Renalase — a new marker or just a bystander in cardiovascular disease: clinical and experimental data

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INTRODUCTION
Renalase, a protein with enzymatic activity, was characterised for the first time in 2005 by Xu et al. [1]. It was described as a flavin adenine dinucleotide-dependent amino oxidase, which was thought to be secreted into the blood by the kidneys, and through its participation in catecholamine metabolism should influence blood pressure [1].

Scientists using the human genomic database (Mammalian Gene Collection Project [MGC]) identified a protein that met the three criteria established in the study. Firstly,
they selected a protein, which was characterised by up to 20% amino acid sequence homology to the already known proteins. The second stage of the study was the selection of the proteins containing protein signal peptides, which are the essential elements of all proteins secreted from cells. Proteins containing transmembrane domain were excluded from further study. About 114 genes fulfilling the mentioned criteria were analysed by Northern blot technique.

Further studies concentrated on the isolation of the protein with limited tissue location and high expression in the kidney in the glomerulus and proximal tubule, and lower expression in cardiomyocytes, skeletal muscle, and liver. The isolated protein was named renalase [1]. The human renalase gene is encoded by a 311 Kbp gene with 10 exons located on chromosome 10 at q23.33 [2]. It exists in humans in seven isoforms (h renalase 1-7), which show tissue specificity. The major isoform of renalase (h-renalase-1) contains 342 amino acids. It includes a signal peptide (flavin-adenine dinucleotide [FAD]) binding domain as also monoamine oxidase domain [3, 4]. H-renalase-1 contains seven exons and encodes a protein with calculated molecular mass of 37.85 kDa. This isoform was found primarily in kidney, cardiomyocytes, liver, intestine, and skeletal muscles [1]. In kidney the highest expression of renalase was observed in renal tubule epithelial cells, glomeruli, mesangial cells, and podocytes [5]. The next papers reported h-renalase-1 mRNA to be detected in peripheral nerves, adrenal glands, central nervous system, and in adipose tissue [6, 7]. The other isoforms of renalase probably have different function. Isoforms 3 and 4 have shortened amino acids domain and thereby they do not have amino acid activity [1, 2, 6, 8]. The mouse renalase gene, characterised by Wang et al. [9] is located on chromosome 19 C1. It is related to h-renalase-1, presenting 72% amino acid identity. Xu et al. [1] reported, in the first published paper relating to renalase, that renalase had an FAD-binding domain and that FAD was a cofactor for its stability and monoamine oxidase (MOA) activity. However, the amino acid sequence of renalase differed from MOA, and renalase shared only 13.2% identity with MOA-A [1]. Xu et al. [1] extrapolated MOA activity of renalase measured using the Amplex Red Monoamide Oxidase Assay Kit, based on the detection of H$_2$O$_2$ in a horseradish peroxidase-coupled reaction [1].

RENALASE AND HYPERTENSION

Hypertension is an important risk factor for cardiovascular diseases (CVD), and its inappropriate treatment may lead to end-stage renal disease [10]. As reported, 20–50% of the adult population suffer from hypertension [11]. Because of the widespread essential hypertension and its consequences, the search for the best medical treatment is of great importance. Many published experimental data concerned the role of renalase in hypertensive animal models. Xu et al. [1] observed in Sprague-Dawley rats a full in blood pressure after intravenous administration of renalase. Besides the hypertensive effect, negative chronotropic and inotropic effects were observed [1]. Wu et al. [12] noticed that renalase knock-out mouse was hypertensive and tachycardiac and had higher catecholamine levels compared to wild type mouse. One year later Desir et al. [13] observed a fall in blood pressure after subcutaneous administration of renalase in 5/6 nephrectomised rats. The same effect was observed after administration of 5 mg/kg enalapril. Fedchenko et al. [14] analysed, with the use of real-time polymerase chain reaction, the levels of renalase mRNA expression in brain, heart, and kidneys of spontaneously hypertensive rats. They observed lower mRNA renalase in brain hemisphere and higher kidney and heart renalase mRNA levels in hypertensive rats (RR systolic > 180 mm Hg) in comparison to rats from the control group. The authors concluded that peripheral and brain renalase mRNA levels change in the opposite directions in hypertensive rats [14].

There are some studies analysing renalase gene polymorphism consisting of the substitution of aspartic acid to glutamic acid at codon 37 of renalase gene. It might influence the function of renalase. The first study in humans showing the correlation between renalase and hypertension, which was a result of single nucleotide polymorphism (SNP) of renalase gene, concerned the Han Chinese population. A total of 2586 subjects were included in the study (1317 with essential hypertension and 1269 healthy volunteers). Zhao et al. [15] described higher incidence of hypertension in subjects with G-allele rs2576178, suspecting this gene to be responsible for essential hypertension. Four years later Stec et al. [16] described association between polymorphism of the renalase gene and hypertension in haemodialysed patients — they observed hypertension more often among patients with a G-allele rs2576178 but also with the G-allele rs10887800. However, in another study in haemodialysis patients, renalase gene rs2576178 polymorphism was not associated with blood pressure [17]. Significant data data was published by Wang et al. [18], describing the association between multiple SNPs in renalase gene (rs919115, rs792205, rs12356177) and blood pressure responses to changes in in dietary salt intake. There is also a study confirming the association of the renalase gene SNP and hypertension in a group of patients with diabetes mellitus type 2 [19]. The presented studies initiated a new direction in the research of the position of renalase in hypertension. Interestingly, a meta-analysis published in 2015, focusing on seven studies exploring SNPs of the renalase gene, indicated no association of renalase gene polymorphism and hypertension [20]. Not many clinical data describing renalase in patients with essential hypertension are available. Wang et al. [21] evaluated blood pressure, serum dopamine levels, and urinary renalase secretion in Chinese adults after salt intake and potassium supplementation. They observed increases in blood pressure, serum dopamine levels, and urinary renalase secretion [21]. The question that Medvedev leaves open is: are...
renal function [30]. A Portuguese group observed also that circulating renalase levels in a group of patients with end stage renal disease, as in transplant recipients, were higher compared to healthy donors. They used, as did Malyszko et al. [29], a commercially available ELISA kit to measure renalase levels [31]. Genetic studies conducted by Stec et al. [16] delivered new information about SNPs in the renalase gene among haemodialysed patients with hypertension. They observed the relationship between hypertension and SNPs in the renalase gene (G allele frequencies of rs2576178 and rs0887800) [16]. An Egyptian study group published data pointing the association of renalase (rs2296545) CC genotype and C allele with CKD. The also observed significantly higher epinephrine levels in CC renalase genotype when compared to GG and CG genotyped in CKD patients [32].

RENALASE AND KIDNEY FUNCTION

Plasma catecholamine levels rise in patients with end-stage renal disease as a result of increased sympathetic activity as well as decreased catecholamine clearance. Increased sympathetic activity in this group of patients worsens their prognosis. Norepinephrine concentration correlates with the survival and the rate of cardiovascular events in patients with end-stage renal disease [25, 26].

Renalase concentration was observed in experimental chronic kidney disease (CKD) model. In the rat model of CKD (5/6 nephrectomised rats) renalase concentration was significantly lower 2–3 weeks after the surgery, and its deficiency was suspected to be the reason for catecholamine levels elevation in 5/6 nephrectomised rats [27]. In another experimental model of CKD on nephrectomised pups, Gosh et al. [7] observed higher norepinephrine levels in the CKD model, suggesting its decreased metabolism. Gu et al. [28] noticed lower renalase expression in the ischaemic kidney model in comparison to healthy one, suggesting that renal blood flow may affect renalase production. There are also some data concerning renalase concentration in patients with end stage renal disease. Xu et al. [1] as well as Wu et al. [12], in small group of patients with end-stage renal disease, measured decreased renalase expression compared to healthy volunteers. Malyszko et al. [29] observed, in a group of 89 kidney transplant recipients, higher renalase concentration than in healthy volunteers. Renalase level was related to kidney function, age, time after transplantation, and blood pressure. These results are in conflict with the data published by Xu et al. [1]. Renalase was measured with the use of different techniques — Xu used Western blot technique with polyclonal antibodies, Malyszko based results on commercially available ELISA assay with monoclonal antibody specific for renalase [12, 29]. Serum renalase was also higher in peritoneal dialysis patients than in healthy control group and its concentration correlated positively with the time of dialysis but there was no correlation between blood pressure control nor residual urinary secretion of renalase and increased blood dopamine level, in this situation, the effect of or only merely events resulting in body response to salt overload [22]. Schlaich et al. [23] observed lower renalase levels in 22 patients with resistant hypertension when compared to a normotensive control group. In a recent study renalase concentration in patients with primary hypertension was significantly higher than in a control group. Plasma renalase concentration correlated with norepinephrine level in hypertensive patients, which was not observed in the control group [24]. Schlaich et al. [23] used the Western blot technique to estimate the renalase concentration in the study population compared to the second study group [24], and they estimated renalase concentration with the use of commercially available enzyme-linked immunosorbent assay.

RENALASE AND CVD

Taking into consideration the relationship between renalase and CVD, there are some experimental studies that should be presented. Gu et al. [28], in the study mentioned in the previous paragraph, besides observing renalase in the CKD model, also created a model of heart failure in rats through the ligation of the left anterior descending coronary artery. This group observed increasing renal renalase expression with the highest level one week after myocardial infarction [28]. There are few clinical studies considering renalase concentration in patients with heart failure. Przybylowski et al. [33] observed higher serum renalase concentration in heart transplant recipients compared to healthy volunteers, but the serum creatinine level appeared to a predictor of renalase concentration. There was no correlation between serum renalase and hypertension nor the presence of diabetes [33]. There are also data showing higher renalase levels in heart failure patients (due to coronary artery disease [CAD] or dilated cardiomyopathy) compared to healthy volunteers. This higher renalase concentration was strongly related to impaired renal function, as observed in heart transplant recipients [34]. He et al. [35] measured renalase concentration in patients with CAD with the use of ELISA kit and interestingly observed statistically significant lower renalase concentrations in patients with multi-branch stenosis as well as in a group with higher Syntax scores compared to those with one-vessel disease and a healthy control group. Renalase level seemed to be a risk factor of CAD, based on logistic regression analysis. There were no differences in kidney function in the study population. The authors concluded that lower renalase concentration may lead to increased sympathetic activity and through this to progression of CAD [35]. Farzaneh-Far et al. [36] found an association between functional missense polymorphism in renalase gene (rs2296545) and cardiac hypertrophy in a Caucasian population. Performing resting and stress echocardiography, they observed that SNP (Glu37Asp) was related to cardiac hypertrophy, ventricular dysfunction, lowered ejection
fraction, and inducible ischaemia in patients with stable CAD. There was no significant association between rs1333049 and blood pressure [36]. Another published prospective cohort observational study based on the Cardiovascular Cohort of “Malmö Diet and Cancer” did not show any effect on blood pressure level and cardiovascular events if two common SNPs of renalase gen (rs2576178 and rs2296545) were taken into account. The hazard ratio for cardiac and cerebrovascular events was not significantly different in carriers of different genotypes. Researchers suggested, that listed polymorphisms are of negligible importance, at least in that population [37].

A recent study by Stec et al. [38] on 309 haemodialysed patients (107 with and 202 without CAD genotyped for two SNPs in the renalase gene [rs10887800 and rs2576178]) rs10887800GG genotype was associated with an increased risk of CAD whereas rs2576178 polymorphism did not influence the risk of CAD. The authors concluded that rs10887800 renalase gene polymorphism could be considered a new genetic risk factor for CAD in this population.

The question about the position of renalase in humans started in 2005 and remains open. Desir et al. [13] suggested that renalase was an amine oxidase that contains a FAD-binding region. Renalase is related to monoamine oxidase A with only 13% of amino acid identity [1]. As mentioned before, renalase is supposed to metabolise circulating in blood catecholamines, but its activity against dopamine, epinephrine, and norepinephrine remains unclear [1, 13]. Moreover, renalase activity measurements need further explanation. Different study groups [1, 31] measured renalase activity with the use of kits designed for measuring MOA activity. The measurement of renalase activity was extrapolated from the reaction based on the detection of H₂O₂ in a horseradish peroxidase-coupled reaction. Boomsma and Tipton [39] deliberated whether or not the method was properly used in those circumstances. Moreover, different scientific groups [40–42] doubt if renalase possesses monoamine oxidase activity. Recombinant human renalase functionally and structurally characterised in vitro contained non-covalently bound FAD with redox features, suggesting dehydrogenase activity. Recombinant renalase was not a catecholamine-degrading enzyme, either through oxidase or NAD(P)H-dependent monoxygenase reactions [40, 43].

Quelhas-Santos et al. [44] designed a study aimed at evaluating the catecholamine-degrading activity of renalase by different technical methods. The rate of resazurin reduction as an indirect measure of renalase oxidase activity measured by Amplex Red Monoamine Oxidase Assay Kit, the consumption of the substrate measured by high-performance liquid chromatography (HPLC) with electrochemical detection, and the formation of the respective catalyse reaction end products as a direct measure of the action of renalase on catecholamines measured by HPLC with photodiode array detection confirmed the position of renalase in the catecholamine deg-

radation process [44]. Published studies suggested also that, independently of renalase enzymatic property, there is also a possibility that renalase acts as a cytokine [3]. In an animal acute kidney injury model renalase decreased kidney damage. Renalase was suggested to interact with a membrane receptor to transmit through the activation of protein kinase [45, 46]. Yin et al. [47] noted that renalase is a cytokine that can mediate cardio-renal protective properties through renalase plasma membrane receptor — a calcium pump participating in Ca²⁺-dependent signalling. Renalase is supposed to protect against renal injury and cardiac remodelling after subtotal nephrectomy through inhibiting inflammation and oxidative stress [47, 48].

Wang et al. [49] proved that endogenous renalase plays an important role in renal protection of delayed ischaemic preconditioning (IPC). The elevated renalase expression was measured for 24 h after renal IPC, which significantly reduced renal tubular inflammation, necrosis, and oxidative stress following renal ischaemia reperfusion injury. Moreover, the authors concluded that hypoxia-inducible factor (HIF)-1α regulates renalase expression in the kidney and contributes to the renal protection of delayed IPC [49].

But if this thesis may totally explain the study of Wu et al. [12], who reported on hypertense and tachycardiac renalase knockout mice, and the data published by Eikels et al. [50], who questioned whether renalase can degrade the circulating catecholamines in the mouse model. This group mentioned that the mouse model of renalase did not contain the N-terminal FAD-binding site [50]. The next question, which needs to be answered, is if experimental studies may be extrapolated to the human population. Beside renalase there is also vascular adhesion protein 1 (VAP-1), a copper-containing semicarbazide-sensitive amine oxidase, which may catalyse the breakdown of primary amines to produce aldehyde, hydrogen peroxide, and ammonia. VAP-1 is secreted by vascular smooth muscle cells, adipocytes, and endothelial cells with functional MAO activity [51, 52]. VAP-1 involved in leukocyte extravasation to sites of inflammation may depend on renalase. VAP-1 was related to renalase in kidney allograft recipients. Patients with CKD and essential hypertension had higher VAP-1 and renalase concentration than healthy volunteers. VAP-1 originates also from similar tissues as renalase [24, 53–55]. Elcioglu et al. [56] observed the association between renalase levels and the presence of simple renal cysts in a group of normotensive patients with proper kidney function. They noticed lower renalase levels in patients with simple renal cysts than in control group, and interestingly renalase levels correlated positively with endothelial dysfunction (evaluated by endothelium-dependent vasodilatation) [56].

A uniform view of the results of research on renalase are also unsettled because of different methods used to determine renalase levels. Western blot analysis, used by Xu et al. [1], is a semi-qualitative method. An ELISA kit, using monoclonal
antibody specific to renalase, should measure the level of h-renalase-1, whether it is active or not. There is a possibility that the antibody in that assay may bind other isoforms or fragments of the renalase causing its higher concentration [57].

CONCLUSIONS

Since 2005 the position of renalase has been changing continually. First described as mono amino oxidase degrading circulating catecholamines renalase seemed to be a new important milestone in the diagnosis and treatment of hypertension. The lack of one validated commercially available test measuring the concentration and activity of renalase prevented progress in studies concerning renalase. Different study groups have published contrary results in similar animal models and groups of patients, and the position of renalase still remains unclear. An increasing number of published studies concerning renalase provide new information but do not clearly position it in the pathophysiology process of cardiovascular events or CKD. The new thesis, placing renalase in the huge group of cytokines, need further studies. The question of whether renalase as a new substance may influence hypertension, or kidney or CVD remains open.

Conflict of interest: none declared

References

31. Quelhas-Santos J, Soares-Silva I, Fernandes-Cerqueira C et al. Plasma and urine renalase levels and activity dur-


