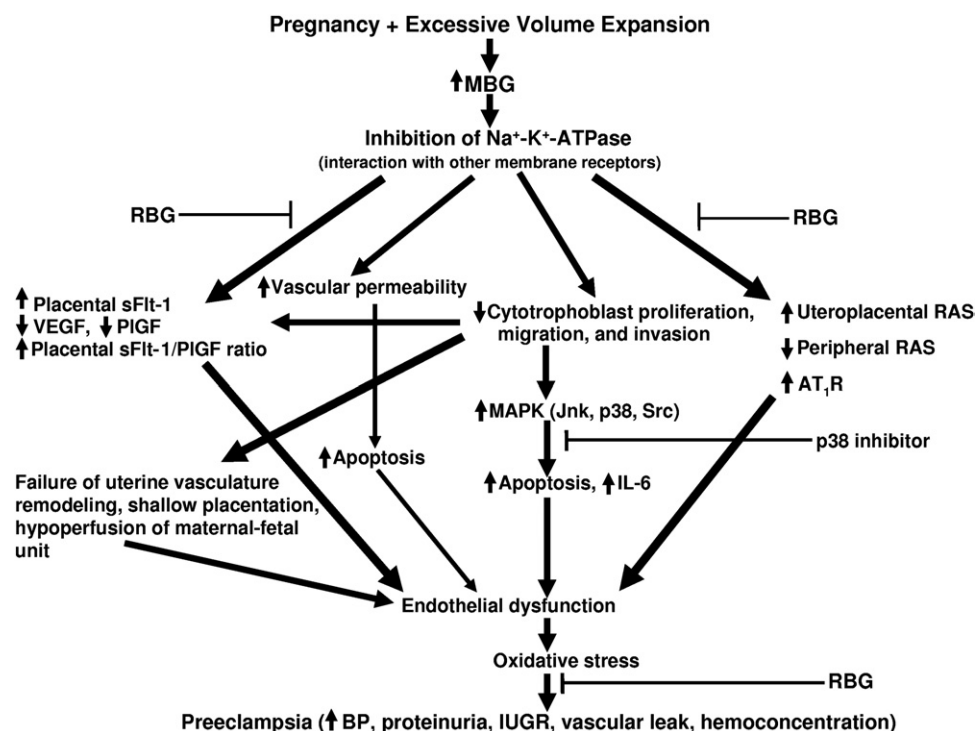
Figure 6. Proposed model of the involvement of increased marinobufagenin (MBG) levels in pre-eclampsia related to initial excessive volume expansion early in pregnancy. MBG causes defective placentation by interfering with cytotrophoblast function, resulting in a lack of vascular remodeling in the uterus. Consequently, hypoperfusion of the maternal-fetal unit eventuates. MBG also causes hypoxia and ischemia leading to an imbalance of pro- and antiangiogenic factors. Additionally, the bufodienolide causes increased vascular permeability resulting in "leak" from the intravascular compartment and consequent hemoconcentration. Its actions also result in enhancement of the uteroplacental renin-angiotensin system (RAS) and angiotensin II type 1 (AT$_1$) receptor function. The cytotrophoblast dysfunction is mediated by alterations in the MAPK (mitogen-activated protein kinase) system, which stimulates apoptosis causing disruption of endothelial cell layers. All these abnormalities culminate in the production of endothelial cell dysfunction and oxidative stress. The actions of MBG can be prevented/ameliorated by resibufogenin (RBG). Inhibition of the activity of the MAPK p38 prevents MBG-induced enhancement of apoptosis. Abbreviations and definitions: BP, blood pressure; IL-6, interleukin 6; Na$^+$-K$^+$-ATPase, adenosine triphosphatase sodium-potassium pump; IUGR, intrauterine growth restriction; PIGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1; VEGF, vascular endothelial growth factor.

Cardiovascular Disease in Chronic Kidney Disease

Chronic kidney disease is a major risk factor for the development of serious cardiovascular disease,[85,86] even in patients with mildly decreased kidney function.[86] Much of the morbidity and mortality of chronic kidney disease is related to cardiac disease, especially congestive heart failure.[87] The involvement of marinobufagenin in cardiac hypertrophy has been studied in a remnant model of chronic kidney failure in the rat.[88,89] In an elegant series of studies by Xie, Shapiro, Bagrov, and their colleagues, it has been determined that in the remnant kidney model, the development of kidney failure is accompanied

by increases in circulating levels of marinobufagenin[90] (Table 2). Additional studies showed that marinobufagenin caused increased fibroblast collagen production and fibrosis in experimental uremic cardiomyopathy.[91] There also were parallel increases in procollagen expression involving protein kinase C and Fli-1.[92] Presumably, the increases in marinobufagenin levels are related to the stimulus provided by chronic volume overload related to kidney failure.[92] Furthermore, immunization against marinobufagenin attenuated the process of cardiac hypertrophy with its attendant impairment in diastolic function. It also decreased cardiac fibrosis and oxidative stress.[90] Very recently, it has been shown that
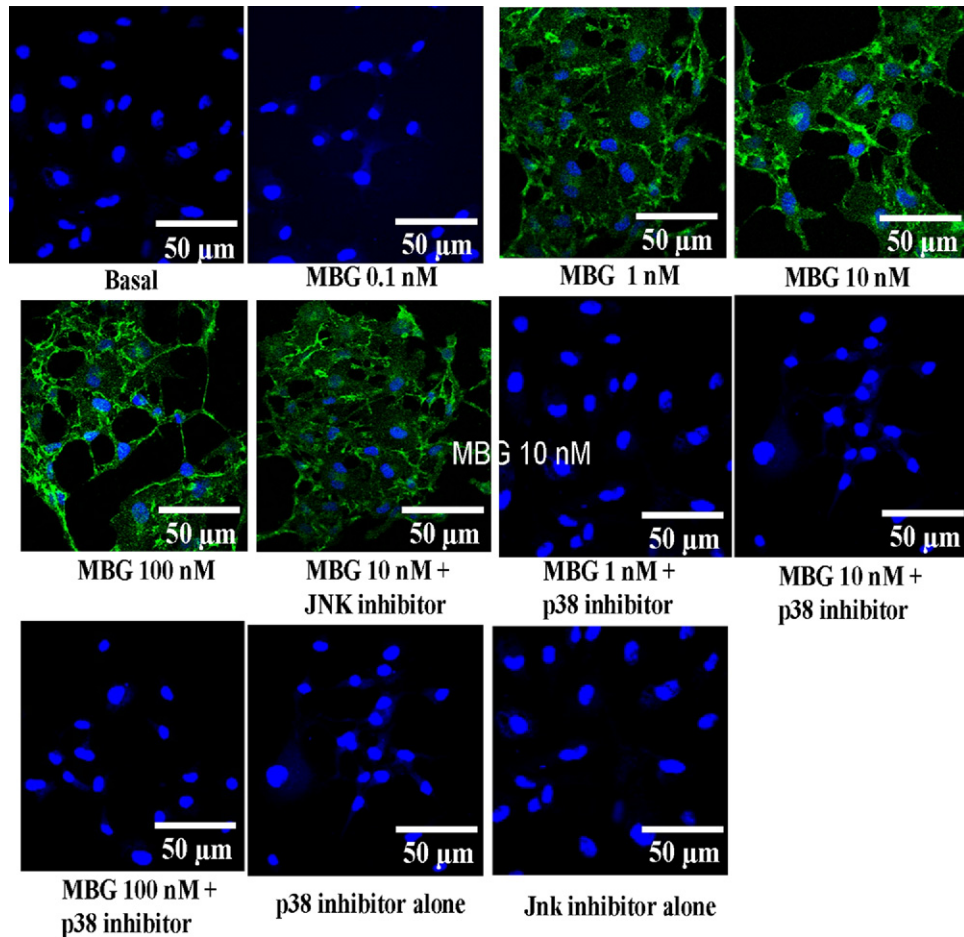
Figure 7. Evaluation of apoptosis. Green staining is annexin V positive, indicating an apoptotic cell; nuclei are stained blue with DAPI (original magnification, ×60). The 1, 10, and 100 nM marinobufagenin (MBG)-treated cells were apoptotic, while 0.1 nM MBG had no effect. Apoptosis was prevented in cells pretreated with a p38 inhibitor, but not by a Jnk inhibitor. Jnk, p38, and pan-caspase inhibitors alone had no effect. Reproduced from Uddin et al[70] with permission of The American Physiological Society.

spironolactone attenuates cardiac fibrosis and cardiomyopathy in both partial nephrectomy and marinobufagenin infusion models. Based on these findings, Tian et al[93] proposed that marinobufagenin might be responsible for some of the cardiac injury that has been attributed to aldosterone and that mineralocorticoid antagonists may produce some of their beneficial effects by antagonism of marinobufagenin signaling through Na^+-K^+-ATPase. Thus, if the animal experimentation described has application to human disease, control of marinobufagenin excess in kidney failure might provide an important therapeutic tool in the control of cardiac disease in chronic kidney disease.

Marinobufagenin in Kidney Disease

Using the experience gathered in their studies of cardiac disease in kidney failure outlined previously, Fedorova et al[94] extended their experiments to the possible involvement of marinobufagenin in the causation of kidney fibrosis. Marinobufagenin infusion induced periglomerular and peritubular accumulation of collagen type I in the renal cortex. This effect of marinobufagenin could be caused by the induction of TGFβ1 (isoform 1 of transforming growth factor β) upregulation by the renin-angiotensin-aldosterone system. Additionally, the profibrotic transcription factor snail, a key regulator of epithelial-mesenchymal transition, was expressed in both

Table 2. Effects of MBG Administration and Immunization Against MBG

	Sham	Partial Nephrectomy	MBG Infusion	Partial Nephrectomy + Immunization
Plasma MBG (pmol/L)	359 ± 16	564 ± 36[a]	546 ± 34[a]	430 ± 36[b]
Urinary MBG excretion (pmol/24 h)	31.3 ± 2.3	60.2 ± 4.5[a]	49.1 ± 3.5[a]	44.7 ± 4.0[b]
Plasma creatinine (mg/dL)	0.30 ± 0.03	0.95 ± 0.12[a]	0.52 ± 0.07	0.95 ± 0.13[a]
Hematocrit (%)	44.5 ± 0.8	38.8 ± 1.3[a]	44.6 ± 0.9	41.1 ± 0.7[a]

Note: Analyses were performed 4 weeks after sham operation (n = 16), partial nephrectomy (n = 20), MBG infusion (n = 14), or immunization against MBG before partial nephrectomy (n = 18).

Abbreviation: MBG, marinobufagenin.

[a]$P < 0.01$ versus sham; [b]$P < 0.05$ versus partial nephrectomy.

Source: Kennedy et al.[90]

cortical and medullary tubular epithelial cells in marinobufagenin-treated kidneys. This suggests that marinobufagenin has a potential causative role in this epithelial-mesenchymal transition.[94] This transition during the pathologic process associated with organ injury and inflammation can lead to the development of fibrosis.[94-97] Strutz et al[98] had observed new expression of fibroblast-specific protein 1 (FSP1) in typically negative tubular epithelial cells at the site of interstitial inflammation, as well as in tubular epithelial cells undergoing transition to fibroblasts. This suggests that epithelial-mesenchymal transition may be mediated through expression of *S100A4*, the gene for FSP1. Should further investigation applying these data to human disease prove successful, marinobufagenin could represent an important target for the prevention/treatment of kidney disease progression.

SUMMARY

The bufadienolides are a group of steroid compounds with structural similarity to their "cousins," the cardenolides. Both groups, included in the class of agents referred to as cardiac glycosides, are believed to function largely through their ability to inhibit the ubiquitous enzyme Na^+-K^+-ATPase. Although originally investigations into their actions focused on their ability to cause vasoconstriction (and therefore hypertension), natriuresis, and cardiac inotropy, it has become clear that they have important functions in the areas of cell growth and differentiation, as well as in pathways of cellular signaling. Moreover, in animal models, antagonism of the best studied bufadienolide, marinobufagenin, has resulted in beneficial effects in a varied group of disorders. These include models of volume expansion–mediated "essential" hypertension, pre-eclampsia, uremic cardiac disease, kidney fibrosis and the progression of chronic kidney disease, and vascular leak syndromes. These observations provide encouragement and impetus for attempts to extend these in vivo and in vitro findings to human disease. They represent a fertile environment in which future developments point toward improvements in our ability to better prevent and manage troublesome disease states.

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