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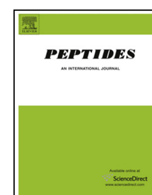

Plasma levels of intermedin (adrenomedullin-2) in healthy human volunteers and patients with heart failure

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ABSTRACT

Intermedin/adrenomedullin-2 (IMD) is a member of the adrenomedullin/CGRP peptide family. Less is known about the distribution of IMD than for other family members within the mammalian cardiovascular system, particularly in humans. The aim was to evaluate plasma IMD levels in healthy subjects and patients with chronic heart failure. IMD and its precursor fragments, preproIMD$_{25-56}$ and preproIMD$_{57-92}$, were measured by radioimmunoassay in 75 healthy subjects and levels of IMD were also compared to those of adrenomedullin (AM) and mid-region proadrenomedullin$_{45-92}$ (MRproAM$_{45-92}$) in 19 patients with systolic heart failure (LVEF < 45%). In healthy subjects, plasma levels (mean + SE) of IMD ($6.3 + 0.6$ pg ml^{-1}) were lower than, but correlated with those of AM ($25.8 + 1.8$ pg ml^{-1}; r = 0.49, $p < 0.001$). Plasma preproIMD$_{25-56}$ ($39.6 + 3.1$ pg ml^{-1}), preproIMD$_{57-92}$ ($25.9 + 3.8$ pg ml^{-1}) and MRproAM$_{45-92}$ ($200.2 + 6.7$ pg ml^{-1}) were greater than their respective bioactive peptides. IMD levels correlated positively with BMI but not age, and were elevated in heart failure ($9.8 + 1.3$ pg ml^{-1}, $p < 0.05$), similarly to MRproAM$_{45-92}$ ($329.5 + 41.9$ pg ml^{-1}, $p < 0.001$) and AM ($56.8 + 10.9$ pg ml^{-1}, $p < 0.01$). IMD levels were greater in heart failure patients with concomitant renal impairment ($11.3 + 1.8$ pg ml^{-1}) than those without ($6.5 + 1.0$ pg ml^{-1}; $p < 0.05$). IMD and AM were greater in patients receiving submaximal compared with maximal heart failure drug therapy and were decreased after 6 months of cardiac resynchronization therapy. In conclusion, IMD is present in the plasma of healthy subjects less abundantly than AM, but is similarly correlated weakly with BMI. IMD levels are elevated in heart failure, especially with concomitant renal impairment, and tend to be reduced by high intensity drug or pacing therapy.

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1. Introduction

There has been much recent interest in the measurement of biomarkers in cardiovascular disease [9]. In the field of heart failure, B type natriuretic peptide (BNP) and its precursor fragment N terminal pro B type natriuretic peptide (NT-proBNP) have become established diagnostic and prognostic markers, and their use has been encouraged in clinical guidelines [44,31]. A variety of alternative or additional markers have been proposed, with previous interest in adrenomedullin (AM) [10,14,35,37] and now renewed focus on its precursor fragment, mid-region proadrenomedullin$_{45-92}$ (MRproAM$_{45-92}$) [20,8,26]. Intermedin (also known as adrenomedullin-2; IMD), a novel peptide independently discovered by 2 research groups in 2004 [38,41], is related to and shares a family of receptors with CGRP and AM [38,41,2]. Proteolytic processing of a larger prepro-IMD precursor yields a series of biologically active C-terminal peptides as well as the precursor fragments, preproIMD$_{25-56}$ and preproIMD$_{57-92}$ (Fig. 1). Exogenous IMD is a potent systemic vasodilator [32,49], influences regional blood flow and water-electrolyte homeostasis [43], augments cardiac contractility and protects against oxidative stress and ischaemic insult in rodents (reviewed in Ref. [2,15]). IMD is detected, but at lower levels than AM, in adult rodent cardiomyocytes [3–5]. Data in humans are scarce but in tissue obtained at autopsy from subjects without known cardiac or renal disease, IMD

Abbreviations: CGRP, calcitonin gene-related peptide; CRT, cardiac resynchronization therapy; TFA, trifluroacetic acid.

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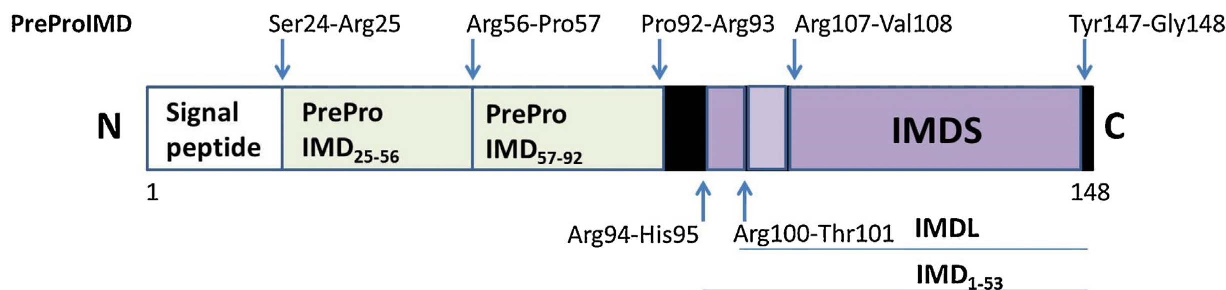

Fig. 1. Structure of the human IMD/AM2 prepro-peptide. Cleavage sites towards the C-terminus of the prepro-IMD molecule (indicated by arrows) give rise to 3 mature IMD/AM2 peptides (IMD_{1-53}, IMD_{1-47}) (IMD long) and IMD_{8-47} (IMD short). The precursor fragment $preproIMD_{25-92}$ is not biologically active and is further cleaved between Arg56-Pro57 to generate two fragments, $preproIMD_{25-56}$ and $preproIMD_{57-92}$.

localized primarily to myocardium and to renal tubular cells [30]. IMD is also expressed in human ventricular cardiac fibroblasts, cardiomyocytes and cardiac microvascular and aortic endothelial cells [33,1,15] and in human renal mesangial and tubular cells (Bell and Metcalfe, unpublished observation) maintained in culture. At mRNA level, expression of IMD is negligible in leukocytes circulating in human blood obtained from healthy subjects but is upregulated markedly in heart failure [27].

Given the vasodilator, positively inotropic, natriuretic and diuretic actions [41,2,15] reported in laboratory studies so far, IMD may have an important role to play in the counter regulatory response to heart failure. Data in humans however are limited. Recently, a number of studies reported on augmentation of IMD levels in the plasma of patients following acute myocardial infarction, correlating with the extent of coronary stenosis and providing a potential prognostic marker for further adverse cardiovascular events [25,36,42,48]. Although such studies included carefully matched controls, relatively little is yet known about characteristics influencing plasma levels of IMD in healthy individuals, how IMD levels are affected by other disease states and clinical interventions, or relate to those of its own precursor fragments or other members of the CGRP/adrenomedullin peptide family. The aim therefore was to measure levels of IMD and the precursor fragments, $preproIMD_{25-56}$ and $preproIMD_{57-92}$, in healthy control subjects, and explore anthropometric influences on these levels. IMD levels in healthy subjects were compared with those measured in a cohort of patients with stable systolic heart failure. Within the heart failure group, the influence of various parameters including intensity of drug therapy and cardiac resynchronization therapy (CRT) on plasma peptide levels was assessed. As comparator, IMD levels were compared with those of AM and mid-region $proAM_{45-92}$ in healthy subjects, and in heart failure patients since these comparators have been found previously to be elevated in such patients [35,37,8].

2. Methods

2.1. Subjects

Two groups were studied. 75 healthy subjects aged 18 years and above were recruited from amongst our clinical and university colleagues. Subjects were excluded if they had cardiac symptoms, a known history of heart failure or any known cardiac disease, known risk factors for cardiac disease including diabetes and hypertension, or were taking any regular medication. 19 patients with heart failure were recruited from the Belfast Health and Social Care Trust heart failure service. All subjects had stable chronic heart failure, left ventricular systolic dysfunction (ejection fraction < 45%) and were receiving maximally tolerated medical therapy (including diuretics, angiotensin converting enzyme inhibitors or angiotensin receptor antagonists, aldosterone receptor antagonists and beta adrenoceptor antagonists as tolerated). A subgroup of patients with heart failure and systolic dysfunction attending for cardiac resynchronization therapy (CRT) were studied within 24 h prior to (baseline) and for up to 12 months after this device-based intervention. Approval was obtained from the Queen's University of Belfast School of Medicine, Dentistry and Biomedical Sciences Research Ethics Committee and Office of Research Ethics Committees for Northern Ireland (ORECNI). The study was performed in accordance with The Declaration of Helsinki and the Belfast Trust Research Governance Framework. All subjects provided informed written consent.

Height and weight were obtained for each subject and body mass index (BMI) calculated. Clinical features, details of LVEF, intensity of medical therapy (those taking 4 or fewer standard therapies for heart failure as listed above) and biochemical parameters including renal function were recorded for the patients. NT-proBNP was determined using the commercially available and validated Elecsys proBNP assay (Roche Diagnostics Ltd.) and analysed on a Roche Modular Analyser (E module).

2.2. Peptide biomarker sampling and analysis

Following venepuncture, 30 ml of blood was immediately collected into chilled bottles containing EDTA and the protease inhibitor aprotinin (0.6TIU (780KIU) ml^{-1} of blood, Apollo Scientific, UK). After immediate centrifugation at $3000 \times g$ for 20 min at 4 °C. the plasma was stored with aprotinin at −80 °C pending analysis. Plasma was defrosted, acidified with an equal volume of trifluoroacetic acid (TFA, 1% v/v, Sigma–Aldrich, UK) and centrifuged at $12000 \times g$ for 20 min at 4 °C. The supernatant was applied onto pre-equilibrated C18 SEP columns (Waters Corporation, UK) eluted with 60% acetonitrile (Riedel de Haen, Germany) in 1% TFA and evaporated to dryness using a combination of centrifugal concentration (Hetero Vac, Scandinavia) and lyophilisation (Edwards, Modalyo). Reconstituted samples were assayed using commercially available radio-immunoassays (RIA; Phoenix Pharmaceuticals Inc., California, USA). Human AM, human $MRproAM_{45-92}$, and human IMD were assayed in all subjects; human $preproIMD_{25-56}$ and human $preproIMD_{57-92}$ were additionally measured in healthy control subjects. Each RIA Exhibits 100% cross-reactivity with its respective peptide antigen and no cross-reactivity with any of the other peptides measured in this study. The AM RIA detects total AM (both glycated and mature forms) and also displays limited cross-reactivity with shorter fragments such as AM_{26-52} (12% cross-reactivity) which represent degradation products of the bioactive peptide. The IMD RIA Exhibits 100% cross reactivity with the 53, 47 and 40 amino acid versions of the human peptide. IMD was measured down to 1 pg ml^{-1} by the use of serial concentration steps. For each of the peptide assays, coefficients of intra-and inter-assay variation were <5% and 10%,

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