Nephrol Dial Transplant (2000) 15: 1212–1216

Nephrology
Dialysis
Transplantation

Original Article

Plasma concentrations of α-melanocyte-stimulating hormone are elevated in patients on chronic haemodialysis

Lorena Airaghi[1], Letizia Garofalo[1], Maria Grazia Cutuli[1], René Delgado[1], Andrea Carlin[1], Maria Teresa Demitri[1], Salvatore Badalamenti[2], Giorgio Graziani[2], James M. Lipton[3] and Anna Catania[1]

[1]Third Division of Internal Medicine, Ospedale Maggiore di Milano IRCCS, Milan, [2]Ospedale Clinicizzato Humanitas, Rozzano, Italy and [3]Zengen Inc., Woodland Hills, CA, USA

Abstract

Background. Clinical and/or laboratory signs of systemic inflammation occur frequently in patients undergoing long-term haemodialysis. It is likely, therefore, that a compensatory release of endogenous anti-inflammatory molecules occurs to limit host reactions. The aim of the present research was to determine if the potent anti-inflammatory peptide α-melanocyte-stimulating hormone (α-MSH), a pro-opiomelanocortin derivative, is increased in plasma of haemodialysis patients. Because endotoxin and cytokines induce α-MSH *in vivo* and *in vitro*, we also measured plasma concentrations of endotoxin, interleukin-6 (IL-6), and tumour necrosis factor α (TNF-α), and the two circulating products of activated monocytes, nitric oxide (NO) and neopterin.

Methods. Thirty-five chronic haemodialysis patients, 20 patients with chronic renal failure not yet on dialysis, and 35 normal controls were included in the study. In the haemodialysis group, blood samples were obtained before and at the end of a dialysis session. Plasma α-MSH was measured using a double antibody radioimmunoassay, and IL-6, TNF-α, and neopterin using specific enzyme-linked immunosorbent assays. Plasma nitrites were determined by a colorimetric method, and endotoxin with the quantitative chromogenic LAL (limulus amoebocyte lysate) method.

Results. Mean plasma α-MSH was higher in haemodialysis patients than in control subjects, with the peptide concentrations being particularly elevated in dialysed patients with detectable endotoxin. High α-MSH concentrations were observed in the pre-dialysis samples, with no substantial change at the end of the dialysis session. Plasma concentrations of IL-6, TNF-α, neopterin, and NO were generally elevated in chronic haemodialysis patients and there was a negative correlation between circulating α-MSH and IL-6. In patients with renal failure not yet on dialysis, mean plasma α-MSH was similar to that of normal subjects.

Conclusions. α-MSH is increased in the circulation of chronic haemodialysis patients and particularly so in case of detectable endotoxaemia. Reduction of renal clearance is unlikely to contribute to the observed rise of the peptide because α-MSH concentration is not increased in patients with chronic renal failure who are not yet on dialysis. It is likely that dialysis-associated endotoxaemia, directly and/or through cytokine release, enhances the production of the anti-inflammatory mediator α-MSH that limits host reactions.

Keywords: endotoxin; interleukin-6; α-melanocyte-stimulating hormone; neopterin; nitric oxide; tumour necrosis factor-α

Introduction

Patients undergoing haemodialysis frequently show clinical and/or laboratory signs of systemic inflammation. This host response, which includes fever and an increase in acute-phase proteins, is believed to be caused by proinflammatory cytokines, such as tumour necrosis factor (TNF) and interleukin-6 (IL-6), which are increased during dialysis [1]. Nitric oxide (NO), another inflammatory mediator released by host cells, markedly increases during haemodialysis-induced hypotensive episodes [2] and can contribute to systemic inflammatory reactions.

Production and actions of proinflammatory mediators are probably modulated by endogenous molecules that counteract their effects on the host. α-Melanocyte-stimulating hormone (α-MSH) is a potent anti-inflammatory peptide with prominent effects in reducing production and actions of mediators of inflammation, including cytokines [3,4]. This molecule is a 13 amino acid pro-opiomelanocortin (POMC) derivative expressed in widespread regions of the

Correspondence and offprint requests to: Anna Catania MD, 3rd Division of Internal Medicine, Ospedale Maggiore di Milano IRCCS, Via F. Sforza 35, I-20122 Milan, Italy.

central nervous system [3] and in peripheral cells, including phagocytes and keratinocytes [5,6]. The anti-inflammatory effects of α-MSH are mainly exerted through antagonism of proinflammatory mediators, including TNF-α, IL-6, and NO [4]. Because intermittent haemodialysis treatment is characterized by enhanced production of all these mediators, it is likely that α-MSH is also released to counteract their proinflammatory effects.

The aim of the present research was to determine possible changes of plasma α-MSH in chronic haemodialysis patients. Because endotoxin and cytokines induce α-MSH *in vivo* and *in vitro*, we measured plasma concentrations of endotoxin, IL-6, and TNF-α, and the circulating levels of two products of activated monocytes, namely NO and neopterin. To determine if any potential increase in α-MSH could be caused by reduced peptide clearance, α-MSH was also measured in patients with severe renal failure who were not yet on dialysis.

Subjects and methods

Patients

Thirty-five patients on regular dialysis, 15 males and 20 females, mean age 57.9 ± 14.4 years (range 23–74) and average dialysis time of 116.2 ± 95 months (range 6–308), were included in the study. All patients were in stable haemodynamic conditions and none were treated with corticosteroids. Dialysis sessions were performed using single-use hollow-fibre dialysers equipped with cellulose-based membranes (14 patients) or synthetic membranes (21 patients). The dialysate was a standard ionic composition, and bicarbonate was used as buffer substrate in all cases.

Blood (4 ml) was withdrawn from the arteriovenous fistula and collected in Vacutainer tubes containing ethylenediamine tetra-acetic acid (EDTA) immediately before and at the end of the haemodialysis session. The dialysis session selected was the one at the end of the 'long' interdialytic period, i.e. 72 h after the previous dialysis session.

To compare plasma concentrations of α-MSH in haemodialysis patients and in patients with severe renal failure not yet undergoing haemodialysis, blood samples were obtained from 20 patients, eight males and 12 females, whose serum creatinine concentration was $\geqslant$416 μmol/l. Control blood samples were obtained from 35 healthy subjects, 21 males and 14 females, mean age 52 ± 2.2 years (range 25–74).

Methods

Plasma α-MSH determination

α-MSH was measured with a double-antibody radioimmunoassay (Euro-Diagnostica AB, Malmö, Sweden). The sensitivity of the assay is 0.5 pg/ml and cross-reactivity with other POMC peptides (adrenocorticotrophic hormone (ACTH) (1–24), ACTH (1–39), β-MSH, γ-MSH) is <0.002%.

Plasma IL-6 and TNF-α determinations

Plasma IL-6 and TNF-α were measured using specific two-site enzyme linked immunosorbent assays (ELISAs) (Biotrak Amersham International, Little Chalfont, Buckinghamshire, UK). The detection limit for IL-6 and TNF-α was <1 pg/ml and <5 pg/ml respectively.

Plasma neopterin determination

Plasma neopterin was measured using a commercial enzyme immunoassay (ELISA) (ELItest Neopterin, Henning GMBH, Berlin, Germany).

Plasma nitrite determination

Plasma nitrites were measured as an estimate of NO production. Nitrates (NO_3^-) were converted into nitrites (NO_2^-) by treatment of serum with nitrate reductase (Boehringer Mannheim Italia SpA, Milan, Italy). After enzymatic reduction, samples were mixed with equal amounts of Griess reagent (sulphanilamide 1%, naphthylethylenediamide 0.1% in phosphoric acid 0.25%). Samples were incubated at room temperature for 10 min and absorbance was measured at 540 nm using a microplate reader.

Plasma endotoxin determination

Blood (3 ml) for endotoxin determination was collected in sterile tubes containing EDTA. Glassware used for the assay was sterilized by autoclaving and dry-heated at 180°C for 4 h. Plasma was obtained by centrifugation at 200 *g* for 10 min. Samples were diluted 1:10 in endotoxin-free water and heated at 70°C for 5 min to remove non-specific inhibitors. The concentration of endotoxin was measured using a quantitative chromogenic LAL (limulus amoebocyte lysate) method (QCL-1000; BioWittaker, Walkersville, MD) according to the manufacture instructions.

Statistical analysis

Values are expressed as means ± SE. Two sample comparisons were performed using Mann–Whitney rank sum test. Spearman rank order correlation was calculated to determine the significance of relations among measures. Probability values <0.05 were considered significant.

Results

In haemodialysis patients, mean concentration of predialytic α-MSH was higher than in control subjects (Figure 1). There was no substantial change in plasma α-MSH at the end of the dialytic session: a small increase of approximately 1 pg/ml, present in all patients, was probably due to haemoconcentration. There was no difference in plasma α-MSH in patients dialysed with cellulosic or synthetic membranes. Finally, there was no difference in plasma α-MSH with regard to dialytic age.

To determine whether increases in α-MSH concentrations were caused by reduced peptide clearance rather then by dialysis procedure, we measured α-MSH in the

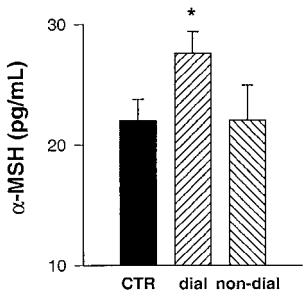

Fig. 1. Plasma concentrations of α-MSH in control subjects ($n=35$), in patients on chronic haemodialysis ($n=35$), and in patients with chronic renal failure not yet on dialysis ($n=20$). Circulating α-MSH was significantly more elevated in patients on chronic haemodialysis than in normal controls and in patients with chronic renal failure not yet dialysed. Bars represent the mean ± SE. *$P<0.05$.

plasma of patients with severe renal failure who were not yet on dialysis. These patients had α-MSH values similar to those of control subjects (Figure 1).

Endotoxin was detectable (≥0.1 UI/ml) in 15/35 (42.8%) plasma samples of haemodialysis patients at baseline, and was undetectable (<0.1 UI/ml) in controls. At the end of the haemodialysis session there was a substantial increase in plasma endotoxin (0.13 ± 0.02 UI/ml at baseline and 0.27 ± 0.05 at the end of the session, $P< 0.001$). Mean concentration of α-MSH was significantly higher in patients with detectable endotoxin at baseline (30.4 ± 2.49 *vs* 23.5 ± 2.27, $P<0.05$; Figure 2).

Although the patients on intermittent haemodialysis had no clinical signs of systemic inflammation such as fever, laboratory measurements indicated an inflammatory status, in that inflammatory mediators were elevated (Table 1). IL-6 and TNF-α, which were generally undetectable in control subjects, were increased in plasma of dialysed patients. Products of activated macrophages, NO_3^- and neopterin, were both higher in blood samples from dialysed patients than in controls. There was a negative correlation between plasma α-MSH and IL-6 ($r=-0.47$, $P=0.01$) (Figure 3).

Table 1. Markers of inflammation in patients on chronic haemodialysis

Group	IL-6 (pg/ml)	TNF-α (pg/ml)	NO_3^- (μmol/l)	Neopterin (nmol/l)
Dialysis	9.4 ± 1.16	9.7 ± 0.68	96.4 ± 15.6	159.1 ± 6.05
Healthy controls	Undetectable	Undetectable	41.9 ± 4.97	7.5 ± 3.5
P			<0.01	<0.01

Values represent the mean ± SE.

Discussion

Results show that the neuropeptide α-MSH, an endogenous modulator of inflammation, is increased in the circulation of haemodialysis patients with detectable plasma endotoxin. This finding is consistent with our prior observation that administration of endotoxin to normal human subjects causes marked release of α-MSH in the circulation [7]. Further, previous research showed that during infectious or inflammatory disorders there are substantial changes in concentrations of circulating and/or local α-MSH [8].

The idea that α-MSH is important in the control of host responses stems from the initial observation that the molecule has antipyretic properties [9,10]. The antipyretic potency of α-MSH in reducing fever caused by endogenous pyrogen is remarkable: more than 20 000 times that of acetaminophen on a molar basis [10]. Fevers caused by endotoxin [11], IL-1 [12], IL-6 [13], and TNF [13], were all inhibited by α-MSH, which also inhibited IL-1- and TNF-induced increases in circulating acute-phase proteins and neutrophils [11]. Further, the peptide inhibited tissue injury in

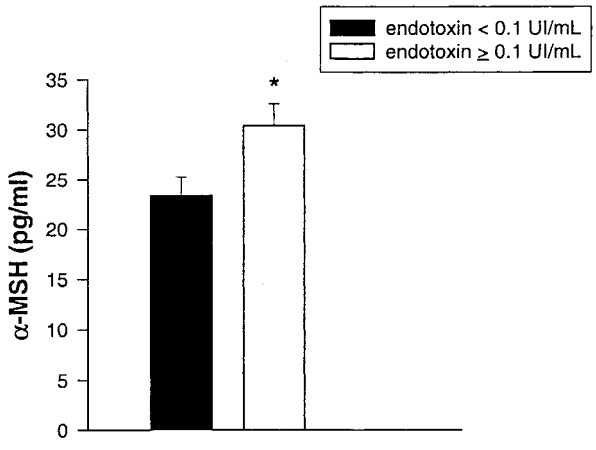

Fig. 2. Circulating α-MSH in haemodialysis patients separated on the basis of endotoxin concentration in plasma before the dialysis session. Subjects with values of endotoxin ≥0.1 UI/ml ($n=15/35$) at baseline had increased concentrations of plasma α-MSH. Bars represent the mean ± SE. *$P<0.05$.

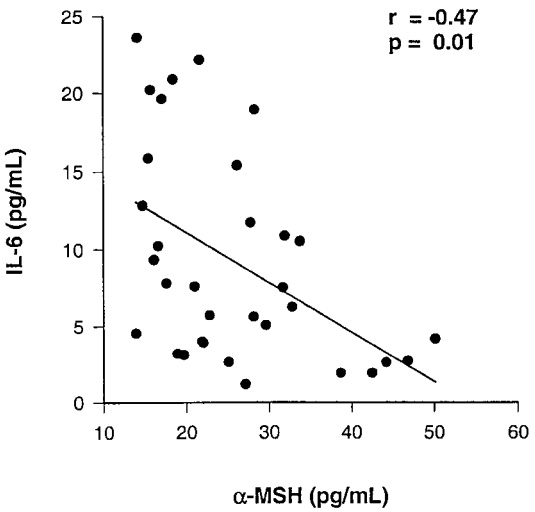

Fig. 3. Correlation between plasma concentrations of α-MSH and IL-6 in haemodialysis patients.

models of systemic inflammation such as acute respiratory distress syndrome and peritonitis caused by caecal ligation and puncture [14] and in ischaemic acute renal failure [15]. Immunoneutralization experiments indicate that endogenous α-MSH is significant in the control of inflammatory host reactions [16]. After blockade of endogenous α-MSH with specific antibodies, IL-1-induced fever was much more prolonged and of greater magnitude.

Experiments *in vitro* showed that α-MSH has broad anti-inflammatory influences in phagocytes [5,17]. Treatment with α-MSH inhibited production of TNF-α [5], neopterin [5], and NO [17] by activated monocytes. The peptide reduced neutrophil chemotaxis induced *in vitro* by IL-8 and by the chemotactic peptide fMetLeuPhe [18]. Most of the inhibitory effects of α-MSH, including inhibition of TNF-α production and anti-chemotactic activity are caused by induction of intracellular cAMP [18], as the α-MSH receptors are G-protein-linked receptors coupled to adenylyl cyclase [4]. Further, recent research indicates that α-MSH inhibits activation of the transcription factor NF-κB [19,20], a central mediator in proinflammatory cytokine transcription. In all these experiments, very small concentrations of α-MSH, even in the femtomolar range, inhibited production of mediators of inflammation. Therefore, small changes in circulating peptide can have marked effects on host cells.

Research on monocytes indicates that anti-inflammatory/anticytokine influences of α-MSH can be exerted in an autocrine fashion [21]. Activated monocyte/macrophages express the pro-opiomelanocortin gene and produce the α-MSH peptide. Further, these cells express the α-MSH receptor MC-1R. Because immunoneutralization of such receptor increases TNF-α production, an autocrine circuit based on the peptide and its receptors probably occurs [21]. Consequently, measurement of circulating peptide may underestimate changes in α-MSH release that occur locally in inflammatory cells. Small increases in the circulating peptide can result from marked production in certain cells such as monocytes, in which the peptide exerts significant autocrine influences on cytokine production.

During haemodialysis, endotoxin can transfer across membranes through backfiltration of dialysate into blood and can stimulate cytokine production by monocytes [22]. Cytokine release can also be induced through direct activation of monocytes by dialysis membranes [23]. Although the trigger for release of α-MSH in haemodialysis patients is unknown, actions of endotoxin and circulating cytokines is probably responsible. Indeed, in experiments *in vitro*, both endotoxin [17] and TNF [5] elicited release of α-MSH from monocytes. That α-MSH was increased mainly in patients with detectable further endotoxin supports this idea. Therefore, it is conceivable that endotoxin, either directly or through cytokine release, induced α-MSH production in haemodialysis patients. Because in patients with end-stage renal failure who were not yet dialysed there was no such increase, it is unlikely that reduced α-MSH clearance augmented its plasma concentrations. The increase in circulating α-MSH was observed in both pre- and post-dialysis samples and did not parallel the dialysis-induced rise in plasma endotoxin. It appears, therefore, that chronic endotoxaemia rather than acute endotoxin absorption induced α-MSH release.

In conclusion, the present observations indicate that the potent anti-inflammatory peptide α-MSH is increased in the circulation of haemodialysis patients, probably to modulate host responses. Indeed, although none of the patients had fever during haemodialysis, there was an increase in pyretic cytokines in a substantial proportion of them. Therefore, it is reasonable to believe that endogenous antipyretic molecules such as α-MSH contribute to reduce effects of inflammatory mediators and limit host reactions.

Acknowledgements. This work was supported by grant 100–01 Ospedale Maggiore di Milano IRCCS and NIH grant NS10046 from the National Institute of Neurological Diseases and Stroke.

References

1. Descamps-Latscha B, Herbelin A, Nguyen AT *et al*. Balance between IL-1β, TNF-α, and their specific inhibitors in chronic renal failure and maintenance dialysis. *J Immunol* 1995; 154: 882–892
2. Yokokawa K, Mankus R, Saklayen MG *et al*. Increased nitric oxide production in patients with hypotension during hemodialysis. *Ann Intern Med* 1995; 123: 35–37
3. Catania A, Lipton JM. α-Melanocyte stimulating hormone in the modulation of host reactions. *Endocrinol Rev* 1993; 14: 564–576
4. Lipton JM, Catania A. Anti-inflammatory influence of the neuroimmunomodulator α-MSH. *Immunol Today* 1997; 18: 140–145
5. Rajora N, Ceriani G, Catania A, Star RA, Murphy MT, Lipton JM. α-MSH production, receptors and influence on neopterin in a human monocyte/macrophage cell line. *J Leukoc Biol* 1996; 59: 248–253
6. Luger TA, Schauer E, Trautinger F *et al*. Production of immunosuppressing melanotropins by human keratinocytes. *Ann N Y Acad Sci* 1993; 680: 567–570
7. Catania A, Suffredini AF, Lipton JM. Endotoxin causes α-MSH release in normal human subjects. *Neuroimmunomodulation* 1995; 2: 248–253
8. Catania A, Airaghi L, Garofalo L, Cutuli M, Lipton JM. The neuropeptide α-MSH in AIDS and other conditions in humans. *Ann N Y Acad Sci* 1998; 840: 848–856
9. Lipton JM, Glyn JR, Zimmer JA. ACTH and α-melanotropin in central temperature control. *Fed Proc* 1981; 40: 2760–2762
10. Murphy MT, Richards DB, Lipton JM. Antipyretic potency of centrally administered α-melanocyte stimulating hormone. *Science* 1983; 221: 192–193
11. Martin LW, Lipton JM. Acute phase response to endotoxin: rise in plasma α-MSH and effects of α-MSH injection. *Am J Physiol* 1990; 259: R768–772
12. Opp MR, Obal F, Kreuger JM. Effects of α-MSH on sleep, behavior, and brain temperature: interactions with IL-1. *Am J Physiol* 1988; 255: R914–922
13. Martin LW, Catania A, Hiltz A, Lipton JM. Neuropeptide α-MSH antagonizes IL-6- and TNF-induced fever. *Peptides* 1991; 12: 297–299
14. Lipton JM, Ceriani G, Macaluso A *et al*. Anti-inflammatory effects of the neuropeptide α-MSH in acute, chronic, and systemic inflammation. *Ann NY Acad Sci* 1994; 741: 137–148
15. Chiao HS, Kohda Y, McLeroy P, Craig L, Housini I, Star RA.

α-Melanocyte-stimulating hormone protects against renal injury after ischemia in mice and rats. *J Clin Invest* 1997; 99: 1165–1172
16. Shih ST, Khorram O, Lipton JM, McCann SM. Central administration of α-MSH antiserum augments fever in the rabbit. *Am J Physiol* 1986; 250: R803–806
17. Star RA, Rajora N, Huang J, Chavez R, Catania A, Lipton JM. Evidence of autocrine modulation of macrophage nitric oxide synthase by α-MSH. *Proc Natl Acad Sci USA* 1995; 92: 8016–8020
18. Catania A, Rajora N, Capsoni F, Minonzio F, Star RA, Lipton JM. The neuropeptide α-MSH has specific receptors on neutrophils and reduces chemotaxis *in vitro*. *Peptides* 1996; 17: 675–679
19. Manna SK, Aggarwal B. α- Melanocyte-stimulating hormone inhibits the nuclear transcription factor NF-κB activation induced by various inflammatory agents. *J Immunol* 1998; 161: 2873–2880
20. Ichiyama T, Zhao H, Catania A, Furukawa S, Lipton JM. α-Melanocyte-stimulating hormone inhibits NF-κB activation and IκBα degradation in human astrocytes and in experimental brain inflammation. *Exp Neurol* 1999; 157: 359–365
21. Taherzadeh S, Sharma S, Chhajlani V *et al.* α-MSH and its receptors in regulation of tumor necrosis factor-α production by human monocyte/macrophages. *Am J Physiol* 1999; 276: R1289–1294
22. Urena P, Herbelin A, Zingraff J *et al.* Permeability of cellulosic and non-cellulosic membranes to endotoxin subunits and cytokine production during *in-vitro* haemodialysis. *Nephrol Dial Transplant* 1992; 7: 16–28
23. Pascual M, Tolkoff-Rubin N, Schifferli JA. Is adsorption an important characteristic of dialysis membranes? *Kidney Int* 1996; 49: 309–313

Received for publication: 30.6.99
Accepted in revised form: 10.3.00