SCIENTIFIC LETTER

Enhanced endothelin-1 degradation by intravenous morphine in patients with congestive heart failure: role of neutral endopeptidase 24.11

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Endothelin-1 (ET-1), as the most potent endothelium dependent vasoconstrictor peptide, contributes to vasoconstriction, decreased ventricular function, and volume retention in congestive heart failure (CHF). Plasma ET-1 concentrations are raised in patients with CHF, correlate with symptoms and with the haemodynamic severity, and are associated with an adverse prognosis.¹ Endothelin receptor antagonism or enhanced ET-1 hydrolysis remains the goal for treating CHF. Neutral endopeptidase 24.11 (NEP), a membrane bound zinc metalloprotease, has been shown to hydrolyse many endogenous peptides including ET-1.² Our previous study demonstrated that morphine could activate neutrophil NEP.³ In this report we will show intravenous morphine provides cardioprotective effects in patients with heart failure by activating NEP and decreasing circulating ET-1.

METHODS

Fifty eight patients who presented with symptoms and signs of CHF and with a final diagnosis of dilated cardiomyopathy (by the World Health Organization criteria) were included for study. All the patients underwent cardiac catheterisation with haemodynamic measurements and coronary angiography to exclude other causes of CHF. They all accepted regular standard treatment for heart failure (oxygen, furosemide 1 mg/kg, and sublingual glyceryl trinitrate 0.5 mg before study) at the emergency department and were randomised into two subgroups according to the different timing of morphine administration. Those treated with protocol 1 (group 1A, $n = 30$) underwent intravenous administration of 3 mg of morphine immediately after the first blood sampling for measurements of neutrophil NEP and ET-1. The second blood sampling was undertaken 10 minutes later. Those treated with protocol 2 (group 1B, $n = 28$) were checked twice for NEP and ET-1 at the same time intervals but were administered morphine immediately after the second blood sampling. Group 2A, as negative control, comprised another 24 patients with dyspnoea but normal cardiac examination, and underwent the same blood sampling and medical treatment as group 1A. Another 18 negative control subjects (group 2B) were treated and sampled as group 1B due to the randomisation process. Plasma ET-1 and peptides with C-terminal tetrapeptide of ET-1 (including ET-1 and its post-degradation C-terminal fragment (CTF)) were measured by using a well established sandwich enzyme immunoassay method (R & D System, Minneapolis, USA). The sensitivity of the method was 0.1 pmol/l and the intra- and inter-assay coefficient of variation were both $< 10\%$. The CTF was thus determined by the concentration measured by this method minus the measured plasma ET-1 concentration. In addition, purified neutrophils in RPMI without additives were analysed for NEP enzymatic activity with an established fluorimetric assay for NEP activities using glutaryl-ala-ala-phe-4-methoxy-2-

nathylamine (Enzyme System Products, Livermore, California, USA) as a substrate.³

RESULTS

Among four age and sex matched subgroups, their clinical backgrounds were similar except for New York Heart Association functional classes ($p < 0.01$) and left ventricular ejection fractions ($p < 0.001$) (table 1). The plasma concentrations of ET-1 were significantly higher in group 1 than in group 2 (table 1). There was no significant difference of plasma ET-1 between group 1A and group 1B patients. In group 1A, the ET-1 decreased significantly at the second blood samplings ($3.2 (0.8) \nu 1.9 (0.7)$ pmol/l, $p < 0.001$) whereas there was no definite change of ET-1 in group 1B. In contrast, the plasma concentrations of CTF were significantly higher in group 1 patients than in group 2. No significant differences existed between group 1A and group 1B patients in regard to initial plasma CTF. In group 1A, the C-terminal degradation products increased significantly at second blood samplings ($0.9 (0.3) \nu 1.9 (0.6)$ pmol/l, $p < 0.001$), whereas there was no definite change in the concentration of those products in group 1B ($0.9 (0.4) \nu 0.9 (0.5)$ pmol/l, $p = \text{NS}$).

There was no significant difference in baseline NEP activities between these two groups. However, the NEP activities at second blood samplings increased significantly in group 1A ($9.44 (1.68) \nu 5.22 (1.40)$ nmol/mg protein, $p < 0.001$), whereas no definite changes were observed in group 2B. The NEP activities at second samplings were also significantly higher in group 1A than in group 1B ($9.44 (1.68) \nu 5.18 (1.70)$ nmol/mg protein, $p < 0.001$). Note that there was also a significant increase in NEP activities in group 2A ($4.76 (1.38) \nu 8.54 (1.88)$ nmol/mg protein, $p < 0.001$) although their ET-1 and C-peptide profiles did not change. These data show that intravenous morphine can increase NEP activities.

DISCUSSION

In this report we have shown that morphine activated NEP and thereafter reduced plasma ET-1 concentration, with a concomitant increase of C-terminal degradation peptides in patients with dilated cardiomyopathy and heart failure. Because of the effects of ET-1 on haemodynamic derangement in patients with CHF, some investigators promoted specific pharmacotherapy aiming at inhibiting the actions of the peptide. For the first time, we have shown that short term use of low dose morphine definitely provides benefits by enhancing hydrolysis of ET-1. According to our previous reports, this is a mu-receptor related action.

Abbreviations: CHF, congestive heart failure; CTF, C-terminal fragment; ET-1, endothelin-1; NEP, neutral endopeptidase

Table 1 Patient clinical characteristics and profiles of endothelin-1, C-terminal fragment, and neutral endopeptidase 24.11

Risk factors	Group 1A (n=30)	Group 1B (n=28)	Group 2A (n=24)	Group 2B (n=18)
Age (years)	58 (12)	59 (14)	55 (16)	56 (18)
Males	18 (60%)	16 (59%)	15 (62%)	12 (66%)
Hypertension	8 (27%)	6 (21%)	4 (17%)	3 (17%)
Diabetes mellitus	3 (10%)	2 (7%)	2 (8%)	2 (11%)
Smoking	12 (40%)	10 (36%)	10 (42%)	8 (44%)
Hyperlipidemia	2 (7%)	1 (4%)	1 (4%)	1 (6%)
Haemoglobin (g/dl)	13.0 (1.8)	13.1 (1.7)	13.4 (1.7)	13.3 (2.0)
Leucocyte ($\times 1000$)*	9.7 (2.5)	9.8 (2.6)	7.1 (1.8)	6.9 (1.8)
Neutrophil (%)	67 (11)	66 (12)	64 (13)	64 (13)
Monocyte (%)	7.4 (3.0)	7.5 (2.9)	7.9 (3.5)	6.2 (3.4)
Platelet ($\times 1000$)	272 (86)	270 (84)	266 (88)	270 (90)
NYHA functional class				
Class I	0	0	24 (100%)	18 (100%)
Class II	0	0	0	0
Class III	22 (73%)	20 (71%)	0	0
Class IV	8 (27%)	8 (29%)	0	0
LVEF (echocardiography)**	22 (8)	22 (10)	62 (10)	64 (12)
LVEF (ventriculography)	20 (6)	21 (8)		
Endothelin-1 (pmol/l)				
Baseline*	3.2 (0.8)	3.3 (0.8)	1.7 (0.6)	1.6 (0.5)
10 min*	1.9 (0.7)	3.4 (0.9)	1.6 (0.6)	1.5 (0.6)
Difference (95% CI)†	1.3 (0.9 to 1.7)	0.1 (0 to 0.5)	0.1 (0 to 0.4)	0.1 (0 to 0.3)
p Value‡	<0.001	0.71	0.73	0.78
C-terminal peptide (pmol/l)				
Baseline*	0.9 (0.3)	0.9 (0.4)	0.2 (0.3)	0.2 (0.2)
10 min*	1.9 (0.6)	0.9 (0.5)	0.4 (0.4)	0.3 (0.3)
Difference (95% CI)†	1.0 (0.6 to 1.4)	0.1 (0 to 0.3)	0.2 (0 to 0.4)	0.1 (0 to 0.2)
p Value‡	<0.001	0.83	0.65	0.55
NEP (nmol/mg protein)				
Baseline*	5.22 (1.40)	5.28 (1.50)	4.76 (1.38)	4.42 (1.68)
10 min*	9.44 (1.68)	5.18 (1.70)	8.54 (1.88)	4.56 (1.92)
Difference (95% CI)†	4.21 (3.58 to 4.92)	0.10 (0 to 0.90)	3.78 (2.99 to 4.57)	0.14 (0 to 1.04)
p Value‡	<0.001	0.27	<0.001	0.64

Values are mean (SD) unless otherwise indicated

*p<0.001 among 4 groups via ANOVA

**p<0.05 among 4 groups via ANOVA

†p<0.01 among 4 groups via ANOVA

‡Baseline versus 10 minutes.

One concern in this study is the effect of NEP activation on natriuretic peptide degradation. Previous animal data showing that opiates increase plasma natriuretic peptides⁴ may suggest inhibition of NEC rather than activation of NEC by morphine. The discrepancies should be partly caused by dose dependent effects. The doses used in animal experiments were 100–1000 times above the doses clinically used. Some of the studies were conducted via intrathecal injection instead of using the intravenous route. The central nervous system will be preferentially affected rather than peripheral tissues. In addition, the balance in the effects of NEP activation on vascular tone depends on the type of predominant substrates degraded and on the extent of NEP involvement in the processing of ET-1.⁵ For example, in the human forearm circulation, certain NEP inhibitors cause vasoconstriction rather than vasodilatation, indicating that vasoconstrictor peptides, such as angiotensin II and ET-1, can be substrates for NEP. Fontana and colleagues⁶ demonstrated that the inhibitory effects of opioid on natriuretic factor actually occurred in those patients with severe heart failure. The diversity in species studied, the pathophysiological conditions, and the treatment modes may explain the discrepancies between some previous reports and our findings.

In conclusion, intravenous morphine activates neutrophil NEP activities and decreases plasma concentrations of ET-1 while concomitantly increasing CTF. The observations may provide further evidence about cardioprotective effects of morphine in patients with CHF.

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