SCIENTIFIC LETTER

Eplerenone inhibits tumour necrosis factor α shedding process by tumour necrosis factor α converting enzyme in monocytes from patients with congestive heart failure

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Eplerenone, a selective mineralocorticoid receptor antagonist, attenuated the expression of systemic proinflammatory molecules in a rat model of congestive heart failure (CHF).¹ However, the mechanisms by which eplerenone reduces proinflammatory molecule expression have not been elucidated. Our previous study was the first to show that expression of tumour necrosis factor α (TNF α) converting enzyme (TACE) in peripheral blood mononuclear cells may be responsible for the release of TNF α in CHF.² To the best of our knowledge, the observation that peripheral monocytes can activate the TACE-TNF α system in CHF is without precedent. The objective of the present study was to examine whether monocytes have a role in the activated TACE-TNF α system and to determine whether eplerenone reduces the TNF α shedding process in monocytes from patients with CHF.

METHODS

Peripheral monocytes were isolated from blood samples at the time of admission (32 patients with CHF and 10 healthy subjects). The origin of left ventricular (LV) dysfunction was idiopathic dilated cardiomyopathy in all cases. Echocardiography was used to determine LV end systolic diameter and LV ejection fraction at the time of admission. Eplerenone (Pfizer Japan Inc) was dissolved in dimethylsulfoxide (Gibco). Isolated monocytes were cultured in RPMI 1640 medium supplemented with 10% heat inactivated fetal calf serum, 10 ng/ml phorbol 12-myristate-13-acetate (PMA; Calbiochem), and vehicle or eplerenone for six hours at 37°C in 5% CO₂. TACE, TNF α , and glyceraldehyde-3-phosphate dehydrogenase mRNA concentrations were quantified by a real time reverse transcriptase polymerase chain reaction method as previously described.² Cultured monocytes were incubated with peridinin chlorophyll protein conjugated CD14 antibody. Intracellular TACE and TNF α were then stained with fluorescein isothiocyanate conjugated antibodies. CD14 positive events were measured on a FACScan flow cytometer (Becton Dickinson). Histograms were generated to measure intracellular TACE and TNF α concentrations by mean fluorescence intensity. TNF- α concentrations in culture supernatant were determined by a slide phase enzyme amplified sensitivity immunoassay kit.

RESULTS

Baseline TACE and TNF α concentrations were higher in patients with CHF than in healthy subjects (all $p < 0.01$). TACE and TNF α concentrations were positively correlated in patients with CHF (mRNA: $r = 0.61$, $p < 0.001$; mean fluorescence intensity: $r = 0.69$, $p < 0.001$). These concentrations in patients with CHF were negatively correlated with LV ejection fraction and positively correlated with LV end systolic diameter. TACE and TNF α generating and secreting capacities were higher in patients with CHF than in healthy

subjects. These capacities were higher in patients with CHF in New York Heart Association class III or IV than in those in class I or II at the time of admission.

Eplerenone inhibited TACE and TNF α in a concentration dependent manner in in vitro PMA stimulated monocytes obtained from patients with CHF (fig 1). Eplerenone (final concentration 10⁻⁵ mol/l) downregulated TACE and TNF α concentrations in unstimulated cultured monocytes from patients with CHF, although it had no effect on the equivalent concentrations in healthy subjects.

DISCUSSION

In the present study, TACE produced by monocytes contributed to alterations in systemic metabolism and the release of TNF α in patients with CHF, leading to the development of CHF. We have also shown that eplerenone has a suppressive effect on in vitro transcription and translation of TACE and TNF α in monocytes from patients with CHF. This suggests that eplerenone may indeed have anti-inflammatory potential against systemic inflammatory reaction in CHF. Eplerenone, a selective mineralocorticoid receptor antagonist, can reduce vascular inflammation.¹ PMA stimulation has shown that eplerenone inhibited TNF α release through TACE in a concentration dependent manner. Therefore, eplerenone can inhibit TACE dependent ectodomain shedding of TNF α by monocytes. Chronic aldosterone treatment leads to activation of reactive oxygen species and proinflammatory phenotype in response to mineralocorticoid receptor activation.³ TACE is activated by agents such as PMA that mimic the effect of reactive oxygen species.² The effect of eplerenone was associated with amelioration of adverse cardiac remodeling by reactive oxygen species and inflammation.⁴ Hansen *et al*⁵ have also reported that spironolactone induced TNF α inhibition was independent of anti-mineralocorticoid activity in cultured human mononuclear cells. Eplerenone may be hypothesised to inhibit transcriptional and translational activity of TACE by blocking reactive oxygen species and may ultimately inhibit ectodomain shedding of TNF- α in monocytes from patients with CHF.

In conclusion, the present study has shown that the clinical severity of CHF is associated with activation of the TACE-TNF α system in monocytes. In addition, the novel mechanism whereby eplerenone inhibits the TNF α shedding process through TACE in monocytes means that eplerenone may have a beneficial role in the treatment of patients with CHF.

Abbreviations: CHF, congestive heart failure; LV, left ventricular; PMA, phorbol 12-myristate-13-acetate; TACE, tumour necrosis factor α converting enzyme; TNF α , tumour necrosis factor α

Figure 1 Effect of eplerenone on tumour necrosis factor α (TNF α) converting enzyme (TACE) and supernatant TNF α concentrations in cultured monocytes from patients with congestive heart failure. *p < 0.05 versus vehicle (0.05% dimethylsulfoxide) alone; †p < 0.05 versus phorbol 12-myristate-13-acetate (PMA) + vehicle. MFI, mean fluorescence intensity.

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