The Cardiotonic Bufodienolide Steroid Marinobufagenin Alters Messenger RNA Concentrations of Soluble Flt-1 in Human Brain Microvascular Endothelial Cell Monolayers

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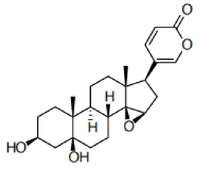
Abstract

Previous studies from this laboratory have provided evidence that, in a rat model of preeclampsia (PE), the cardiotonic bufodienolide, marinobufagenin (MBG), is not only a biomarker but an etiologic factor. Concentrations of MBG in blood and urine are increased in women with PE compared to those with normal pregnancies. Additionally, MBG has been determined in both *in vivo* and *in vitro* studies to cause vascular leak. Those studies included direct evidence that MBG causes hyperpermeability of endothelial monolayers. Because PE patients may exhibit neurologic abnormalities often associated with cerebral edema, we examined the effects of MBG on endothelial monolayers obtained from human brain microvasculature. Since PE has also been determined to alter concentrations of factors that affect angiogenesis, we investigated the effects of treatment with 10^{-8} M MBG on primary cultures of endothelial cells from human brain microvasculature on concentrations of mRNAs encoding the VEGF receptor called soluble Flt-1 (Flt-1 variant 2 mRNA) and Flt-1 mRNA variant 3, as well as NF kappa B (NFKB1), a substance often involved in tissue injury. MBG depressed the concentrations of soluble Flt-1 mRNA within 2 h, with the largest effect (reduced to ½) at 12 h compared to vehicle treated controls. The effect was reversed at 24 h, when MBG-treated endothelial cells contained 2.4-fold higher soluble Flt-1 mRNA. Throughout the time course, Flt-1 variant 3 and NFKB1 mRNA levels were unchanged. These studies are currently being extended with microarray analyses of the global transcriptome of MBG-treated and control cultures of endothelial cells from human brain microvessels.

Introduction

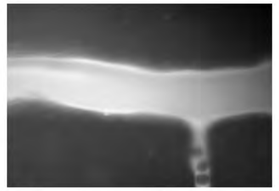
1. Marinobufagenin (MBG) is a cardiotonic steroid that increases 5-fold in the urine of pregnant women with preeclampsia compared to normal pregnant women.

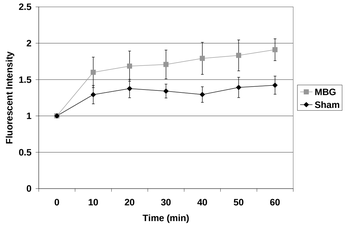


Marinobufagenin

Introduction (continued)

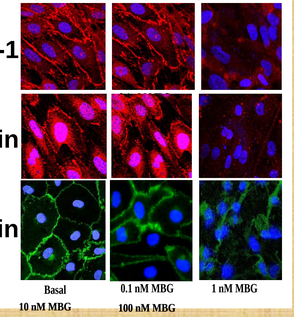
2. MBG causes a “vascular leakage” syndrome in a rat model of preeclampsia**. FITC-albumin leaks from post-capillary venules more rapidly with MBG treatment.





3. MBG (1 nM and higher doses) increases the permeability of monolayer cultures of human brain microvascular endothelial cells (hBMEC) on a membrane insert to FITC-albumin by 60% within 30 min. (not shown)

4. MBG disrupts tight junctions in monolayers of human brain microvascular endothelial cells, as seen by scattered immunostaining of proteins composing the tight junctions between endothelial cells from human brain microvasculature after 24 h of MBG treatment. Nuclei are counter-stained with DAPI.

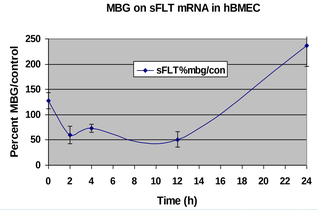


Hypothesis

MBG alters expression of specific genes in human brain microvascular endothelial cells (hBMEC) whose protein products regulate tight junctions.

Results

1. Expression of soluble FLT (sFLT1), FLT1var3 and NFKB genes was quantified with PCR normalized to GAPDH, PPIA & RPLP0 mRNAs. hBMEC cells were treated with 10 nM MBG for 2, 4, 12, and 24 h. Only sFLT mRNA levels were regulated by MBG.



2. Microarrays were performed to interrogate the global transcriptomes of hBMEC cells treated with 10 nM MBG for 12 hours or not (controls).

Gene Name	Symbol	Fold change (MBG/Con)
MOST SIGNIFICANT UP-REGULATED of 55		
Similar to alkaline ceramidase 2		8
Copine VIII	CPNE8	18
Enkurin	ENKUR	18
Transmembrane protein 207	TMEM207	11
ATPase aminophospholipid transporter	ATP8B3	17
Proprotein convertase	PCSK6	14
MAX interactor 1	MXI1	10
DNA methyltransferase 3-like	DNMT3L	10
MOST SIGNIFICANT DOWN-REGULATED of 123		(-Con/MBG)
Tubulointerstitial nephritis antigen	TINAG	-6
NLR family, pyrin domain containing	NLRP3	-4
Late cornified envelope 2D	LCE2D	-4
Serine protease 42	PRSS42	-7
Melanocortin 5 receptor	MCR5	-5
Retinaldehyde binding protein 1	RLBP1	-7
Calpain 13	CAPN13	-5
Fibroblast growth factor receptor 2	FGFR2	-5
Glutamate receptor inotropic N-methyl,D-aspartate 2C	GRIN2C	-5
Integrin beta 2	ITGB2	-3.5
Integrin alpha 2B	ITGA2B	-3.5
Interleukin 1 family member 10	IL1F10	-3.5

Summary of Conclusions

MBG regulates the expression of several genes in endothelial cells. Most, like soluble FLT, the secreted VEGF receptor, were strongly down-regulated at early time points from 4 to 12 h.

It remains to be seen whether the partial antagonist of MBG, resibufogenin, will antagonize all of MBG’s effects on gene expression in endothelial cells.

Acknowledgements

Support of this work was provided by Grant-in-Aid from Dialysis Clinic, Inc, Nashville, Tenn., and the Jules B. Puschett Endowment fund.

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