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ORIGINAL ARTICLE

Myocardial and anti-inflammatory effects of chronic bosentan therapy in monocrotaline-induced pulmonary hypertension

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KEYWORDS
Pulmonary hypertension;
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Abstract
Introduction and Objectives: Endothelin-1 antagonists are increasingly used in the treatment of pulmonary hypertension despite the lack of knowledge of their myocardial and systemic effects. We assessed the right ventricular myocardial and systemic effects of endothelin-1 antagonists in monocrotaline-induced pulmonary hypertension.
Methods: Male Wistar rats (180–200 g, n=57) randomly received 60 mg/kg monocrotaline or vehicle subcutaneously. Two days later, bosentan was randomly started (300 mg/kg/day) by oral route in a subgroup of monocrotaline-injected rats, while the other monocrotaline-injected and control rats received vehicle. At 25–30 days, invasive hemodynamic assessment was performed under anesthesia, arterial blood samples were collected for gas analysis and plasma was extracted for quantification of endothelin-1, cytokines, nitrates and 6-keto-prostaglandin $F_{1\alpha}$. Right ventricular myocardium was collected for assessment of cyclooxygenase and nitric oxide synthase activity and gene expression.
Results: The monocrotaline group developed pulmonary hypertension, low cardiac output, right ventricular hypertrophy and dilation, changes in gene expression and inflammatory activation that were attenuated in the group treated with bosentan. From a functional point of view, this group had improved right ventricular function and preserved ventriculo-vascular coupling, without deterioration in arterial gas parameters or systemic hypotension. In molecular terms, they showed reduced endothelin-1 and cytokine levels, decreased right ventricular inducible nitric

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oxide synthase and cyclooxygenase-2 activity and increased nitrate plasma levels compared with the non-treated group.
Conclusions: In this study we demonstrate that besides attenuating pulmonary hypertension, bosentan has beneficial hemodynamic, myocardial and anti-inflammatory effects.

PALAVRAS-CHAVE
Hipertensão pulmonar;
Antagonistas da endotelina-1;
Função ventricular direita;
Bosentan

Efeitos miocárdicos e anti-inflamatórios da terapêutica crónica com bosentan na hipertensão pulmonar experimental

Resumo
Introdução e objetivos: Os efeitos miocárdicos e sistémicos dos antagonistas da endotelina-1 na hipertensão pulmonar são ainda pouco conhecidos. Procurámos avaliar os efeitos miocárdicos ventriculares direitos e sistémicos, no que concerne à ativação inflamatória, dos antagonistas da endotelina-1 no modelo de hipertensão pulmonar induzida pela monocrotalina.
Métodos: Ratos Wistar machos (180-200 g, n=57) receberam aleatoriamente 60 mg/kg de monocrotalina ou veículo, via subcutânea. Um subgrupo aleatório destes animais passou a receber bosentan 300 mg/kg/dia por via oral, dois dias após, enquanto os restantes animais do grupo monocrotalina e o grupo controlo receberam veículo. Aos 25-30 dias procedeu-se à avaliação hemodinâmica invasiva, colheita de sangue arterial, de plasma para quantificação de endotelina-1, citoquinas, nitratos e 6-ceto-prostaglandina F1α, bem como de ventrículo direito para avaliação génica e da atividade das ciclo-oxigénases e síntases do óxido nítrico.
Resultados: O grupo monocrotalina desenvolveu hipertensão pulmonar, dilatação e hipertrofia ventricular direita, bem como diminuição do débito cardíaco, alterações da expressão génica ventricular direita e ativação inflamatória, que foram atenuadas no grupo monocrotalina tratados com bosentan. Do ponto de vista funcional, salienta-se que este grupo apresentou melhoria da função ventricular direita com preservação do acoplamento ventrículo-vascular, sem deterioração da gasometria ou hipotensão sistémica e, em termos moleculares, diminuição dos níveis plasmáticos e da expressão ventricular direita de endotelina-1 e citocinas, diminuição da atividade das síntases do óxido nítrico induzível e da ciclo-oxigénase-2.
Conclusão: Demonstrámos que o bosentan, para além de atenuar a hipertensão pulmonar, pode ter efeitos hemodinâmicos benéficos por ação anti-inflamatória, protetora, no miocárdio ventricular direito.

List of abbreviations

6-keto-PGF$_{1\alpha}$	6-keto-prostaglandin F$_{1\alpha}$
ACE	angiotensin-converting enzyme
AU	arbitrary units
BNP	B-type natriuretic peptide
CO	cardiac output
COX	cyclooxygenase
E_A	effective arterial elastance
EDPVR	end-diastolic pressure-volume relationship
E_{es}	end-systolic elastance
ET-1-1	endothelin
FiO_2	fraction of inspired oxygen
IL-6	interleukin-6
iNOS	inducible nitric oxide synthase
IVS	interventricular septum
LV	left ventricle/left ventricular
MCT	monocrotaline
mRNA	messenger ribonucleic acid
NO	nitric oxide
NOS	nitric oxide synthase
PAH	pulmonary arterial hypertension
PCR	polymerase chain reaction
PGI_2	prostaglandin I_2 or prostacyclin
PH	pulmonary hypertension
P_{max}	maximum pressure
pO_2	oxygen partial pressure
RT	reverse transcription
RV	right ventricle/right ventricular
TNF-α	tumor necrosis factor-α

Introduction

Pulmonary hypertension (PH) is defined on hemodynamic grounds as mean pulmonary arterial pressure >25 mmHg. It is a syndrome with different etiologies and represents the most serious chronic disease of pulmonary circulation.