ELSEVIER

The Journal of Heart and Lung Transplantation

http://www.jhltonline.org

Endothelin-1 antagonism and nitric oxide augmentation prevents cyclosporine-induced vasomotor impairment

Danny Ramzy, MD, PhD, Jack Wallen, MD, PhD, Mitesh V. Badiwala, MD, Laura C. Tumiati, BSc, Elissa Tepperman, MSc, Heather J. Ross, MD, Diego H. Delgado, MD, and Vivek Rao, MD, PhD

From the Heart Transplant Program, Toronto General Hospital, University Health Network, Division of Cardiac Surgery, University of Toronto, Toronto, Ontario, Canada.

KEYWORDS:
cyclosporine;
vascular function;
nitric oxide;
endothelium
transplant;
heart

BACKGROUND: We previously demonstrated that cyclosporine (CyA) impairs endothelial function as a result of alterations in nitric oxide (NO) and endothelin-1 (ET-1) regulation. Bosentan (BOS), an ET-1 antagonist, and tetrahydrobiopterin (BH_4), an eNOS cofactor, may reduce endothelial dysfunction by improving ET-1/NO homeostasis.

METHODS: Lewis rats received intraperitoneal injections of CyA with BOS or with BOS+BH_4 daily for 2 weeks. Control (Con) animals received saline injections. Thoracic aortic segments were assessed for endothelial-dependent (E_{dep}) and -independent (E_{ind}) relaxation ($E_{max\%}$) after exposure to acetylcholine and sodium nitroprusside. Vessel sensitivity to ET-1–induced vasospasm was evaluated.

RESULTS: CyA use resulted in impaired E_{dep} vasorelaxation when compared with Con, whereas BOS and BH_4 treatment preserved E_{dep} vasorelaxation. CyA significantly altered E_{ind} vasorelaxation, whereas BOS and BH_4 therapy attenuated CyA-induced effects. Compared with Con, CyA and BH_4 exposure demonstrated increased sensitivity to ET-1 vasospasm. BOS therapy abrogated the CyA and BH_4-induced sensitivity to vasospasm. CyA treatment resulted in higher 8-isoprostane levels compared with Con. CyA-mediated vascular dysfunction is characterized by impaired NO and ET-1 homeostasis.

CONCLUSIONS: Our study suggests potential therapeutic strategies to prevent endothelial dysfunction as combined therapy with ET-1 antagonism and NO augmentation completely abrogated CyA-induced vascular injury.

J Heart Lung Transplant 2011;30:77-85

© 2011 International Society for Heart and Lung Transplantation. All rights reserved.

Cyclosporine (CyA) has been implicated in the development of endothelial dysfunction and cardiac allograft vasculopathy (CAV).[1,2] Previous studies by our group have suggested that alterations in nitric oxide (NO) and endothelin-1 (ET-1) regulation may be a possible mechanism of CyA-induced endothelial dysfunction.[3,4] CyA exposure resulted in endothelial nitric oxide synthase (eNOS) downregulation and ET_A receptor upregulation.[3] Sudhir et al and Diederich et al also reported that CyA therapy leads to NO impairment and altered vasomotor function.[4,5] Buetler et al demonstrated that CyA may generate reactive oxygen species (ROS),[6] and Takeda found that CyA can alter ET receptor expression.[7] NO and ET-1 regulation may therefore play an important role in CyA-induced vasomotor dysfunction.

The enzyme NOS can generate NO or ROS depending upon its configurational state. NO is produced exclusively when the enzyme is in its dimeric form, whereas, in monomeric form, NOS generates ROS. NOS requires the cofactor tetrahydrobiopterin (BH_4) for dimerization. We have previously demonstrated that BH_4 therapy limits CyA-induced endothelial dysfunction.[8] However, this protection comes at a cost of increased sensitivity of ET-1 vasospasm.

ET-1 has several deleterious effects on the vasculature. We have previously demonstrated that bosentan (BOS), an ET-1 antagonist, can limit endothelial injury after transplantation.[9] Gupta et al and Yamaguchi et al observed that BOS can attenuate oxidative stress and prevent CAV.[10,11]

Reprint requests: Vivek Rao, MD, PhD, Division of Cardiovascular Surgery, Toronto General Hospital, 4N-464, 200 Elizabeth Street, Toronto, ON M4G 2C4, Canada. Telephone: 416-340-3562. Fax: 416-340-3337.

E-mail address: vivek.rao@uhn.on.ca

1053-2498/$ -see front matter © 2011 International Society for Heart and Lung Transplantation. All rights reserved.
doi:10.1016/j.healun.2010.08.001

In our investigation we evaluate the ability of BOS to prevent CyA-induced endothelial dysfunction by improving NO–ET-1 homeostasis and by limiting ROS injury. We also aim to evaluate the potential of BOS to limit BH_4-induced vasospasm. We intentionally employed a non-surgical, non-transplant model to exclude the obvious confounding effects of inflammation or immune-mediated injury.

Methods

Animal care conformed to the Canadian Council on Animal Care Guide to the Care and Use of Experimental Animals (NIH Publication No. 86-23, revised 1996). Male Lewis rats (200 to 300 g, $n = 8$ per group) were administered the drug of interest: saline control, CyA (5 mg/kg/day), BOS (100 mg/kg), and/or BH_4 (25 mg/kg/day) via peritoneal injection for a period of 14 days. The dose of CyA was chosen to produce a therapeutic plasma level throughout the 2-week exposure, whereas the BOS dose has been previously shown to result in complete inhibition of the ET_A and ET_B receptors.[9] Similarly, the BH_4 dose was validated in previous studies.[8]

On Day 15, rats were anesthetized and median sternotomy was performed. Hearts were excised for myocardial analysis and segments of thoracic aorta (TAo) procured for assessment of endothelial function and for biochemical assessment. Prior to tissue excision, 2 ml of blood from the right ventricle was collected for analysis of ET-1 and CyA levels.

In a separate series of experiments, rats were followed for a total of 4 weeks. Four groups of animals were compared: Group 1, 4-week exposure to CyA; Group 2, 4-week exposure to CyA with BOS/BH_4 exposure for the latter 2 weeks; Group 3, 2-week exposure to CyA with BOS/BH_4 exposure alone for the next 2 weeks; and Group 4, 2-week exposure to CyA followed by no drug exposure for 2 weeks.

Endothelial function assessment

Endothelial-dependent and -independent vascular relaxation was assessed in isolated segments of TAo. Five-millimeter segments of aorta were employed for the assessment of vascular function using a small-vessel myograft for isometric tension recording. After mounting the vessel on a pressure transducer, maximum vasoconstriction was achieved with exposure to phenylephrine (100 nmol/liter; Sigma Co, St. Louis, MO). Endothelial-dependent relaxation was assessed by incremental exposure to acetylcholine (ACh; 10 nmol/liter to 10 μmol/liter; Sigma Co). Endothelial-independent relaxation was assessed using incremental exposure to sodium nitroprusside (SNP; 10 nmol/liter to 5 μmol/liter; Sigma Co.). Complete vasomotor data for all groups are presented in the figures to visualize the dose-dependent effects of each intervention. In addition, maximum endothelial relaxation ($E_{max\%}$) was calculated by determining the percent maximal relaxation from phenylephrine-induced vasoconstriction. After SNP washout, sensitivity to vasospasm was assessed by incremental exposure to ET-1 (0.05 to 10 nmol/liter; Sigma Co.) and maximum concentration ($C_{max\%}$) calculated as the maximum increase in tension from baseline. ED_{50}, defined as the concentration required to achieve half-maximum vasorelaxation or vasoconstriction, was compared between groups.

Plasma measurements

Plasma ET-1 levels were quantified using a commercial enzyme-linked immunoassay (ELISA; Biomedica, Vienna, Austria). Absorption was read by an ELISA reader at a wavelength of 405 nm against a reference of 630 nm. CyA levels were determined at the Toronto Medical Laboratories using high-pressure liquid chromatography.

Assessment of oxidative injury

8-Isoprostane is the stable end-product of arachidonic acid oxidation generated by ROS injury. Determination of 8-isoprostane levels from TAo tissue was performed using a commercially available kit (Cayman Chemical Co, Ann Arbor, MI). Baseline assessments were made on aortic segments harvested from control animals not subjected to intraperitoneal injections and the percent change from these baseline values was calculated to compare differences between groups.

Western blot analysis

Western blot determined protein expression of inducible NOS (iNOS), and eNOS, with the use of protein-specific monoclonal antibodies (Biosciences, Mississauga, ON, Canada), and ET_A and ET_B receptor (Rc) expression with the use of protein-specific polyclonal antibodies (Chemicon, Temecula, CA) as previously described.[3] Comparisons between groups were performed using computerized densitometric analysis with commercially available software (BioRad, Hercules, CA). Protein determination was determined by Bradford's method.

Statistical analysis

Statistical analysis was performed with the SAS statistical software program, version 8.2 (SAS Institute, Cary, NC). Continuous data were analyzed by analysis of variance and expressed as mean $\pm$ SD.

Results

All animals survived until day of killing. CyA levels were within the normal clinical range (55 to 60 ng/ml) in all groups with no significant effect observed during exposure to BH_4 or BOS.

Endothelial function

Endothelial-dependent vasorelaxation was impaired after CyA treatment. Figure 1a–c depicts the cumulative dose-response curves to ACh from each group. A significant interactive effect was seen between groups and ACh concentration ($p < 0.0001$). CyA-treated animals displayed a significantly impaired response to ACh when compared with control. BOS therapy restored a normal ACh dose response in CyA-treated rats. Combined treatment with BOS+BH_4 demonstrated improved endothelial-dependent

Download English Version:

https://daneshyari.com/en/article/2971523

Download Persian Version:

https://daneshyari.com/article/2971523

Daneshyari.com