Beta-receptor blockade attenuates atherosclerosis progression following traumatic brain injury in apolipoprotein E deficient mice

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Abstract

Traumatic brain injury (TBI) is associated with cardiovascular mortality in humans. Enhanced sympathetic activity following TBI may contribute to accelerated atherosclerosis. The effect of beta-adrenergic receptor blockade on atherosclerosis progression induced by TBI was studied in apolipoprotein E deficient mice. Mice were treated with metoprolol or vehicle following TBI or sham operation. Mice treated with metoprolol experienced a reduced heart rate with no difference in blood pressure. Six weeks following TBI, mice were sacrificed for analysis of atherosclerosis. Lesion thickness was analyzed at the level of the aortic valve and found to be increased in mice receiving TBI with vehicle treatment but was reduced in TBI mice receiving metoprolol. The increase in lesion thickness was associated with increased percent macrophage immunostaining area of intima which was also reduced with metoprolol. No effect of metoprolol on lesion thickness or macrophage content was observed in mice receiving only sham operation. In conclusion, accelerated atherosclerosis following TBI is reduced with beta-adrenergic receptor antagonism. Beta blockers may be useful to reduce vascular risk associated with TBI.

Introduction

Traumatic brain injury (TBI) is a common cause of morbidity and mortality worldwide [1]. In addition to neurocognitive deficits, TBI is associated with other comorbidities, including cardiovascular complications. In the acute setting, cardiovascular effects of TBI include stress-related cardiomyopathy [2], arrhythmias [3], and ECG changes [4], all of which are associated with increases in catecholamines. Chronic cardiovascular effects, including increased coronary artery calcification and cardiovascular mortality have also been demonstrated [5]. Preclinical studies in mice support a causal relationship between TBI and atherosclerosis [6] which may be related to enhanced sympathetic activity. Chronic increases in sympathetic activity following TBI have been identified in studies of both rodents and humans [7–9]. Therapeutic blockade of beta-adrenergic receptors with beta-blockers have been shown to improve neurological and functional outcomes in humans following TBI [10, 11]. Beta-blockers may also be useful in reducing other comorbidities that may be associated with TBI. The current preclinical study was designed to determine whether the beta-receptor antagonist, metoprolol, would be effective in preventing the increased atherosclerosis observed following TBI in hyperlipidemic mice.

Results

Effect of TBI and metoprolol on baseline parameters in ApoE$^{-/-}$ mice

At 10 weeks of age, *ApoE*$^{-/-}$ mice were started on a western diet to induce hyperlipidemia and accelerate the development of atherosclerosis. Four weeks later mice underwent a TBI or sham control procedure. Mice recovered quickly from the procedure and demonstrated grossly normal activity and eating behavior.

Twenty-four hours following injury, mice were given metoprolol or placebo in the drinking water. Body weights were not different between the TBI ± metoprolol and sham groups ± metoprolol of mice 6 weeks following the procedure (Table 1) indicating the TBI procedure did not impair feeding or cause illness. There were also no significant differences in total cholesterol between the 2 groups of mice (Table 1). Tail-cuff plethysmography was used to measure blood pressure and pulse 3 weeks following injury in non-anesthetized mice. To ensure reliable and stable blood pressure measurements, mice were first trained for seven consecutive days and all blood pressure measurements were performed in the morning. Pulse rate was reduced in TBI and sham-operated mice receiving metoprolol versus placebo but not different as a function of TBI vs sham-operation (Table 1). No differences in systolic blood pressure were observed among the groups (Table 1).

Table 1

Effect of TBI and metoprolol on baseline parameters in *ApoE*$^{-/-}$ mice

	Sham			**TBI**			***Sham v TBI***
	- Met	**+ Met**	*P value*	**- Met**	**+ Met**	*P value*	*-Met P value*
Body weight (g)	32.8 ± 3.7	28.9 ± 3.1	*0.0964*	30.1 ± 2.1	28.4 ± 2.6	*0.1895*	*0.279*
Blood Pressure (mm Hg)	113 ± 33	105 ± 30	*0.7364*	126 ± 35	85 ± 11	*0.0666*	*0.709*
Pulse Rate (bpm)	589 ± 37	536 ± 67	*0.0366*	562 ± 42	510 ± 48	*0.0382*	*0.2283*
Total cholesterol (mg/dL)	607 ± 49	619 ± 20	*0.5818*	502 ± 21	536 ± 17	*0.2932*	*0.1581*

The mean value ± standard deviation are listed for mice with (+ Met) or without (- Met) metoprolol (2 mg/mL) for 6 weeks following TBI (n=4 per group). P values were determined using two-tailed student's t-test.

Effect of TBI and metoprolol on atherosclerosis in ApoE$^{-/-}$ mice

Quantitation of atherosclerosis performed at the level of the aortic sinuses revealed increased I/M ratio in mice subjected to TBI compared to sham-operated mice (Fig. 1). However, this TBI-induced increase in atherosclerosis was attenuated in mice treated with metoprolol (Fig. 1). No effect of metoprolol on atherosclerosis was observed in sham-operated mice.

Plaque area occupied by MAC3 positive staining cells was higher in the TBI-treated mice compared to sham-treated mice and this was also reduced in TBI mice treated with metoprolol (Fig. 1).

Discussion

Cardiovascular complications are increased following brain injury [5, 12] and these effects may be mediated by catecholamine surges as chronic and paroxysmal sympathetic hyperactivity are common after traumatic brain injury [13]. Sympathoadrenal activation following TBI has been shown to induce a coagulopathy and endotheliopathy as evidenced by biomarkers and these effects have been associated with a poor prognosis [14]. In a preclinical model, TBI was shown to accelerate atherosclerosis in hyperlipidemic mice [6].

Pharmacologic blockade of adrenergic receptors, which mediate effects of sympathetic hyperactivation, with beta blockers have been shown to improve neurologic outcome following TBI [10, 11]. Since a murine model demonstrating effects of TBI on a relevant vascular endpoint has been previously established [6], the current study was designed to determine whether therapy with beta blockers might be effective in preventing TBI-induced accelerated atherosclerosis.

The CCI model has been commonly used in mice to explore pathways in post-traumatic brain injury [15]. As in humans, cascades are activated subsequent to injury that lead to chronic systemic effects involving apoptosis [16], inflammation [17] and oxidative stress [18, 19]. The mortality rate associated with the CCI model in rodents is low, so long term survival studies are feasible. Pathophysiological changes have been shown to occur even 1 year after CCI, including ongoing neurodegeneration, microglial activation [20], and neurologic compensatory responses [21]. The model is therefore useful to analyze effects of TBI on vascular disease processes.

Atherosclerotic-prone Apolipoprotein E deficient mice are widely employed to study genetic and environmental factors involved in atherosclerosis [22]. Vascular endpoints are accelerated with a western diet [22–25], allowing the current study to focus on a timepoint of 6 weeks following the TBI.

Consistent with a previous study [6], atherosclerosis was increased in mice subjected to TBI. This effect was not associated with increases in blood pressure or pulse. Previous studies have demonstrated effects of TBI on leukocyte activation and biomarkers of endothelial adhesiveness [6], although the precise mechanism(s) for atheroprotection remains to be proven. In the current study, therapy with the β-adrenergic antagonist, metoprolol, prevented the increase in atherosclerosis following TBI. This was associated with reduction of pulse rate in both TBI and sham-operated mice. Hemodynamic effects are unlikely the sole mechanism for attenuation of atherosclerosis as pulse and blood pressure were not different between TBI and sham-operated animals. Additional studies are necessary to uncover the mechanism(s) responsible for beta-blocker-mediated atheroprotection following TBI, however given

benefits in neuroprotection and possible vascular protection, the threshold may be lowered for using this class of drugs in patients following TBI.

Methods And Materials

Animals

Male apolipoprotein E–deficient (*ApoE*$^{-/-}$) on the C57BL6/J strain background were purchased from Jackson Laboratory (Bar Harbor, Maine) at 8 weeks of age. Mice were housed under specific pathogen-free conditions in static microisolator cages with tap water ad libitum in a temperature-controlled room with a 12:12-hour light/dark cycle. At 10 weeks of age mice were started on a Western diet (TD88137, Harlan, WI) and at 14 weeks of age, mice were randomly allocated to the CCI or sham procedures, with or without metoprolol. Mice receiving metoprolol were grouped in same cage as drug was supplied in water source. Metoprolol or vehicle control was administered via the drinking water at a concentration of 2 mg/mL for 6 weeks following TBI. Jintao Wang was aware of cage allocation following TBI procedure.

Ethics Statement

All animal use protocols complied with the Principle of Laboratory and Animal Care established by the National Society for Medical Research and were approved by the University of Michigan Committee on Use and Care of Animals. This study is reported in accordance with ARRIVE guidelines as set by the National Centre for the Replacement Refinement and Reduction of Animals in Research [26].

Model of TBI

To induce TBI, male *ApoE*$^{-/-}$ mice were anesthetized with 2% isoflurane and placed in a stereotactic frame (Kopf,Tujunga,CA, USA) as previously described [27,28]. Briefly, a 5 mm circular craniotomy, centered between the bregma and lambda, was made and then a controlled cortical impact (CCI) was delivered to the midline at an impact speed of 3.00 m/s, tissue displacement of 1.1 mm, and impact duration of 50 ms. Following impact, the circular bone fragment from the craniotomy was glued back to the cranial window. The sham procedure was identical except for craniotomy and delivery of the CCI.

Blood Pressure Measurement

Blood pressure and pulse rate were measured 3 weeks after CCI or sham operation in non-anesthetized, trained mice by tail plethysmography using the BP-2000 Blood Pressure Analysis System (Visitech System, Apex, NC) as previously described [23].

Histological Analysis

Quantification of atherosclerosis, the primary outcome, was performed as previously described [23]. Briefly, mice were euthanized under pentobarbital anesthesia (i.p., 100 mg/kg), and arterial trees were perfused at physiological pressure and fixed in 10% zinc formalin. Paraffin-embedded hearts, which included aortic valves, were sectioned for lesion analysis. A series of 5 μm sections were obtained at the level of the aortic sinus and 4 cross sections were analyzed from each mouse. Sections were stained with hematoxylin and eosin for quantification of lesion area normalized by adjacent medial area of aorta to control for possible tangential sectioning [23,29]. The lesion area was defined as the area between the endothelial cell layer and internal elastic lamina.

For plaque composition analysis, macrophages were quantified with an antibody to MAC3 (1:100, BD Biosciences, San Jose, CA). Negative controls consisted of tissues handled identically to experimental samples except that the primary antibody was omitted. The detection system was streptavidin-HRP and endogenous peroxidase was quenched with hydrogen peroxide. Sections were counterstained with hematoxylin. Positive staining area was analyzed from three fields in each section and expressed as percentage of the total area. All images were analyzed by automated detection of positive stained area using Nikon MetaMorph software with observer blinded to treatment allocation.

Measurements of Plasma Samples

Plasma samples were collected via terminal heart puncture bleeding 6 weeks after TBI or sham operation. Total cholesterol was measured using Infinity cholesterol enzymatic-colorimetric kit (Thermo Fisher, #TR13421).

Statistical analysis

All data are presented as mean ± standard deviation. Statistical analysis was carried out using GraphPad Prism. Shapiro-Wilk normality test was used for normal distribution testing. Results were analyzed using 2-tailed t-tests for comparison between two groups. Sample size was determined by power calculation based on variability of atherosclerosis previously established in this model [6]. No animals were excluded from analysis.

Declarations

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Author contributions:

JV contributed to the manuscript writing, data acquisition and analysis. JW contributed to study design, manuscript writing, data acquisition, and analysis. EJS, CG, DL contributed to study design, data acquisition, and analysis. DTE contributed to study conception, design, manuscript writing, and final approval of submitted version. DTE is the guarantor of this work, had full access to all the data, and takes full responsibility for the integrity of data and the accuracy of data analysis. All authors reviewed the manuscript.

Data availability:

For original data, please contact deitzman@umich.edu.

Additional information:

The authors have no competing financial interests to declare.

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Figures

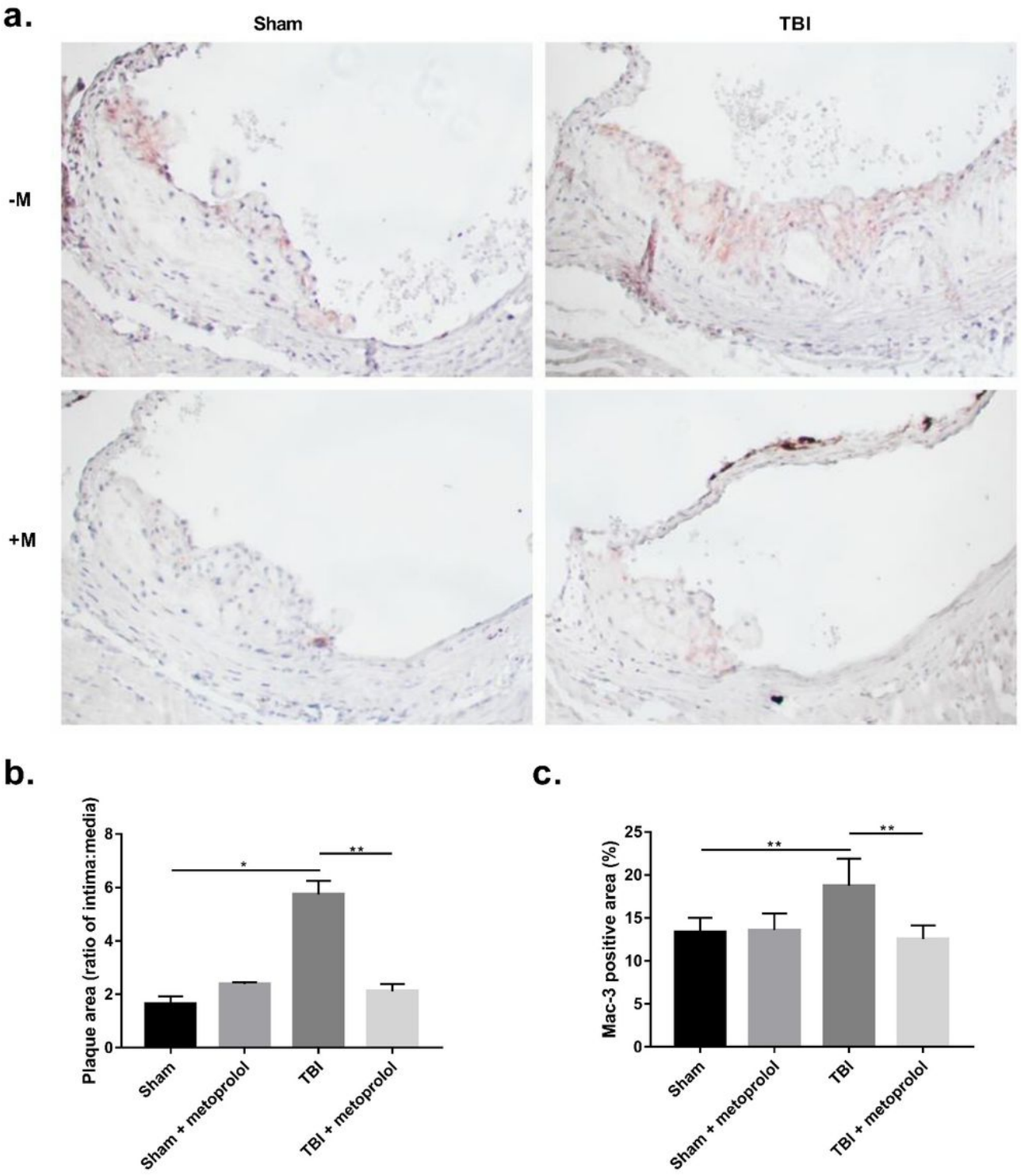

Figure 1

Plaque area and MAC3 immunostaining in response to TBI and metoprolol

A) Representative images of sections of the aortic root stained with anti-MAC3 antibody for $APOE^{-/-}$ mice subjected to Sham or TBI surgeries with (+M) or without (-M) metoprolol (2 mg/mL) for 6 weeks following the surgeries. **B)** Quantification of intima:media ratio. **C)** Quantification of MAC3 staining as percent of

the plaque area. All data are presented as mean ± standard deviation. Results were analyzed using 2-tailed t-tests for comparison between two groups (n=4, *=$p<0.05$, **=$p<0.01$).