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Eplerenone mimics features of the alternative activation in macrophages obtained from patients with heart failure and healthy volunteers

Krzysztof Łabuzek, Sebastian Liber*, Łukasz Bułdak, Joanna Krupej-Kędzierska, Grzegorz Machnik, Magdalena Bobrzyk, Bogusław Okopień

Department of Internal Medicine and Clinical Pharmacology, Medical University of Silesia, Medyków 18, PL 40-752 Katowice, Poland

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ABSTRACT

Alternative activation of macrophages plays protective role in cardiac remodelling in heart failure and the activity of mineralocorticoid receptor may determine the phenotype of these cells. We examined the influence of eplerenone, aldosterone, and IL-4 on descriptors of alternative activation in blood monocytes collected from 19 patients with heart-failure and 20 healthy volunteers. "Heart failure" macrophages in comparison with "healthy" macrophages had increased mineralocorticoid activity, NO and reactive oxygen species production, expression of iNOS mRNA and protein, but decreased expression of arginase I and mannose receptor proteins, and activity of MnSOD and CuZnSOD. Aldosterone increased mineralocorticoid activity, NO and reactive oxygen species production, iNOS mRNA and protein expression, MnSOD and CuZnSOD activity. Eplerenone attenuated the effects of aldosterone on all but MnSOD and CuZnSOD variables. Eplerenone alone increased the production of NO, MnSOD and CuZnSOD activity, arginase I gene and protein expression, and mannose receptor gene and protein expression, but decreased mineralocorticoid activity only in "heart failure" macrophages. The latter suggests altered function of mineralocorticoid receptor in heart failure. Increased mineralocorticoid activity accounts for increased NO production, iNOS gene and protein expression but does not explain the increased basal reactive oxygen species production and decreased markers of alternative activation in "heart failure" macrophages. In the lack of change in basal mineralocorticoid activity, eplerenone increases markers of alternative activation in a mineralocorticoid receptor-independent manner. Because of changes in iNOS and NO variable, eplerenone induced qualitatively different activation of macrophages from that obtained with IL-4.

1. Introduction

Eplerenone, along with spironolactone, belongs to a class of mineralocorticoid receptor antagonists, which are also known as aldosterone antagonists. In contrast to spironolactone, eplerenone has no active metabolites, is much more specific for mineralocorticoid receptor, and does not bind to any other nuclear receptors. It is indicated as a treatment for disorders of the renin-angiotensin-aldosterone axis, such as hepatic cirrhosis, heart failure, and arterial hypertension (Kolkhof and Borden, 2012). Aldosterone is a mineralocorticoid hormone that is synthesised by the cortex of the adrenal gland. It is the ligand of a nuclear receptor known as the mineralocorticoid receptor. Mineralocorticoid activity is classically described in relation to its effects on the kidney epithelium, namely salt and water retention. However, in recent years, there has been increased interest in the extrarenal actions of this hormone, especially in the heart, blood vessels and adipose tissue (Gilbert and Brown, 2010).

Most research devoted to the aldosterone axis focuses on the interactions between mineralocorticoid activity and parenchymal cell behaviour; frequently, a dysregulation of sodium-potassium ATPase and intracellular oxidative equilibrium is implied. Several recent studies have shown that inflammation, macrophage activity, and macrophage phenotype may be the downstream effects of aldosterone (Fraccarollo et al., 2008; Frieler et al., 2011; Usher et al., 2010). Importantly, it was shown that selective macrophage depletion in animal model of heart infarct abrogated the beneficial effects of eplerenone on infarct expansion (Fraccarollo et al., 2008).

Previously, we showed that eplerenone promotes alternative activation in macrophages obtained from healthy volunteers (Łabuzek et al., 2013). Alternative activation produces macrophages with an anti-inflammatory phenotype that opposes the pro-inflammatory and toxic phenotype (Liu and Yang, 2013). Alternative

* Corresponding author.
E-mail address: sebastian.liber@gmail.com (S. Liber).

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activation is induced by interleukin-4 (IL-4), and is distinguished by high levels of arginase I and mannose receptor expression and low levels of inducible nitric oxide synthase expression, as well as low levels of nitric oxide and reactive oxygen species. Dysregulation of the macrophage phenotype is thought to affect the progression of atherosclerosis and heart infarct expansion (Fraccarollo et al., 2008; Khallou-Laschet et al., 2010).

In this study, we analysed the influence of aldosterone and its antagonist eplerenone on human monocyte-derived macrophages obtained from patients with heart failure in comparison to those obtained from healthy volunteers. We investigated how heart failure modifies the phenotype of macrophages in the basal state and the plasticity of these cells' phenotype in response to modulators of mineralocorticoid receptor and IL-4. Phenotypes of these cells and their activity were assessed with commonly used indices of alternative activation, toxic molecule production, and radical scavenging capability.

2. Materials and methods

2.1. Subjects

All study participants (patients and volunteers) were fully informed of the study purpose and the potential risks associated with blood sampling. The study was performed in accordance with the 1964 Helsinki Declaration, and the local ethics committee accepted the protocol. All study groups were similar as regard to age, sex and weight (Table 1). None of the subjects had prior use of mineralocorticoid receptor antagonists including eplerenone and spironolactone.

2.1.1. 19 Patients (aged 55–76 years) met all inclusion criteria such as

1. Body Mass Index (BMI) 18.4–29.9 kg/m^2.
2. Stable congestive heart failure NYHA II or III.

Table 1
The baseline characteristics of cell donors.

	Congestive heart failure	Healthy	*P*
Patient characteristics			
Number of patients	19	20	
Age (years)	63.1 ± 7.5	59 ± 6.9	0.094
Men/women	8/11	10/10	
BMI (kg/m^2)	26 ± 3.2	28.8 ± 1.2	0.39
Stable coronary artery disease (%)	57.8	0	
Stable cerebrovascular disease (%)	10.5	0	
Arterial hypertension	42.1	0	
NYHA II/III (number of patients)	13/6	0	
EF (%)	40.7 ± 3.1	54.5 ± 3.8	< 0.001
Medication			
Beta blocker	100	0	
ACE-I/ARB	100	0	
Diuretics	100	0	
Antiplatelet therapy	100	0	
Variables			
Fasting glucose (mg/dl)	97.5 ± 12.9	90.4 ± 8.6	0.022
HbA1c	5.9 ± 0.4	5.4 ± 0.3	0.031
Total cholesterol (mg/dl)	179 ± 32	186 ± 24	0.749
LDL(mg/dl)	101 ± 14	105 ± 10	0.155
HDL(mg/dl)	38 ± 4	47 ± 6	0.039
TG(mg/dl)	140 ± 9	125 ± 23	0.064
hsCRP(mg/l)	2.1 ± 0.4	1.1 ± 0.6	0.005

3. Left Ventricular Ejection Fraction (LVEF) < 45% established on the basis of echocardiography.
4. Pharmacotherapy with: beta-blockers, ACE-I or ARB, loop diuretics and acetylsalicylic acid.
5. Informed consent to participate in the study.

2.1.2. Twenty volunteers (aged 55–76 years) met all inclusion criteria such as

1. Body Mass Index (BMI) 18.4–29.9 kg/m^2.
2. Absence of any active disease or history of any chronic disorder.
3. Negative drug history.
4. Informed consent to participate in the study.

2.1.3. All subjects (patients and volunteers) were excluded if they met at least one of the following criteria

1. Usage of any drugs, except for: beta-blockers, ACE-I or ARB, loop diuretics and acetylsalicylic acid.
2. Any acute and chronic inflammatory processes within 4 weeks before blood sampling.
3. Within the last 6 months before the start of blood sampling:
   - myocardial infarction (STEMI and NSTEMI) or unstable angina pectoris,
   - treatment for myocardial revascularisation and coronary artery bypass grafting or percutaneous transluminal coronary angioplasty,
   - stroke or TIA,
   - chronic heart failure in the class I or IV,
   - arterial hypertension (according to ESC/ESH),
   - clinically significant heart failure and cardiac arrhythmias affecting haemodynamic function,
   - diabetes,
   - malignancies including lymphoma and leukaemia in the last 5 years before the study,
   - liver failure and other active diseases such as hepatitis B, C or cirrhosis,
   - endocrine disorders requiring hormone substitution with glucocorticoids or thyroid hormones,
   - overuse of psychotropic drugs, psychostimulants, alcohol and cigarette smoking,
   - chronic kidney disease (III–V) or dialysis, and
   - history of chemotherapeutic and cytostatic treatment.
4. Obesity (BMI > 29.9 kg/m^2).
5. Malabsorption syndromes.
6. Poor patient compliance.
7. Abnormal laboratory tests: hsCRP > 5 mg/l, creatinine > 1.2 mg/dl or GFR < 60 ml/min, ALT and AST > 40 IU/ml, bilirubin > 1 mg/dl, fasting glucose > 100 mg/dl or 2 h oral glucose tolerance test glucose ≥ 140 mg/dl, Hb < 12 g/dl or > 16 g/dl, RBC < 3.5 M/μl or > 5.5 M/μl, WBC < 3.5 K/μl or > 10 K/μl, PLT < 140 K/μl or > 400 K/μl, TC > 200 mg/dl, LDL > 115 mg/dl, and TG > 150 mg/dl.
8. Smoking.

2.2. Cell cultures and drug treatment

Fifteen millilitre of peripheral blood was collected between 8.00 and 10.00 a.m. to avoid circadian fluctuations of the studied parameters. Mononuclear cells were separated by histo-opaque density gradient centrifugation using protocol previously described by Fløe et al. (1991) and Okopień et al. (2005). Monocytes/macrophages were isolated by negative immunomagnetic