

A Rationale for Epigenetic Repurposing of Hydralazine in Chronic Heart and Kidney Failure

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Abstract

The hydrazinophthalazines-derivates (syn. hydrazine) hydralazine and dihydralazine were approved by the FDA as anti-hypertensives in 1953 – and because they are both effective and safe they are among the oldest drugs which have kept their place in clinical practice. While hydrazine was originally discovered as potent vasodilator which lowered blood pressure and increased renal perfusion, it received renewed recognition in the 1980s due to its effectiveness for the treatment of heart failure and further reconsideration in the 2000s due to its effectiveness to reverse epigenetic DNA methylation in cancer. In light of recent advances in the understanding of cardio-renal interactions and contribution of epigenetics to chronic heart and kidney failure, we here re-visit rationales for use of hydrazine in clinical care.

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Hydrazinophthalazines-Derivates Hydralazine and Dihydralazine

The hydrazinophthalazines-derivates hydralazine ($C_8H_8N_4$, syn. 1(2H)-phthalazinone, hydrazine, 1-hydrazinophthalazine, hydralazine mono-hydrochloride, hydralazine hydrochloride) and dihydralazine ($C_8H_{10}N_6$, syn. 1,4-phthalazinedione, 2,3-dihydro-, dihydrazone, 1,4-dihydrazinonaphthalazine, dihydralazine sulfate, dihydrazinophthalazin) were discovered more than 50 years ago as potent vasodilators which lowered blood pressure and increased renal perfusion [1-3]. While the two substances are considered fully interchangeable regarding their biological activity, they have slightly different pharmacokinetics. Dihydralazine's half-life of 4.8 hours is almost twice as long as the half-life of hydralazine-hydrochloride (2.5 hours) and dihydralazine is typically taken on a twice daily regimen compared to three- or four-times a day regimen with hydralazine [4]. There is some confusion in the literature, because "hydralazine", "hydrazine" and "dihydralazine" are being used synonymously, whereas distinct substances are being historically used in different countries: While in the U.S. the hydrazinophthalazines-derivate hydralazine is being used (marketed as "Apresoline"), in Europe dihydralazine ("Nepresol") is available. For practical purposes we will adhere here to the term "hydrazine" if no further specification between hydralazine and dihydralazine is warranted.

Hydrazine for Anti-hypertensive Therapy

Hydrazine was first introduced to clinical application in 1952 for its blood pressure lowering effect (which had been consistently documented in animal studies) [1]. Of note, despite being already in clinical use, it was first tested in a controlled, double-blinded clinical study in 1964, which confirmed its safety and effectiveness [5]. Through observational studies, evidence for the benefit of combination therapy of hydrazine with an inhibitor of the beta-adrenergic system and a diuretic emerged, and by the late 1970s such "triple therapy" was standard of care for the treatment of essential hypertension [6, 7]. Hydrazine has kept its role as third-line anti-hypertensive in patients with severe hypertension and due to its benign side effects it still has its place in pregnancy hypertension [8, 9]. Due to its reliable vasodilatory effect it is

still used intravenously to control blood pressure in ICU setting [9]. In summary, despite being brought to clinical use in pre-modern clinical trial times, hydrazine has kept its role in clinical practice for over 50 years, which is unparalleled by any other anti-hypertensive drug.

Hydrazine Therapy for Chronic Heart Failure

The concept that pharmacologic reduction in systemic vascular resistance could improve cardiac performance and outcomes for patients with chronic heart failure led to clinical testing of hydralazine. The Veterans Affairs Vasodilator-Heart Failure Trials (V-HeFT I and II) evaluated the efficacy of vasodilators in chronic heart failure patients: Whereas in V-HeFT I, the combination of hydralazine and isosorbide dinitrate (BiDil) provided a beneficial effect on prognosis in heart failure, V-HeFT II demonstrated superiority of the angiotensin converting enzyme inhibitor (ACEI) enalapril over BiDil on 2-year survival [10]. However, BiDil exerted the most favourable short term impact on exercise performance and left ventricular ejection fraction. Since then seven clinical trials revealed reduced all-cause mortality upon BiDil therapy compared to placebo [11]. However, when compared to, ACEIs were associated with even lower all-cause mortality and cardiovascular mortality than hydralazine plus isosorbide dinitrate therapy [11]. Notably, the African American Heart Failure Trial (A-HeFT) heart failure study established that hydralazine administered at 200-300 mg/day plus isosorbide dinitrate in self-declared African Americans was more effective than ACEIs (making hydralazine the first race-based prescription drug in the United States), but failed again to demonstrate substantial superiority in Caucasians as compared to enalapril [12, 13]. In summary, hydrazine exerts beneficial effect in patients with heart failure [11]. Because hydralazine plus isosorbide dinitrate at fixed dose in Caucasians and Asians was inferior to treatment with optimum doses of ACE inhibitors, renin angiotensin aldosterone system (RAAS) inhibition became gold-standard treatment. As the underlying mechanisms of beneficial effect of hydrazine were not fully understood, all studies concluded that combination therapy of hydrazine with ACE inhibitors should be undertaken, but such trials have not been performed [14].

Hydrazine and Kidney Function

First studies on systemic functions of hydrazine documented increased renal blood flow upon hydrazine administration in both hypertensive and normotensive subjects [1]. Such increased renal blood flow is linked to increased cardiac output. Increased hydrazine-induced renal perfusion does not translate to immediate increase of glomerular filtration rate (GFR) in subjects with normal kidney function, and lowering of blood pressure in patients with malignant hypertension and chronic kidney disease through hydrazine administration is associated with stable GFR as well. Studies in patients with both chronic heart failure and chronic kidney disease documented even increased GFR upon hydralazine administration [15, 16]. In the 1980s several studies reported a less-severe long-term GFR-decline upon hydralazine therapy in animal studies and small patient cohorts [17-19]. Since

emergence of several conflicting reports regarding a potential benefit of hydrazine on kidney function, anti-hypertensive hydrazine therapy has been widely considered to be “neutral” on kidney function [20]. However, our group recently reported anti-fibrotic reno-protective efficacy of hydrazine in several murine models of chronic kidney disease and also through retrospective analysis of patients with complicated hypertension [21]. Notably, such reno-protective effects were observed both in mice and patients at doses which were below standard anti-hypertensive regimen [21]. Because so-called DNA promoter de-methylation was achieved at both low-dose and standard dose hydralazine therapy, and because aberrant DNA promoter methylation causally contributes to progression of kidney (and heart) failure these studies suggested that beneficial effect of hydralazine was due to its epigenetic biological activity (as further detailed below) [21-24].

Hydrazine-Induced Promoter De-Methylation

While the molecular mechanisms through which hydrazine exerts its biological effects are still not fully understood, it has been established that one of its actions is to induce promoter methylation of select genes. Promoter methylation refers to a prototypical epigenetic mechanism in which methylated cytosine residues clustered within cytosine-guanidine rich “CpG islands” in proximal promoter regions effectively silence transcription of affected genes [25]. Such promoter methylation (syn. DNA-methylation or promoter CpG island methylation) plays an important role in gene imprinting, cell differentiation, but also in adaptation to environmental factors and numerous diseases [26]. Studies on monozygous twins proved that aberrant methylation of select genes can be solely responsible for causing cancer [27, 28]. Not surprisingly, aberrant promoter CpG island methylation also contributes to progression of chronic heart and kidney failure [29, 30]. Such aberrant promoter CpG island methylation in context of chronic heart and kidney failure does not affect random genes, but few select genes are consistently hypermethylated. In this regard, the gene encoding for Ras-Gap-like protein 1 (RASAL1) is hypermethylated in both chronic heart and kidney failure [31, 32]. RASAL1 converts the active form of the oncoprotein Ras-GTP to inactive Ras-GDP, and hence RASAL1 depletion through promoter methylation causes increased Ras signaling activity [33]. Such increased Ras-GTP activity contributes to progression of fibrosis – a pathological scarring process – in heart and kidney and also liver and lung, making demethylation of aberrantly methylated (RASAL1) promoters an attractive therapeutic target [22, 29, 34]. In addition to contributing to fibroblast activation, aberrant DNA methylation involves other cell types as well, such as cardiomyocytes and macrophages in chronic heart failure and podocytes and tubular epithelial cells in chronic kidney disease. In animal studies of chronic fibrosis, therapeutic de-methylation through administration of the nucleoside analogue 5’azacytidine (Vidaza) inhibited progression of fibrosis in various organs including kidney and heart [22, 35-37]. While 5’azacytidine is in clinical use for its de-methylating activity in refractory myelodysplastic syndrome, its genotoxicity puts its potential decade-long use in chronic heart and kidney disease to question [38].

Hydrazine possesses similar de-methylating activity as 5’azacytidine, without being genotoxic. While the nucleoside

analogue 5'azacytidine exerts its de-methylating activity by causing DNA damage which is (mostly) repaired with unmethylated DNA, hydrazine induces an endogenous demethylation mechanism. Hydrazine induces so-called Tet protein-mediated hydroxymethylation of methylated DNA (the mechanism which also removes methylation marks in germline cells), which ultimately results in replacement of methylated cytosine residues with naked cytosine [21]. Tet proteins specifically are recruited to genes with CXXC motifs within their promoter regions. RASAL1 possesses such CXXC motif, making it a specific target of hydrazine-induced demethylation [29].

Broad de-methylating activity of dihydralazine has been long known and there are several ongoing clinical trials in cancer patients in which this de-methylating activity is being utilized [39, 40]. In these studies 3X 25 mg/day hydralazine were determined as optimum de-methylating dose, which is in line with the optimum dose of 1-2 times 25 mg/day dihydralazine which were identified in our studies (dihydralazine has a longer half-life as compared to hydralazine-HCl) [39]. Hydrazine exerts its de-methylating activity via induction of the endogenous de-methylating enzyme Tet3 (a member of the ten-eleven family of zinc finger proteins), making it substantially better tolerable as compared to traditional de-methylating drugs such as 5'azacytidine, which are also used in the cancer field. Importantly, such optimum de-methylating anti-fibrotic activity is achieved at doses (25-50 mg/day) which are substantially lower than standard anti-hypertensive regimen (100 to 200 mg/day) [21, 40]. This is important, as use of low dose dihydrazine reduces likelihood of hypotension, even as add-on to existing renin-angiotensin-aldosterone inhibitory therapies.

Hydrazine and RAAS Inhibitors

Since the discovery of angiotensin converting enzyme (ACE)-inhibitors captopril [41] and enalapril [42], clinical use of hydrazine was inversely correlated to the use of inhibitors of the renin angiotensin aldosterone system (RAAS): In direct comparison, long-term anti-hypertensive effects of enalapril and captopril were more efficient in lowering blood pressure in hypertensive patients [20]. After numerous controlled double-blinded multi-center clinical trials revealed decreased mortality upon long-term anti-hypertensive RAAS-inhibitor treatment they rose to prominence [43, 44], whereas trials of comparable dimensions were not done for hydrazine. In chronic kidney disease associated with proteinuria, RAAS-inhibitors were proven to pre-empt decline glomerular filtration rate (GFR) of in various experimental models and also in large controlled clinical trials [45, 46]. Fixed combination hydralazine hydrochloride and isosorbide dinitrate ("BiDil") was proven to be effective in treatment of chronic heart failure, but FDA approval was initially not granted due to failure to prove superiority to ACE-inhibitor therapy (and upon further review approved for the use in self-identified African Americans as the first "race-specific" drug) [47]. Today, ACE-inhibitors (and angiotensin receptor blockers) are first-line therapies for essential hypertension, for chronic heart failure and for patients with chronic proteinuric kidney disease. As chronic heart and chronic kidney failure are common co-morbidities, RAAS inhibitors are among the most commonly used drugs in both cardiology and nephrology [48]. Nevertheless, a fundamental difference between RAAS inhibitors and hydrazine is that ACE inhibitors (and angiotensin receptor blockers) reduce GFR (by preventing angiotensin II-induced vasoconstriction of the efferent arteriole, which is the driving force for ultrafiltration and thereby maintaining or increasing GFR in the face of a reduced plasma flow) - whereas

hydrazine vasodilatory activity is unspecific (and does not affect proteinuria) [1-49]. Even though it was demonstrated that decline of GFR does not enhance progression of chronic kidney disease [50], it does often limit use of RAAS inhibitors at optimum doses (i.e., due to ensuing hyperkalemia) or reduces GFR below tolerable threshold in advanced stages of chronic kidney disease (CKD). The consequence in clinical practice is, that target doses proven to be beneficial in chronic heart failure (i.e., 10 mg ramipril or twice daily 160 mg valsartan) are often not realized [51, 52]. The benefit of sub-optimal doses has not yet been systematically proven for chronic heart failure (especially not in comparison with hydrazine, which does not require dose reduction in CKD). ESC guidelines for management of chronic heart failure arbitrarily suggest reduction of RAAS inhibitor dosage by 50% with serum creatinine levels greater than 3 mg/dl [53]. Effectiveness of half-optimal doses of RAAS inhibitors for chronic heart or kidney failure has not been compared to optimum doses of hydrazine, yet.

A Rationale for Epigenetic Repurposing of Hydralazine in Chronic Heart and Kidney Failure

As outlined above, it is conceivable that hydrazine deserves reconsideration for treatment of chronic heart and kidney failure due to both its traditional vasodilatory function and also due to its promoter-demethylating activity. In light of proven effectiveness of RAAS inhibitors at optimal dosage in chronic heart failure and proteinuric kidney diseases and their limitations to achieve such optimal doses due to low GFR, hyperkalemia and hypotension, utility of hydrazine may lie mostly in scenarios in which it is added to existing RAAS therapeutic regimen (at optimal or especially at sub-optimal doses). Such value of add-on hydrazine therapy may lie in further improvement of cardiac function, increased renal perfusion, and additionally in the reversal of aberrant DNA promoter methylation. Specifically its epigenetic potential may add a novel therapeutic modality to existing therapy. Attraction of hydrazine-mediated epigenetic therapy lies also in the potential of therapy stratification (aberrant DNA methylation can be detected in blood) and possibility of monitoring therapeutic effectiveness (through measurement of possible de-methylation in blood samples). Due to its potential – improved cardiac function (both systolic and diastolic), increased renal perfusion and correction of aberrant DNA methylation – and its safety (which has been established in over 60 years of clinical practice and which led to preferable use of hydrazine in pregnancy hypertension), there is much to gain at moderate risk, and hydrazine may deserve a fair trial to re-assess its place in cardio-renal medicine.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contribution

MZ wrote the manuscript. EMZ wrote the manuscript.

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