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Low-dose copper infusion into the coronary circulation induces acute heart failure in diabetic rats: New mechanism of heart disease

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ABSTRACT

Diabetes impairs copper (Cu) regulation, causing elevated serum Cu and urinary Cu excretion in patients with established cardiovascular disease; it also causes cardiomyopathy and chronic cardiac impairment linked to defective Cu homeostasis in rats. However, the mechanisms that link impaired Cu regulation to cardiac dysfunction in diabetes are incompletely understood. Chronic treatment with triethylenetetramine (TETA), a Cu^{2+}-selective chelator, improves cardiac function in diabetic patients, and in rats with heart disease; the latter displayed ~3-fold elevations in free Cu^{2+} in the coronary effluent when TETA was infused into their coronary arteries. To further study the nature of defective cardiac Cu regulation in diabetes, we employed an isolated-perfused, working-heart model in which we infused micromolar doses of Cu^{2+} into the coronary arteries and measured acute effects on cardiac function in diabetic and non-diabetic-control rats. Infusion of $CuCl_2$ solutions caused acute dose-dependent cardiac dysfunction in normal hearts. Several measures of baseline cardiac function were impaired in diabetic hearts, and these defects were exacerbated by low-micromolar Cu^{2+} infusion. The response to infused Cu^{2+} was augmented in diabetic hearts, which became defective at lower infusion levels and underwent complete pump failure (cardiac output = 0 ml/min) more often ($P < 0.0001$) at concentrations that only moderately impaired function of control hearts. To our knowledge, this is the first report describing the acute effects on cardiac function of pathophysiological elevations in coronary Cu^{2+}. The effects of Cu^{2+} infusion occur within minutes in both control and diabetic hearts, which suggests that they are not due to remodelling. Heightened sensitivity to the acute effects of small elevations in Cu^{2+} could contribute substantively to impaired cardiac function in patients with diabetes and is thus identified as a new mechanism of heart disease.

1. Introduction

Cu plays crucial roles in many biological processes including anti-oxidant defence, oxygen utilization, energy metabolism, and the cross-linking of collagen and elastin. It is an essential trace nutrient in all known organisms, but excess free Cu (present as Cu^{II} in the extracellular space) is toxic. After absorption from the gut, it becomes bound to Cu-transporting proteins which deliver it to cells, where it is taken up via specific cell-membrane Cu transporters/channels [1,2]. Cu atoms are subsequently delivered to specific intracellular destinations via chaperones, thus activating Cu enzymes which catalyse numerous crucial biochemical reactions [3]. Cardiac tissue requires a substantial amount of Cu to sustain oxidative phosphorylation and generate the large amounts of ATP required for muscle contraction as well as peptide hormone biogenesis, protection against oxidative stress, and other critical functions [4,5].

Abbreviations: Cu^{I}, univalent Cu; Cu^{II}, divalent Cu; Cu^{2+} (aq), aqueous Cu^{2+} ion; CI, confidence interval; DCM, diabetic cardiomyopathy; ECM, extracellular matrix; LMEM, linear mixed-effects model; REML, restricted maximum likelihood; SEM, standard error of the mean.

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Defects in Cu homoeostasis, for example those caused by dietary Cu insufficiency, can impair Cu supply to mitochondria and cause severe cardiomyopathy [6]; so too can defects in proteins that mediate intracellular Cu transport, for example those caused by mutations in *SCO2* (the synthesis of cytochrome *c* oxidase 2 gene), which encodes a chaperone essential for Cu incorporation into cytochrome *c* oxidase (CcO) [7]. On the other hand, excessive amounts of Cu in the cardiac tissues can also be toxic, possibly due to elevated levels of free (or 'chelatable') Cu ions: these readily react with H_2O_2 or $O_2^{\bullet-}$ to catalyse the production of highly-toxic ROS, particularly $HO^{\bullet}$, which can damage lipids, proteins, and DNA [8]. Myocardial Cu is tightly regulated to ensure adequate intracellular supply whilst minimising risk from effects of catalytically-active Cu^{II} ions [9].

There is a strong link between Cu deficiency and cardiomyopathy. For example, Cu deficiency can cause the development of a cardiomyopathy characterized by cardiac hypertrophy, rhythm disturbances, cardiac fibrosis, and distorted myofibrils [6,10–12] Cardiac hypertrophy in Cu-deficient animals is due, at least in part, to structural alterations in the mitochondria accompanied by altered expression of mitochondrial proteins, and increased numbers and enlargement of mitochondrial volume with extensive disruption of ultrastructure [4,6,13,14]. Furthermore, defective myocardial Cu transport can also impair mitochondrial function via deficient activation of subunits CO1 and CO2 in CcO, thus impairing the production of ATP via defective oxidative phosphorylation [15]. Animals consuming Cu-deficient diets have decreased myocardial ATP and phosphocreatine, with elevated ribose 5-phosphate and phosphocholine, consistent with impaired ATP synthesis [6], and also show numerous electrocardiographic abnormalities [6,14,16,17]. Lastly, chronic Cu deficiency can also contribute to cardiac failure by altering myocardial expression of genes, including those which encode various contractile proteins, Ca^{2+}-regulatory proteins, extracellular matrix (ECM) collagen proteins, and others [18].

Defective Cu regulation is implicated in the pathogenesis of DCM [19–23]. In patients, chronic hyperglycemia impairs Cu regulation, and elevates Cu balance [20], urinary Cu excretion [20] and plasma Cu, particularly in those with cardiovascular or renal disease [24,25]. Plasma Cu also increases with age [26], and has been identified as an independent risk factor for coronary heart disease [27]. The molecular mechanisms implicated in systemic Cu overload in diabetes differ from those in Wilson's disease, in which Cu overload is caused by mutations in *ATP7B*, which encodes a Cu-transporting ATPase: these mainly cause damage in the liver, eyes and CNS [28], although rare cardiac manifestations also occur [29,30]. Imbalance between intracellular and extracellular Cu pools may elevate plasma Cu in diabetes [22,23,31,32], and altered regulation of Cu-transporting ATPases is implicated in the diabetic complications [33] including diabetic cardiomyopathy (DCM) [23]. Hyperglycemia may increase free Cu^{II} in the extracellular space by impairing the Cu-binding properties of ceruloplasmin and albumin, possibly via non-enzymatic glycation [34–36], causing increased levels of Cu^{II} bound to ECM components such as collagen. The advanced glycation endproducts (AGEs), whose production is enhanced in diabetes, can forge cross-links between long-lived fibrous proteins [37] and act as localized endogenous chelators to increase tissue-Cu binding in the ECM [38–40]. Elevated catalytically-active Cu^{II} in the ECM could overwhelm anti-oxidant defences, such as those catalysed by extracellular superoxide dismutase (SOD3) [33], causing enhanced ROS production through Fenton or Haber–Weiss chemistry, thus elevating oxidative stress and promoting TGF-β-evoked fibrosis [20,32,41]. Moreover, multiple defects in the intracellular pathways of myocardial Cu transport have been demonstrated in a widely-used rat model of diabetes [23]. Such defects can lead to accumulation of catalytically-active Cu^{II} in the cardiac ECM, which is proposed as an important catalyst of cardiovascular damage in diabetes [21,22].

Physiological Cu occurs in one of two valence states: Cu^{I}, localized mainly inside cells, especially in mitochondria, where it is covalently bound to Cu proteins and perhaps other structures [42,43] and comprises ~95% of total body-Cu [44]; and Cu^{II}, which is largely present in the extracellular space, and comprises the remaining ~5% [44]. In biology, almost all Cu is covalently bound to proteins, usually through amino-acid residues whose side-chains contain N- or S-atoms (e.g. lysine, histidine, arginine, cysteine, or methionine), or to small molecules such as histidine/histidine dimer [44]. Free cytoplasmic Cu is said to be essentially undetectable (<0.1 pM) [45]. Covalently-bound Cu ions are either Cu^{I} (univalent) or Cu^{II} (divalent), reflecting the nature of their binding to adjacent molecules (other than water); very low levels of Cu^{I} (aq) ions are present in the extracellular space because of their intrinsic instability via disproportionation ($2Cu^{+} \rightarrow Cu^{2+} + Cu$) [46]. When Cu^{II} is present as free aqueous ions, where each Cu is centrally bound inside an enveloping hydration shell of coordinated water molecules, such ions are designated as aqua ions, or Cu^{2+}(aq): hereinafter, Cu^{2+} is defined to mean Cu^{2+} (aq).

Free tissue Cu^{II} can be demonstrated by treating with a selective chelator such as triethylenetetramine, which is employed as a second-line treatment for Wilson's disease as its dihydrochloride salt, trientine [19,22]. When Cu is extracted from a tissue such as the coronary arteries by this method, its identity as Cu^{II} can be proven: first, by its ability to bind to triethylenetetramine, a Cu^{II}-selective chelator [20,47]; and second by electron paramagnetic resonance (EPR) spectroscopy, since Cu^{II} but not Cu^{I} is EPR active [19]. Cu^{II} thus demonstrated can be designated as 'chelatable', and is considered as a surrogate measure for catalytically-active 'free' Cu^{II}. Cu^{II} bound to pathogenic sites, such as those in AGE-modified proteins [39], is thought to undergo instantaneous reduction to Cu^{I} by reducing agents in the extracellular fluid, such as ascorbate ions, triggering reactions with ROS [32,44,48,49]. When free Cu^{II} is elevated, it is bound at molecular sites other than those in physiological Cu proteins, so that it is catalytically active, retaining the ability to catalyse $HO^{\bullet}$ formation from H_2O_2 via Cu^{I}-catalysed Fenton chemistry (a simplified schema of which is shown in Eq. (1)), or from $O_2^{\bullet-}$ and H_2O_2 via the Cu^{I}-catalysed Haber–Weiss reaction (Eq. (2)) [22,32,50].

$$H_2O_2 + Cu^{I} \rightarrow Cu^{II} + HO^{\bullet} + OH^{-} \quad (1)$$

$$H_2O_2 + O_2^{\bullet-} + Cu^{I} \rightarrow Cu^{II} + O_2 + HO^{\bullet} + OH^{-} \quad (2)$$

H_2O_2 reacts in the presence of free Cu to form $O_2^{\bullet-}$, which in turn can react with NO to yield peroxynitrite ($ONOO^{-}$), thereby lowering the bioavailability of NO and impairing NO-mediated vascular smooth-muscle relaxation [51,52]. This effect of Cu could contribute to impaired NO-mediated vascular relaxation in various circumstances [51,53].

Diabetic rats have deficient total myocardial Cu (mainly Cu^{I}) [23,54], whereas their $[Cu^{II}]_{ECM}$ is increased by ~3-fold [19]. Recent studies show that this cardiac Cu imbalance is probably caused by a diabetes-induced defect in the activity of the high-affinity Cu transporter, Ctr1, which causes the imbalance between the two compartments [23]. Similar imbalance of Cu pools between intracellular and extracellular compartments occurs in other circumstances, for example in Menkes' disease, a genetic disorder of Cu homoeostasis caused by mutations in *ATP7A*, which encodes a second Cu-transporting ATPase [32]. Heart failure in diabetes may thus be explained in part by defective distribution of the two Cu valence states and the myocardial damage that ensues. Related processes may also occur in other organs such as the arteries [41] and kidneys [55].