Involvement of the Bufodienolides in the Pathogenesis and Potential Therapy of Preeclampsia, the Acute Respiratory Distress Syndrome and Traumatic Brain Injury

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Abstract

The purpose of this review is to provide detailed information on the evidence for marinobufagenin (MBG) as a predictive and causative factor in preeclampsia (PE), the acute respiratory distress syndrome (ARDS) and traumatic brain injury (TBI). In addition, evidence is provided that resibufogenin (RBG), the antagonist of MBG is effective in the treatment of all three diseases. Results from experiments conducted on animal models and in human subjects indicate that patients with PE, ARDS and TBI have increased urinary and serum MBG levels. In PE patients, MBG is elevated in the early stages of pregnancy. In ARDS, MBG was elevated in serum samples of hyperoxic rats. MBG levels were also elevated in concussed athletes and in rat studies in which TBI was induced. In the animal models, all three disease processes were prevented/treated by the administration of RBG. Human trials of MBG as a predictor of PE, ARDS and TBI are underway as are studies of RBG as a therapy with respect to its usefulness and safety. Early detection of PE will significantly reduce its effects on pregnancy. ARDS, which has a high mortality rate, would benefit from studies on employing RBG. TBI patients can be diagnosed much more quickly than currently possible utilizing MBG as an early indicator. Furthermore, RBG may serve as a therapy.

Keywords: Marinobufagenin; Resibufogenin; Preeclampsia; Acute Respiratory Distress Syndrome; Traumatic Brain Injury

Introduction

The members of the investigative group involved in the discussions in this review began their studies with an examination of the pathogenetic processes involved in the pregnancy-specific illness, preeclampsia (PE). As their investigation proceeded, they became aware that their findings, especially those related to the pathogenetic role of a group of steroid hormones, the “cardiotonic steroids” or “cardiac glycosides” [1] might be applicable as well to the pathogenesis of three other disease processes. These include, thus far, the acute respiratory distress syndrome (ARDS) and the neurotrauma disorders, traumatic brain injury (TBI) and the post-traumatic stress disorder (PTSD). The research efforts involved in the investigations of these processes have produced a pattern of tissue injury in three separate organ systems that appear to share the common denominators of inflammation, vascular damage and “leak” and involvement with the members of the bufodienolide family of agents. The latter represent a group of hormones which share the ability to inhibit the actions of the ubiquitous enzyme, sodium/potassium ATPase (Na/K ATPase) [1]. Our investigative studies began with an evaluation of the potential roles of the bufodienolides in these three seemingly very different disease processes. All three of these entities appear to involve the bufodienolides, marinobufagenin (MBG) and resibufagenin (RBG), in their pathophysiology and, perhaps, their treatment. We begin with a discussion of PE.

Figure 1: Endogenous Cardiac Glycosides. (Cardenolides (left column), Bufadienolides (right column)).
As pregnancy proceeds, and beginning at about 6-8 weeks, blood volume begins to increase. While red cell mass also increases, its growth lags behind that of body volume (Figure 2).

Accordingly, the hematocrit value falls, such that as the pregnancy proceeds, a so-called “anemia of pregnancy” is noted, as depicted in Table 1. However, as shown also in Table 1, the hematocrit of preeclamptic patients is elevated compared to that of normal pregnant patients. Thus, the “leak” of fluid from the vascular space of PE patients has proceeded [2].

Table 1: Representative values for the hematocrit determination in non-pregnant, normal pregnant and preeclamptic women.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>AVERAGE HEMATOCRIT VALUES</th>
</tr>
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<tbody>
<tr>
<td>Non-pregnant</td>
<td>42%</td>
</tr>
<tr>
<td>Normal pregnant</td>
<td>35%</td>
</tr>
<tr>
<td>Preeclamptic</td>
<td>39%</td>
</tr>
</tbody>
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Hamlyn and his collaborators [2] and Morrow, et al. [3] determined that an endogenous inhibitor of the sodium pump circulates in human plasma and that its concentration correlates with blood pressure. These substances, the cardiotonic steroids, act on the sodium pump, present in all cells. In addition to human plasma, the cardiac glycoside, ouabain, the precursor of digoxin (Figure 1) has also been found in the adrenal gland and in the hypothalamus [1-3]. These steroids appear to be synthesized primarily in both the zona glomerulosa and fasciculata of the adrenal cortex [4] as well as, perhaps, in the placenta and brain [1]. MBG exhibits a significant affinity for the ouabain-resistant α1 subunit of the Na+/K+ ATPase [5]. Because the α1 isoform of MBG is the major form of the glycoside in the kidney, the major effect of MBG in the kidney to inhibit the enzyme plays a major role in regulating sodium transport in this organ and, therefore, in the body. Thus, the inhibition of the Na+/K+ ATPase pump is importantly involved in the mechanism by which MBG regulates alterations of sodium excretion/reabsorption in the renal tubular system [6].

Other Hypertensive States

Approximately 90-95% of hypertensive patients are classified as “essential,” while the remaining 5-10% are the result of secondary causes including such disease states as chronic kidney disease, pheochromocytoma, primary hyperaldosteronism, etc. [7] In turn, in patients with essential hypertension, the primary pathophysiologic event is related either to excessive volume expansion (a function of the excessive retention of salt and water), or vasoconstriction. The relationship between blood flow (Q), vascular resistance to flow (R) and blood pressure (P) is: P = Q x R. Solving for pressure, the formula becomes: P = Q x R. Evidence that excessive volume expansion is an important causative mechanism in PE has been provided by experiments performed by Chesley and his associates [8,9]. These workers determined that PE patients infused with saline demonstrated reduced excretion of sodium in the urine compared with both normal pregnant patients and those with pregnancy-related hypertension (Figure 3) [10].

Thus, it has been hypothesized that PE patients are sodium retentive, and, therefore, volume expanded. In addition, examination of the hematocrit values of PE patients compared to their normal pregnant counterparts (Table 1) reveals the following: although both groups of patients demonstrate the reduction in hematocrit associated with the greater accumulation of fluid than the increment in red cell mass (Figure 2), PE patients demonstrate a higher hematocrit value than do normal pregnant patients (Table 1). These observations indicate that PE patients are not only volume expanded but are also hemoconcentrated. They give evidence of a “vascular leak” as indicated by a comparison of their hematocrit values compared to those of patients undergoing normal pregnancy (Table 1). Evidence has been marshalled that this “vascular leak” is a consequence of the secretion and elaboration of MBG from early in the preeclamptic state [11].
Furthermore, evidence has been developed which indicates that MBG levels are elevated in human PE compared to those of normal pregnancy [11,12]. This increase in MBG begins to occur in early pregnancy as demonstrated in an animal model of preeclampsia (Figure 4). Furthermore, the administration of MBG to animals from early in gestation results in the development of a syndrome which resembles human PE [13]. The introduction of volume expansion in normal rat pregnancy results in MBG levels above those seen in the urine of animals undergoing normal (rat) pregnancy (Figure 4).

In this model, not only do the animals become hypertensive and proteinuric, but they also deliver fewer pups than do normal rats and approximately 18% of those pups are developmentally abnormal (Figure 5) [14-16]. If, on the other hand, RBG, the antagonist of MBG, is administered from early pregnancy to rats destined to become "preeclamptic," the entire syndrome of rat "preeclampsia" is prevented [17,18].

Summary

In summary, 1) the preeclamptic picture in the animal model can be induced either by volume expansion or by the administration of MBG from early in pregnancy. 2) MBG causes hyperpermeability of the endothelial cell layer of the vasculature [24]. 3) This circulating "cardiotonic" steroid also interferes with the process of cell proliferation in the uterine mucosa. 4) The antagonist of MBG, RBG, (Figure 7) if given from early in the gestation period prevents the "preeclamptic" picture in the rat model [25]. 5) Urinary MBG levels are elevated in approximately 85% of patients with PE, compared to normal pregnant patients. These findings, taken together, strongly support the view that the bufodienolides are important in the production of human preeclampsia. 6) Furthermore, our experimental results suggest that RBG may prevent the PE syndrome, if given from early in pregnancy [25].
The Acute Respiratory Distress Syndrome

The acute respiratory distress syndrome (ARDS) is a pathophysiological abnormality resulting from inflammation and increased permeability of the alveolar endothelial and epithelial cell barrier [24]. It has a central pathogenesis in common with preeclampsia (PE), a syndrome characterized by volume expansion [25]. In PE, excessive volume expansion interferes with the functioning of the cytotrophoblast cells resulting in vascular leakage of the endometrium. Interestingly, hyperpermeability of the pulmonary vasculature also causes ARDS. ARDS is a life-threatening condition identified by hypoxemia, dyspnea and the presence of bilateral pulmonary opacities [26]. Certain risk factors such as septic shock, trauma and exposure to toxic chemicals potentiate the occurrence of ARDS [27-30]. The current mortality rate in ARDS varies between 40 and 52% depending upon the severity of toxic exposure and the patient’s previous health status [31]. However, ventilator measures utilized to attempt to better oxygenate these patients often lead to alveolar damage [32-34].

The disruption of the alveolar-capillary membrane is central to the pathogenesis of ARDS. The loss of integrity of the epithelial and endothelial cell membranes results in the exudation of protein-rich fluid into the air spaces of the lungs producing a picture of pulmonary edema [24]. During the initial phase of ARDS, polymorphonuclear neutrophils along with monocytes and macrophages mediate the involvement of pro-inflammatory cytokines that include interleukin-8 and tumor necrosis factor-α [35-37]. Stimulation of these factors potentiates the enhancement of permeability of the epithelial and endothelial membranes. A close resemblance can be found to preeclampsia in which increased vascular permeability is the hallmark of its pathogenesis [25].

The disruption of vascular endothelial cadherin (VE-cadherin) causes the breakdown of the endothelial barrier and the disruption of its agonist- TNF (tumor necrosis factor), thrombin and vascular endothelial growth factor (VEGF) [38,39]. In fact, the microarray analysis of genetic expression in cytotrophoblast (CTB) cells treated with MBG show down-regulation of the soluble VEGFR transcript, sflt by 59%. Concomitantly, we have seen that MBG increases the permeability of endothelial cells in a concentration dependent manner (Figure 8) [40].

The similarity between the mechanism of action of MBG in previously studied disorders such as preeclampsia and its pathophysiologic role in the disruption of vascular integrity has stimulated interest in the study of the pathogenesis of ARDS.

In an animal model of PE, the increased excretion of MBG well before the onset of the manifestations of the illness has provided evidence for the consideration of MBG as a biomarker [24]. We have previously shown that MBG is significantly elevated in ICU patients diagnosed with ARDS (Figure 9) [41].

Figure 7: Structures of marinobufagenin (MBG) and its antagonist, resibufogenin (RBG).

Figure 8: The bar graph shows the permeability coefficient of monolayer human brain endothelial cells (HBMECs) exposed to increasing concentrations of MBG. As noted, MBG increased the permeability of HBMECs in a concentration dependent manner.

Figure 9: This plot shows that urinary MBG levels (pg MBG/mg creatinine) were significantly elevated in ARDS patients compared to control ICU patients.
if the elevated level of MBG is sustained throughout the duration of the syndrome. In a study by Thickett et al, it was noted that VEGF levels in epithelial cell lining fluid (ELF) are reduced in early ARDS but that they are elevated because of increasing levels in patients with resolving lung injury [43]. These evidences have generated an interest in studying the role of MBG as a pathogenetic factor and a biomarker for ARDS.

The ability to reverse the increased permeability of the alveolar-capillary membrane and the removal of protein-rich fluid in the air sacs and interstitial spaces in ARDS are considered important methods to improve oxygenation, shortening the duration of mechanical ventilation and increasing the likelihood of survival for ARDS patients [44,45]. Multiple biomarkers have been employed in preclinical and clinical trials to identify patients most likely to develop ARDS. However, only a few have shown promise in evaluating the response to treatment [46]. RBG, the antagonist of MBG, has been studied in both PE and ARDS. RBG is a bufadienolide which differs from MBG only in the absence of an hydroxyl group in the -5 position of the molecule. RBG is a proven antagonist to MBG and its role in the prevention and progression of disorders characterized by inflammation are extensively noted [1].

MBG has been found to be elevated in serum samples of hyperoxic rats. The hematoxylin and eosin (H&E) stains of hyperoxic rat lungs show low recruitment of alveoli, the presence of large distended airspaces and the infiltration of proteins in interstitial lung spaces indicating pulmonary edema and inflammation (Figure 10) [41].

When RBG was administered to hyperoxic rats, the level of MBG was significantly reduced in conjunction with an improvement in the histoarchitecture of the lungs [41]. Moreover, there is a relatively lower recruitment of neutrophils in alveoli and alveolar ducts. These changes in the presence of neutrophils can be attributed to translocation of these cells across the endothelial membrane that correlates with lung injury [47]. The emigration of neutrophils from airspaces upon the administration of RBG (Figure 4) indicates a possible reversal in the pathogenetic state of the hyperoxic lungs. These changes include reduction in the inflammation and permeability of the alveolar capillary membranes [41].

### Traumatic Brain Injury

Traumatic brain injuries (TBIs) are a growing public health concern. Any disruption in normal brain function resulting from trauma to the head is defined either as traumatic brain injury (TBI) or concussion. In fact, 30% of all injury-related deaths and disability in the United States are attributed to TBI [48]. According to the CDC, in 2010, TBI contributed to approximately 50,000 deaths with TBI directly or indirectly involved in 280,000 hospitalizations and 2.2 million emergency department visits 48. The leading causes of TBI are falls, blunt trauma, motor vehicle accidents and assaults. Falls account for nearly 40% of cases of TBI exhibiting a bimodal age distribution of cases; 0 - 14 years and >65 years. The pathophysiology of TBI involves cellular (endothelial vascular changes), metabolic (biomarker changes) and calcium ion changes. This has also been seen in experimental concussion in an animal model accompanied by axonal injury [48,49]. During the phase of recovery, the concussed brain is at risk for greater damage with a repeat blow [50,51]. Cases of increased dysfunction and disability after a second concussion are also seen in young children and adolescents [48,49]. This raises questions as to the utility of employing neurocognitive testing (NCT) assessments as evidence of complete recovery from an initial concussion. Sport-related TBI has increased in annual concussion rates due to increased awareness and reporting.

The disordered histologic picture of hyperoxic lungs also correlates with the infiltration of neutrophils into the alveolar air spaces. In some studies, the presence of neutrophils has been described during the early phases of the syndrome (Figure 11) [35,36].

![Figure 10: H&E stains: A comparison between, A. Rats exposed to room air given only sesame oil intraperitoneally: B. Rats exposed to room air given RBG in sesame oil: C. Rats exposed to hyperoxia for 48 hours and given sesame oil compared to :D. Rats exposed to hyperoxia for 48 hours and given RBG. RBG given in hyperoxic rats show improved alveoli recruitment, lower lung edema helping in the healing of the perivascular injury, and interstitial inflammation.](image1.png)

![Figure 11: Immunohistochemistry demonstrating neutrophil recruitment. A. Rats exposed to room air given sesame oil: B. Rats exposed to room air given RBG in sesame oil: C. Rats exposed to hyperoxia for 48 hours and given sesame oil: D. Rats exposed to hyperoxia for 48 hours and given RBG in sesame oil. The administration of RBG revealed the lower recruitment of neutrophils compared with rats with hyperoxia.](image2.png)
MBG was measured several weeks further post-concussion [65]. In Figure 12, are shown the values of the MBG concentrations in 110 concussed athletes. The pre-training values of MBG and post-concussed samples showed a marked difference. The difference was also seen in the symptom score and the MBG values as well as the NCT test results [65].

![Graph showing MBG levels in concussed athletes](Image)

**Figure 12:** MBG levels in concussed athletes (filled circles) compared to those obtained prior to spring training (filled diamonds).

In previous experiments on rats in which TBI was induced, MBG levels were elevated when compared to controls and these levels came to normal after the rats were given resibufagenin (RBG) (the antagonist to MBG) 24 hours after concussion [64]. Histology performed in rats to which RBG was administered showed reduced gliosis and vascular damage [64]. Studies have shown that MBG results in increased endothelial cell layer permeability through apoptotic changes [19,64]. The oxidative stress caused by the MBG was shown to be prevented in the rat PE model, by the administration of RBG [23].

**Summary**

MBG levels are elevated in concussed athletes and in the studies in which TBI was induced in rats. MBG causes vascular leak through the blood brain barrier and results in further damage to the brain tissue. From the animal experiments and the histologic observation and results from the concussed athletes, MBG was found to be an excellent biomarker to evaluate the progression of inflammation in TBI and can be used in association with imaging modalities to monitor the recovery of TBI patients. In TBI induced rats, urinary MBG was elevated compared to that obtained in controls and was reduced to normal levels in rats treated with RBG, 24 hours after the contusion. RBG reduced gliosis and vascular injury and prevented scar formation. Studies of the possible involvement of MBG and RBG in PTSD are underway.

**References**


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Pathophysiology

Since TBI symptoms are often the result of cellular damage, they may be related to inflammatory processes at work [62,63]. They may often involve vascular leak across the blood brain barrier [40]. This damage results in an increase in the level of the biomarker, MBG. Activation of MBG then upregulates apoptosis, resulting in an alteration in gap junctions and further brain damage [62-65] and is accompanied by evidence of inflammation [66,67]. MBG disrupts the integrity of the human brain endothelial cell (HBMEC) monolayer [40], thus increasing permeability [20]. Experiments performed in this laboratory have demonstrated that an increase in the VEGF receptor was regulated by MBG in HBMEC [40]. MBG was found to increase the permeability of the endothelial monolayer cells and was responsible for gene expression effects [40]. Previous studies have shown VEGF to be important for endothelial cell function in the blood brain barrier [66]. Examination of two of its receptor transcripts (i.e. FVt3 and sFLT) was conducted. These encode proteins, which result in an accumulation of VEGF at the injury site and also in vascular leak at other sites. MBG also regulated numerous gene products [40], which are involved in cell adhesion. ENKUR mRNA was the only gene confirmed to encode proteins, which result in normal in microarrays [40].

The activity of MBG in blood and urine have shown promise in the PTSD determination as well (Puschett JB, et al. unpublished observations). In addition, determinations of the bufadienolide, marinobufagenin (MBG) in blood and urine have shown promise in the PTSD determination as well (Puschett JB, et al. unpublished observations).

**MBG as a Biomarker in concussed subjects**

Our investigation included measuring urinary concentrations of MBG at various time intervals before and after concussion and measuring ELISA on these samples with polyclonal antibodies. The value of MBG obtained was plotted versus the symptom score. The symptom score was obtained at several time points during the first week to 10 days post-concussion until the score returned to baseline.


