Gynecology & Obstetrics
ISSN: 2161-0932

Chen, et al., Gynecol Obstet 2018, 8:1
DOI: 10.4172/2161-0932.1000460

Review Article Open Access

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

Qingzheng Chen, Kamran Abbas, Muppala Raju and Jules B. Puschett*

Texas A&M Department of Medicine, College of Medicine and College of Veterinary Medicine and Biosciences, College Station, Texas, USA

***Corresponding author**: Jules B. Puschett, Texas A&M Department of Medicine, College of Medicine and College of Veterinary Medicine and Biosciences, College Station, Texas, USA, Tel: (713) 320-5432; E-mail: jbpuschett@aol.com

Recieved date: January 17, 2018; **Accepted date**: January 29, 2018; **Published date**: January 31, 2018

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: Marinobufogenin; 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.

Preeclampsia

Shown in Figure 1 are the chemical structures of the related, but different, two groups of agents, the cardenolides and the bufodienolides, which make up the two groups of compounds collectively called the "cardiotonic steroids" (or, "cardiac glycosides"). These two clusters of steroid hormones differ structurally in that the cardenolides possess 5 lactone rings whereas the bufodienolides have 6 such components (Figure 1).

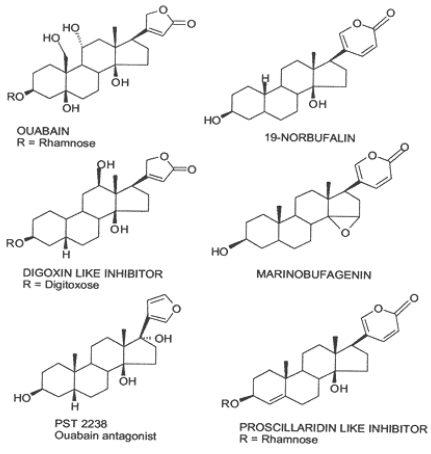

Figure 1: Endogenous Cardiac Glycosides. (Cardenolides (left column), Bufadienolides (right column).

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).

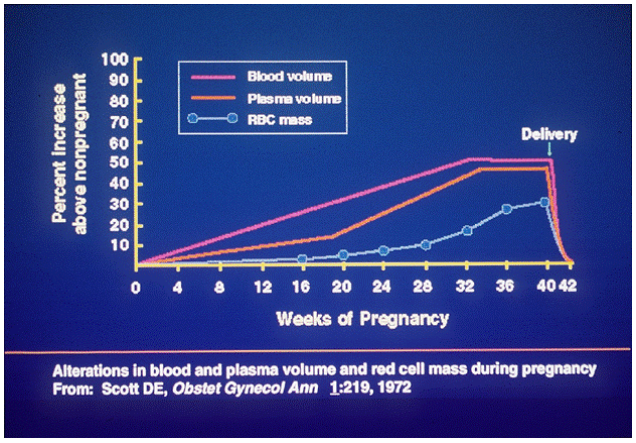

Figure 2: Alterations in blood and plasma volume and red cell mass during pregnancy.

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].

GROUP	AVERAGE HEMATOCRIT VALUES
Non-pregnant	42%
Normal pregnant	35%
Preeclamptic	39%

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

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: Q=P/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].

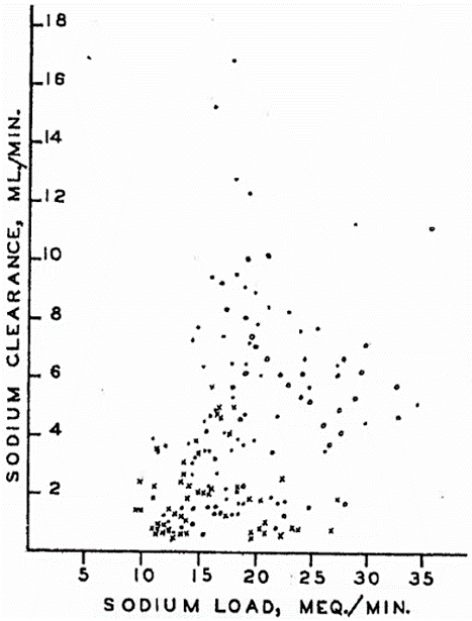

Figure 3: The relationship between the filtered load of sodium and its renal clearance. Open circles=normal pregnant women; solid circles = normal non-pregnant women; x = preeclamptic patients.

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).

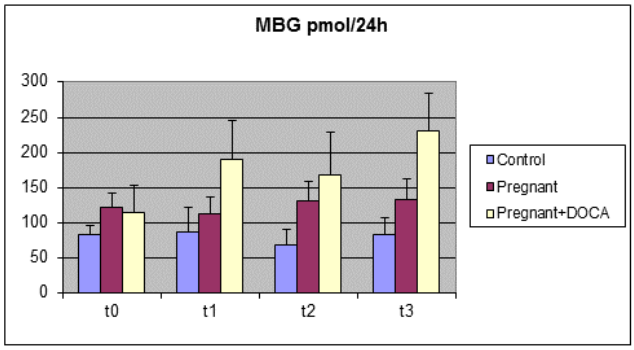

Figure 4: MBG levels in a rat model of preeclampsia. t0 = time at which pregnancy was established; t1=3-5 days of pregnancy; t2=7-10 days of pregnancy; t3=18-20 days of pregnancy just prior to delivery. At time t1, MBG values are already elevated. They remain elevated throughout the remainder of pregnancy in both the normal pregnant animals and those in which volume expansion was produced, leading to the rat version of preeclampsia. MBG levels remained elevated in those animals which became "preeclamptic" as a result of volume expansion. The latter state was induced by replacing the tap water provided with saline solution and the weekly injection of desoxycorticosterone acetate (DOCA).

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].

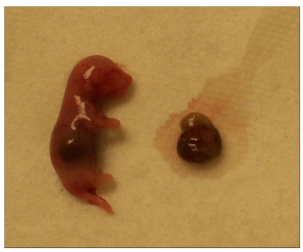

Figure 5: Comparison of the development in a normal pup (on the left) and an abnormal one (on the right).

The causation of vascular leak by MBG involves the induction of vascular endothelial cell monolayer hyperpermeability by the mechanism of altered apoptotic signaling [19]. Studies performed in endothelial cell monolayers have revealed that the action of MBG was attenuated by ERK, p38 and caspase inhibition [20]. MBG significantly decreased the phosphorylation of ERK 1/2 and activated the phosphorylation of Jnk and p38. In addition, MBG increased the expression of caspases 3/7, 8 and 9, indicating activation of apoptosis of the endothelial cell junctions. This effect was prevented by a pan caspase inhibitor [20].

Additionally, MBG inhibits the proliferation and migration of both cytotrophoblast and CHO cells [21] further interfering with the maturation process (Figure 6). Finally, rats in which PE had been produced by the administration of DOCA and the replacement of tap water with saline as drinking water, demonstrated increased superoxide production by NADPH oxidase, superoxide degradation of BH4 and uncoupled eNOS which contributed to endothelial dysfunction [22]. Furthermore, RBG administration prevented oxidative stress in a rat model of human PE [23].

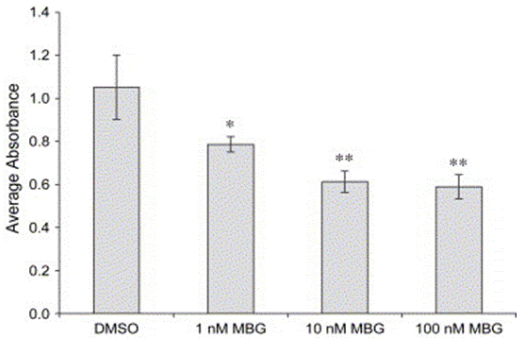

Figure 6: Inhibition of cell proliferation by MBG. Serum-starved SGHPL-4 cells were treated with DMSO (vehicle) or 1, 10 or 100 nM MBG in the presence of 10% FBS for 48 h at 37°C and cell proliferation was measured using the Cell Titer96 Aqueous Assay. Cell proliferation was significantly inhibited in MBG-treated cells as compared to DMSO-treated groups ($^{*}p<0.05$, $^{**}p<0.001$). The mean was calculated from the average of 8 replicates per experimental condition and the results presented are the mean ± sem from a representative experiment. The experiment was performed a total of 3 times.

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].

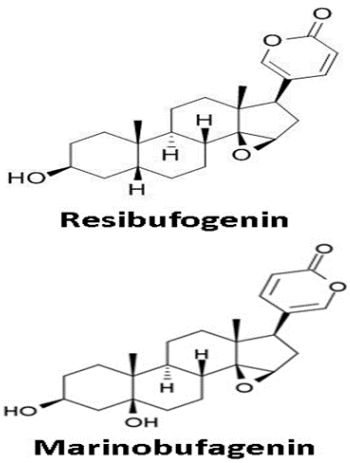

Figure 7: Structures of marinobufagenin (MBG) and its antagonist, resibufogenin (RBG).

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].

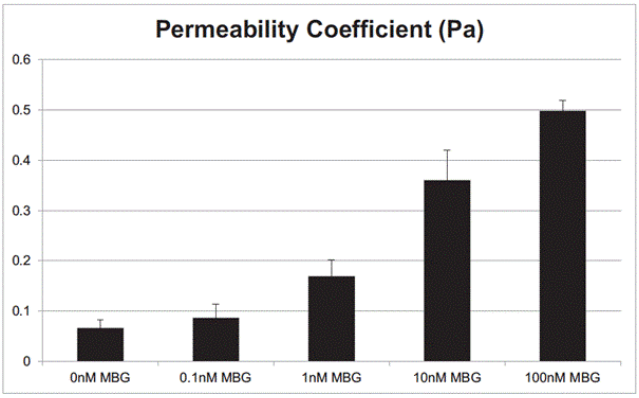

Figure 8: The bar graph shows the permeability coefficient of monolayer human brain endothelial cells (HBMECs) exposed to increasing concentrations of MBG. As noted, MBG increased the permeability of HBMECs in a concentration dependent manner.

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].

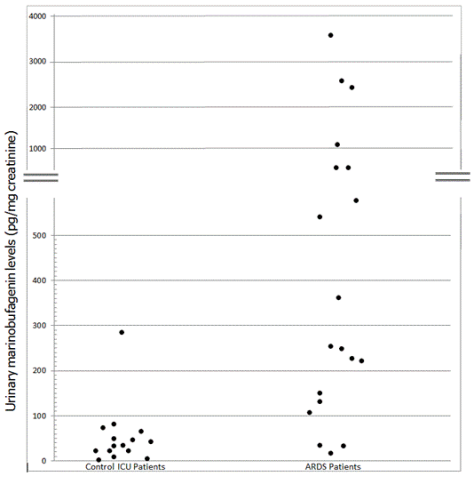

Figure 9: This plot shows that urinary MBG levels (pg MBG/mg creatinine) were significantly elevated in ARDS patients compared to control ICU patients.

However, further investigations are required to document that MBG is indeed elevated before the onset of the first clinical insult in the lungs occurs as described in the epidemiologic parameters of the Berlin Conference [42]. Moreover, it would be interesting to determine

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].

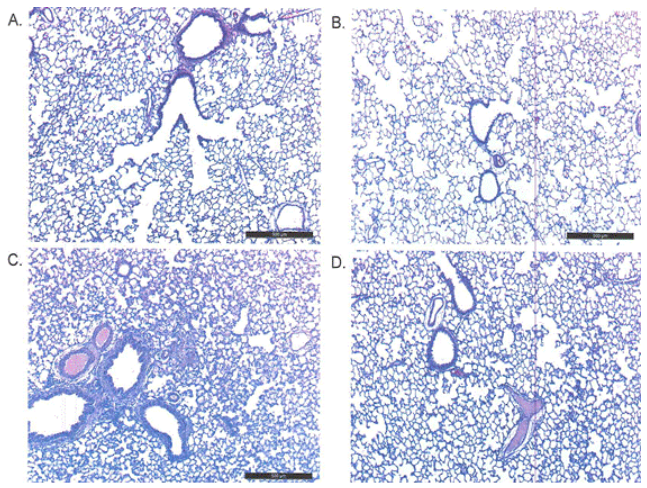

Figure 10: H&E stains: A comparison between, A. Rats exposed to room air given only sesame oil intraperitonealy: B.Rats exposed to room air given RBG in sesame oil: C. Rats exposed to hyperoxia for 48 hours and given sesame oil compared to :D. Rats exposed to hyperoxia for 48 hours and given RBG. RBG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

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].

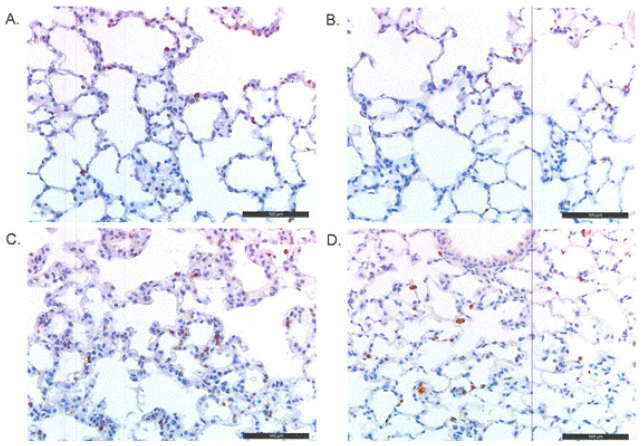

Figure 11: Immunohistochemistry demonstrating neutrophil recruitment. A. Rats exposed to room air given sesame oil: B. Rats exposed to room air given RBG in sesame oil: C. Rats exposed to hyperoxia for 48 hours and given sesame oil: D. Rats exposed to hyperoxia for 48 hours and given RBG in sesame oil. The administration of RBG revealed the lower recruitment of neutrophils compared with rats with hyperoxia.

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

[52,53]. Studies indicate that athletes with TBI may become symptom free in approximately 7 days after an injury [54]. A NCT may indicate deficits still present, but the importance of a positive NCT with no symptoms of TBI is unknown [55,56]. Newer imaging modalities such as functional magnetic resonance imaging (fMRI), positron emission tomography (PET) and single photon emission computed tomography (SPECT) can detect minor structural abnormalities but their clinical relevance is still unclear [57-61]. However diffusion tensor imaging (DTI) studies have shown progress in detecting lingering anatomic abnormalities (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).

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. FLTv3 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 product upregulated, confirmed by PCR. The ENKUR protein was shown to interact with calmodulin and transient receptor cation channel proteins [67]. qPCR also confirmed that on the HBMEC, MBG downregulated ITGA2B, GRIN2C, FERMT1, and TMEM207 genes, as earlier identified in microarrays[40]. These genes encode for surface receptors on cells through which they attach to fibronectin (ITGA2B), and encode for proteins which are calcium channels. These proteins bind glutamate to maintain calcium ion equilibrium (GRIN2C). Upregulation of MBG leads to the accumulation of Ca_2^+ intracellularly due to glutamate excitotoxicity leading to the sequestration of mitochondria with high Ca_2^+ levels [68]. In turn these changes lead to the production of reactive oxygen species [69]. The FERMT1 gene encodes for a protein involved with signaling and the attachment of integrins and actin cytoskeletons. The ESR1 gene encoding the estrogen receptor alpha in HBMEC was downregulated because of MBG [40]. The identity of the receptor protein that binds MBG is unknown.

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. 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].

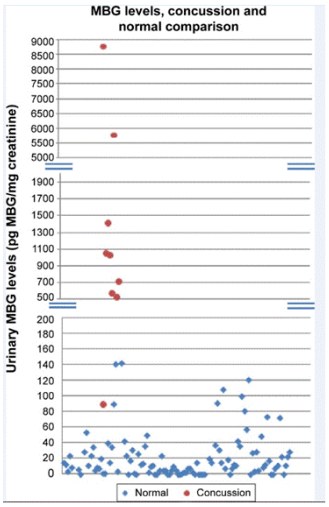

Figure 12: MBG levels in concussed athletes (filled circles) compared to those obtained prior to spring training (filled diamonds).

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

1. Schoner W, Scheiner-Bubis G (2007) Endogenous and exogenous cardiac glycosides: Their roles in hypertension, salt metabolism and cell growth. Am J Physiol Cell Physiol 293: C509-C536.

2. Hamlyn JM, Ringel R, Schaeffer J, Levinson PD, Hamilton BP, et al. (1982) A circulating inhibitor of Na+/- K+ ATPase associated with essential hypertension. Nature 300: 650-652.
3. Morrow JS, Cianci CD, Ardito T, Mann AS, Kashgarian M (1989) Ankyrin links fodrin to the a-subunit of Na, K-ATPase in Madin-Darby canine kidney cells and in intact renal tubule cells. J Cell Biol 108: 455-465.
4. Laredo J, Hamilton JP, Hamlyn JM (1995) Secretion of endogenous ouabain from bovine adrenal cells. Role of zona glomerulosa and zona fasciculata. Biochem Biophys Res Commun 212: 487-493.
5. Blanco G, Mercer RW (1998) Isozymes of the Na-K-ATPase: heterogeneity in structure, diversity in function. Am J Physiol 275: 633-650.
6. Federova OV, Bagrov AY (1997) Inhibition of Na/K-ATPase from rat aorta by two endogenous Na/K pump inhibitors ouabain and marinobufagenin. Evidence of interaction with different a-subunit isoforms. Am J Hypertens 10: 929-935.
7. Chesley LC (1972) Plasma and red cell volumes during pregnancy. Am J Obstet Gynecol 112: 440-450.
8. Chesley LC (1958) The renal excretion of sodium in women with preeclampsia. Clin Obstet Gynecol 1: 317-323.
9. Chesley L, Zalenti C, Rein A (1958) Excretion of sodium loads by nonpregnant and pregnant normal hypertensive and preeclamptic women. Metabolism: Clinical and Experimental 7: 575-588.
10. Agunanne E, Horvat D, Harrison R, Uddin MN, Jones R, et al. (2011) Marinobufagenin levels in preeclamptic patients: a preliminary report. Am J Perinatol 2011; 28: 509-514.
11. Uddin MN, McLean LB, Hunter FA, Horvat D, Severson J, et al. (2009) Vascular leak in a rat model of preeclampsia. Am J Nephrol 30: 26-33.
12. Gonick HC, Ding Y, Vaziri ND, Bagrov AY, Federova AV (1998) Simultaneous measurement of marinobufagenin, ouabain and hypertension-associated protein in various disease states. Clin Exp Hypertension 20: 617-627.
13. Lopatin EA, Aliamazian EK, Dimitrius RI, Shpen DR, Federova OV, et al. (1999) Circulating bufodienolides and cardenolides sodium pump inhibitors in preeclampsia. J Hypertens 17: 1179-1187.
14. Ianosi-Irimie M, Vu HV, Whitbred JM, Pridjian CA, Nadig JD, et al. (2005) A rat model of preeclampsia. Clin Exp Hypertens 27: 605-617.
15. Agunanne EE, Uddin MN, Horvat D, Puschett JB (2010) Contribution of angiogenic factors in a rat model of pre-eclampsia. Am J Nephrol 32: 332-339.
16. Puschett JB, Kumar B, Abbas MMK (2014) Differing effects of resibufagenin on cinobufatalin-versus-marinobufagenin-induced preeclampsia in a rodent model. Am J Perinatol 32: 803-808.
17. Vu H, Ianosi-Irimie M, Danchuk S, Rabon E, Nogawa T, et al. (2006) Resibufagenin corrects hypertension in a rat model of human preeclampsia. Exp Biol Med 231: 215-220.
18. Uddin MN, Horvat D, Childs EW, Puschett JB (2009) Marinobufagenin causes endothelial cell monolayer hyperpermeability by altering apoptotic signaling. Am J Physiol Regul Integr Comp Physiol 296: 1726-1734.
19. Uddin MN, Horvat D, Glaser SS, Danchuk S, Mitchell BM, et al. (2007) Marinobufagenin inhibits proliferation and migration of cytotrophoblasts and CHO cells. Placenta 29: 266-273.
20. LaMarca HL, Morris CA, Pettit GR, Nagowa T, et al. (2006) Marinobufagenin impairs first trimester cytotrophoblast differentiation. Placenta 27: 984-988.
21. Mitchell BM, Cook LG, Danchuk S, Puschett JB (2007) Uncoupled endothelial nitrate oxide synthase and oxidative stress in a rat model of pregnancy-induced hypertension. Am J Hypertens 20: 1297-1304.
22. Uddin MN, Agunanne E, Horvat D, Puschett JB (2010) Resibufagenin administration prevents oxidative stress in a rat model of human preeclampsia. Hypertension and Pregnancy 31: 70-78.
23. Pugin J, Verghese G, Widmer MC (1999) The alveolar space is the site of intense inflammatory and profibrotic reactions in the early phase of acute respiratory distress syndrome. Crit Care Med 27: 304-312.
24. Puschett JB (2012) Marinobufagenin predicts and resibufogenin prevents preeclampsia: a review of the evidence. Am J Perinatol 29: 777-785.
25. Matthay MA, Zimmerman GA (2005) Acute lung injury and the acute respiratory distress syndrome: four decades of inquiry into pathogenesis and rational management. Am J Respir Cell Mol Biol 33: 319-327.
26. Doyle RL, Szaflarski N, Modin GW, et al. (1995) Identification of patients with acute lung injury. Predictors of mortality. Am J Respir Crit Care Med 152: 1818-1824.
27. Heffner JE, Zamora CA (1990) Clinical predictors of prolonged translaryngeal intubation in patients with the adult respiratory distress syndrome. Chest 97: 447-452.
28. Fowler AA, Hamman RF, Good JT (1983) Adult respiratory distress syndrome: risk with common predispositions. Ann Intern Med 98: 593-597.
29. Hudson LD, Milberg JA, Anardi D (1995) Clinical risks for development of the acute respiratory distress syndrome. Am J Respir Crit Care Med 151: 293-301.
30. Rubenfeld GD, Caldwell E, Peabody E, Weaver J, Martin DP, et al. (2005) Incidence and outcomes of acute lung injury. N Engl J Med 353: 1685-1693.
31. Calfee CS, Eisner MD, Ware LB (2007) Trauma-associated lung injury differs clinically and biologically from acute lung injury due to other clinical disorders. Crit Care Med 35: 2243-2250.
32. Pelosi P, Bottino N, Chiumello D (2003) Sigh in supine and prone position during acute respiratory distress syndrome. Am J Respir Crit Care Med 167: 521-527.
33. Meade MO, Cook DJ, Guyatt GH (2008) Ventilation strategy using low tidal volumes, recruitment maneuvers, and high positive end-expiratory pressure for acute lung injury and acute respiratory distress syndrome: a randomized controlled trial. JAMA 299: 637-645.
34. Bachofen M, Weibel ER (1982) Structural alterations of lung parenchyma in the adult respiratory distress syndrome. Clin Chest Med 3: 35-56.
35. Bachofen M, Weibel ER (1977) Alterations of the gas exchange apparatus in adult respiratory insufficiency associated with septicemia. Am Rev Respir Dis 116: 589-615.
36. Donnelly SC, Haslett C, Reid PT (1997) Regulatory role for macrophage migration inhibitory factor in acute respiratory distress syndrome. Nat Med 3: 320-323.
37. Vestweber D, Winderlich M, Cagna G (2009) Cell adhesion dynamics at endothelial junctions: VE-cadherin as a major player. Trends Cell Biol 19: 8-15.
38. Corada M, Mariotti M, Thurston G (1999) Vascular endothelial-cadherin is an important determinant of microvascular integrity in vivo. Proc Natl Acad Sci USA 96: 9815-9820.
39. Ing NH, Berghman L, Abi-Ghanem D (2014) Marinobufagenin regulates permeability and gene expression of brain endothelial cells. Am J Physiol Regul Integr Comp Physiol 306: 918-924.
40. Abbas MM, Patel B, Chen Q (2017) Involvement of the bufadienolides in the detection and therapy of the acute respiratory distress syndrome. Lung 195: 323-332.
41. Force ADT, Ranieri VM, Rubenfeld GD (2012) Acute respiratory distress syndrome: the Berlin Definition. JAMA 307: 2526-2533.
42. Thickett DR, Armstrong L, Christie SJ (2001) Vascular endothelial growth factor may contribute to increased vascular permeability in acute respiratory distress syndrome. Am J Respir Crit Care Med 164: 1601-1605.
43. Jiang X, Ingbar DH, O'Grady SM (1998) Adrenergic stimulation of Na+ transport across alveolar epithelial cells involves activation of apical Cl- channels. Am J Physiol 275: 1610-1620.
44. Matthay MA, Folkesson HG, Verkman AS (1996) Salt and water transport across alveolar and distal airway epithelia in the adult lung. Am J Physiol 270: 487-503.
45. Blondonnet R, Constantin JM, Sapin V (2016) A pathophysiologic approach to biomarkers in acute respiratory distress syndrome. Dis Markers 3501373.

46. Worthen GS, Haslett C, Rees AJ (1987) Neutrophil-mediated pulmonary vascular injury. Synergistic effect of trace amounts of lipopolysaccharide and neutrophil stimuli on vascular permeability and neutrophil sequestration in the lung. Am Rev Respir Dis 136: 19-28.
47. Faul M, Xu L, Wald MM, Coronado VG (2010) Traumatic brain injury in the United States: emergency department visits, hospitalizations, and deaths. Atlanta (GA): Centers for Disease Control and Prevention, National Center for Injury Prevention and Control.
48. Prins ML, Hales A, Reger M (2010) Repeat traumatic brain injury in the juvenile rat is associated with increased axonal injury and cognitive impairments. Dev Neurosci 32: 510-518.
49. Shrey DW, Griesbach GS, Giza CC (2011) The pathophysiology of concussions in youth. Phys Med Rehabil Clin N Am 22: 577-602.
50. Barkhoudarian G, Hovda DA, Giza CC (2011) The molecular pathophysiology of concussive brain injury. Clin Sports Med 30: 33-48.
51. Lincoln AE, Hinton RY, Almquist JL, Lager SL, Dick RW (2007) Head, face, and eye injuries in scholastic and collegiate lacrosse: a 4-year prospective study. Am J Sports Med 35: 207-215.
52. Hootman JM, Dick R, Agel J (2007) Epidemiology of collegiate injuries for 15 sports: summary and recommendations for injury prevention initiatives. J Athletic Training 42: 311-319.
53. Meehan WP, D'Hemecourt P, Comstock RD (2010) High school concussions in the 2008-2009 academic year: mechanism, symptoms, and management. Am J Sports Med 38: 2405-2409.
54. McCrea M, Barr WB, Guskiewicz K (2005) Standard regression-based methods for measuring recovery after sport-related concussion. J Int Neuropsychol Soc 11: 58-69.
55. Lovell M, Collins M, Bradley J (2004) Return to play following sports-related concussion. Clin Sports Med 23: 421-441.
56. McCrory P (2009) Sport concussion assessment tool 2. Scand J Med Sci Sports 19: 452.
57. Kelly AB, Zimmerman RD, Snow RB (1988) Head trauma: comparison of MR and CT-experience in 100 patients. AJNR Am J Neuroradiol 9: 699-708.
58. Zhang K, Johnson B, Pennell D (2010) Are functional deficits in concussed individuals consistent with white matter structural alterations: combined fmri & dti study. Exp Brain Res 204: 57-70.
59. Levin HS, Wilde E, Troyanskaya M (2010) Diffusion tensor imaging of mild to moderate blast-related traumatic brain injury and its sequelae. J Neurotrauma 27: 683-694.
60. Henry LC, Tremblay S, Boulanger Y (2010) Neurometabolic changes in the acute phase after sports concussions correlate with symptom severity. J Neurotrauma 27: 65-76.
61. Hinson HE, Rowell S, Schreiber M (2014) Clinical evidence of inflammation driving secondary brain injury: a systematic review. J Trauma Acute Care Surg. 78: 184-191.
62. Ling JM, Klimaj S, Toulouse T, Mayer AR (2013) A prospective study of gray matter abnormalities in mild traumatic brain injury. Neurology 81: 2121-2127.
63. Shapiro L, Foresti M, Arisi G (2011) Marinobufagenin diagnoses and resibufogenin ameliorates traumatic brain injury. In: American Society for Clinical Pharmacology and Therapeutics. (Abstract), Meeting Program Booklet: 78.
64. Oliver J, Abbas K, Lightfoot JT (2015) Comparison of neurocognitive testing and the measurement of marinobufagenin in mild traumatic brain injury: A preliminary report. J Exper Neurosci 9: 67-72.
65. Argaw AT, Asp L, Zhang J, Navrazhina K, Pham T, et al. (2012) Astrocyte-derived VEGF-A drives blood-brain barrier disruption in CNS inflammatory disease. J Clin Invest 122: 2454-2468.
66. Beech DJ (2007) Canonical transient receptor potential. Handb Exp Pharmacol 5: 109-123.
67. Nicholls DG (1985) A role for the mitochondrion in the protection of cells against calcium overload? Prog. Brain Res 63: 97-106.
68. Maciel EN, Vercesi AE, Castilho RF (2001) Oxidative stress in Ca (2+)-induced membrane permeability transition in brain mitochondria. J Neurochem 79: 1237-1245.
69. Goult BT, Bouaouina M, Harburger DS, Bate N, et al. (2009) The structure of the N-terminus of kindlin-1: a domain important for alphaiibbeta3 integrin activation. J Mol Biol 394: 944-956.