

Special Article – Molecular Biomarkers

Kallistatin: A Novel Biomarker for Hypertension, Organ Injury and Cancer

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Abstract

Kallistatin has pleiotropic effects in vasodilation and inhibition of inflammation, angiogenesis, oxidative stress, fibrosis, and cancer progression. Kallistatin administration by gene or protein delivery is observed to offer protection against a large number of pathological conditions in animal models, such as hypertension, cardiovascular and organ damage, arthritis, sepsis, influenza virus infection, tumor growth and metastasis. However, injection of a neutralizing Kallistatin antibody into hypertensive rats aggravates cardiovascular and renal injury in association with increased inflammation, oxidative stress and tissue remodeling. Thus, animal studies show that kallistatin treatment exerts beneficial effects against hypertension, organ damage and cancer development. Moreover, serum kallistatin levels are markedly reduced in several animal models of hypertension and cardiac, cerebral and renal injury. Importantly, kallistatin levels in circulation, body fluids or tissues are significantly lower in patients with liver disease, septic syndrome, diabetic retinopathy, severe pneumonia, inflammatory bowel disease, and cancer of the colon and prostate. Furthermore, reduced plasma kallistatin levels are associated with adiposity and metabolic risk in apparently healthy African American youths. The focus of this review is to highlight circulating kallistatin as a potential new biomarker for human diseases.

Keywords: Kallistatin; Hypertension; Inflammation; Vascular injury; Organ damage; Infection; Sepsis; Cancer

Abbreviations

eNOS: endothelial Nitric Oxide Synthase; EPC: Endothelial Progenitor Cell; HMGB1: High Mobility Group Box-1; I/R: Ischemia/Reperfusion; KBP: Kallikrein-Binding Protein; LPS: Lipopolysaccharide; MI: Myocardial Infarction; NO: Nitric Oxide; ROS: Reactive Oxygen Species; SHR: Spontaneously Hypertensive Rat; SOCS3: Suppressor of Cytokine Signaling 3; SPTBN1: β II-Spectrin; STZ: Streptozotocin; TGF- β : Transforming Growth Factor- β ; TLR4: Toll-Like Receptor 4; TNF- α : Tumor Necrosis Factor- α ; VEGF: Vascular Endothelial Growth Factor

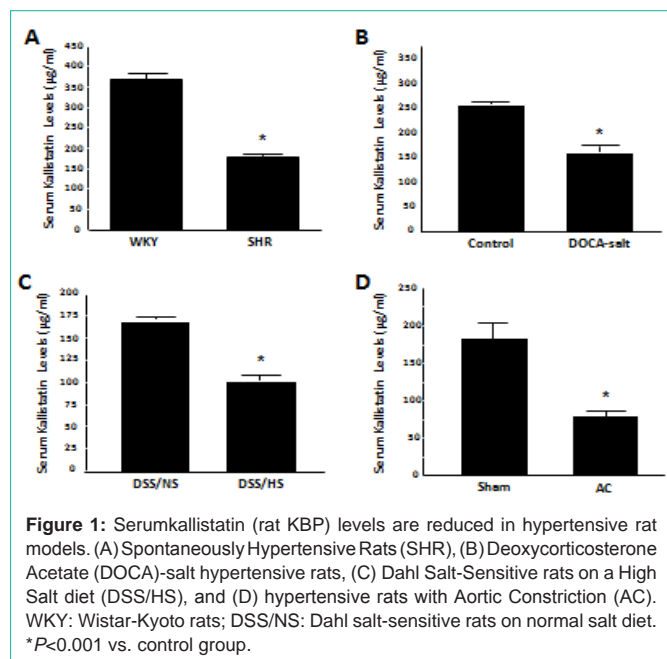
Introduction

Kallistatin is an endogenous protein that was first discovered in human plasma as a tissue Kallikrein-Binding Protein (KBP) [1]. Tissue kallikrein is a serine proteinase that releases vasodilating kinin peptides from kininogen substrate [2]. The tissue kallikrein-kinin system is involved in mediating beneficial effects in hypertension as well as cardiac, cerebral and renal injury [3]. KBP was later identified as a serine proteinase inhibitor (serpin) because of its ability to inhibit tissue kallikrein activity, and was subsequently named “kallistatin” [4-9]. Kallistatin is mainly expressed in the liver, but is also present in the heart, kidney and blood vessel [9-11]. Kallistatin protein contains two structural elements: an active site and a heparin-binding domain [12-14]. The active site of kallistatin is crucial for complex formation with tissue kallikrein, and thus tissue kallikrein inhibition [6]. Kallistatin sheparin-binding domain, however, is essential for antagonizing signaling pathways mediated by Vascular Endothelial Growth Factor (VEGF), Tumor Necrosis Factor (TNF)- α , High

Mobility Group Box-1 (HMGB1), and Transforming Growth Factor (TGF)- β [15-18]. Through its structural elements, kallistatin is able to modulate a wide spectrum of biological activities, such as blood pressure, angiogenesis, inflammation, apoptosis, fibrosis, and cancer.

Reduced kallistatin levels are associated with hypertension

Kallistatin is expressed in tissues relevant to cardiovascular function, and has consequently been shown to have vasodilating properties [19]. Spontaneously Hypertensive Rats (SHR) display markedly reduced circulating kallistatin levels compared to normotensive rats, indicating that kallistatin may be involved in maintaining normal blood pressure [5,20]. In addition to SHR, salt- and surgically-induced hypertensive rats exhibit decreased serum kallistatin levels, as measured by specific Enzyme-Linked Immunosorbent Assay (ELISA) [21] (Figure 1). Kallistatin has been demonstrated to stimulate vasodilation, as an intravenous injection of purified kallistatin induced a rapid and transient reduction of blood pressure in both normotensive and hypertensive rats [19]. Vasorelaxation in isolated aortic rings was also observed upon kallistatin administration [19]. Neither the blood pressure-lowering effect nor the vasorelaxation ability of kallistatin was abolished by icatibant (Hoe140, a kinin B2receptor antagonist), indicating that kallistatin-mediated vasodilation is unrelated to the tissue kallikrein-kinin system [19]. Furthermore, over expression of rat kallistatin in transgenic mice resulted in significantly lower blood pressure as compared to control mice [22]. Likewise, gene delivery of human kallistatin caused a protracted blood pressure reduction in SHR [23]. These studies demonstrate that kallistatin is a novel vasodilating



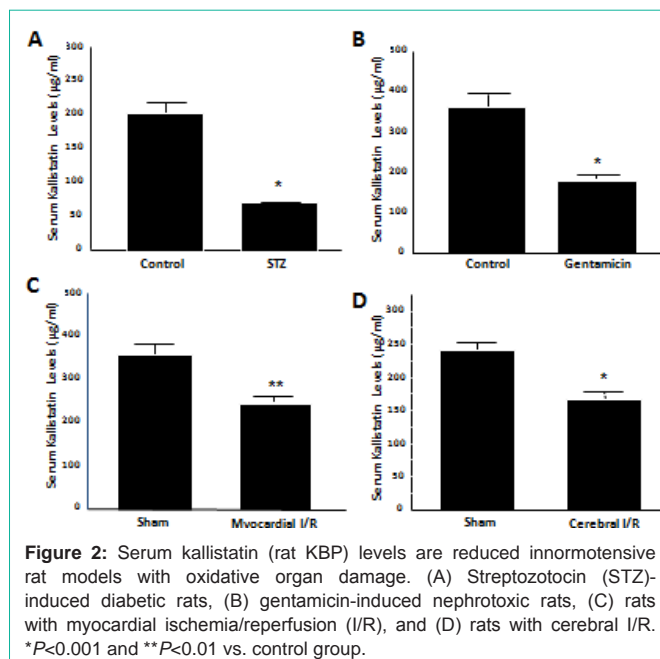
agent; and that circulating kallistatin levels are associated with the development of hypertension.

Decreased kallistatin levels during inflammation and organ injury

Kallistatin is considered to be a negative acute-phase protein. Circulating kallistatin as well as hepatic expression levels are rapidly reduced within 24 hours after Lipopolysaccharide (LPS)-induced endotoxemia in mice [24]. Serum kallistatin levels are also significantly lower in rat models with organ damage, such as Streptozotocin (STZ)-induced diabetes, gentamicin-induced renal toxicity, cardiac Ischemia/Reperfusion (I/R) and cerebral ischemic stroke, as measured by ELISA [21] (Figure 2). Similarly, circulating kallistatin levels are markedly decreased in patients with septic syndrome and liver disease [25]. However, kallistatin exhibits potent anti-inflammatory activity. For example, kallistatin gene delivery suppressed inflammatory responses and joint swelling in arthritic rats [26]. In addition, kallistatin administration into rat heart improved cardiac function and inhibited inflammatory cell infiltration after acute myocardial I/R and chronic Myocardial Infarction (MI) [27,28]. Kallistatin treatment also protected against renal injury, inflammation and oxidative stress in hypertensive rats [29,30]. Moreover, kallistatin gene transfer attenuated mortality, inflammation, and liver and skin damage in mice with streptococcal infection [31]. In cultured endothelial cells, kallistatin inhibited TNF- α -induced NF- κ B activation and inflammatory gene expression [16]. Furthermore, kallistatin inhibited vascular inflammation by stimulating endothelial Nitric Oxide Synthase (eNOS) expression and activation, and thus NO formation, in endothelial and Endothelial Progenitor Cells (EPCs) [30,32,33]. Therefore, kallistatin is an anti-inflammatory agent, and its administration inhibits inflammatory responses and organ damage.

Reduced kallistatin levels in cancer development

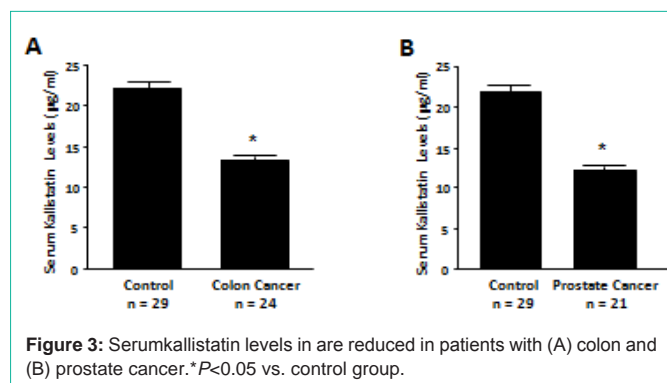
Kallistatin protective role in cancer progression has been



documented in animal models and cultured cells [34-41]. Injection of the kallistatin gene into an urine model of pre-established breast cancer xenografts resulted in significant suppression of tumor growth and blood vessel numbers [34]. Systemic kallistatin gene delivery into mice markedly decreased tumor metastasis into lungs, which was accompanied by reduced angiogenesis and inflammation; kallistatin treatment also enhanced survival of tumor-bearing mice [41]. Moreover, up-regulation of kallistatin by SPTBN1 (β II-spectrin) reduced hepatocellular carcinoma progression in mice through suppression of Wnt signaling [42]. Kallistatin was also shown to inhibit Wnt-induced motility and invasion of cultured breast cancer cells through interaction with the Wnt co-receptor low-density Lipoprotein Receptor-related Protein 6 (LRP6) [43]. Additionally, kallistatin induced apoptotic cell death in human colorectal and breast cancer cells *in vitro* [40,44]. These findings indicate that kallistatin attenuates cancer development by inhibiting angiogenesis, inflammation, cancer cell growth, migration and invasion, and by inducing cancer cell apoptosis. Moreover, serum kallistatin levels are significantly decreased in patients with colon and prostate cancer, as measured by ELISA [25] (Figure 3). Thus, circulating kallistatin levels may serve as a predictive biomarker for cancer progression.

Kallistatin levels inversely correlate to oxidative stress

The pathogenesis of hypertension and cardiovascular and renal diseases is tightly linked to increased oxidative stress and reduced NO bioavailability [45]. Time-dependent elevation of circulating oxygen species are associated with reduced kallistatin levels in animal models of hypertension and cardiovascular and renal injury [29,46]. Stimulation of NO formation by kallistatin may lead to inhibition of oxidative stress and thus multi-organ damage. In rat models of myocardial I/R, MI, and hypertension, attenuation of organ injury by kallistatin gene transfer was accompanied by decreased ROS production and increased eNOS and NO levels [27-30]. Conversely, endogenous kallistatin depletion by neutralizing antibody increased oxidative stress and aggravated cardiovascular and renal damage in



hypertensive rats [21]. Moreover, kallistatin through its anti-oxidant activity protected against CCl_4 -induced liver fibrosis in rats as well as LPS-induced acute lung injury in mice [47,48]. Furthermore, kallistatin reduced H_2O_2 -mediated oxidative stress and NADPH oxidase expression in rat corneal epithelium and human hepatic Stellate cells [47,49]. NO has been shown to inhibit of NAD (P)H oxidase activity [50]; thus, stimulation of NO formation by kallistatin may lead to the attenuation of oxidative stress. Indeed, kallistatin was demonstrated to lower superoxide production and NAD(P)H oxidase activity provoked by TNF- α , H_2O_2 , or angiotensin II via NO formation in cultured renal cells, cardiomyocytes, myofibroblasts, endothelial cells and EPCs [16,28-30,32,33]. Collectively, these studies show that kallistatin protects against the pathogenesis of hypertension and cardiovascular and renal diseases by anti-oxidant activity in conjunction with NO production. Therefore, an inverse relationship exists between kallistatin levels and oxidative stress.

Kallistatin is depleted in septic shock

A human kallistatin gene polymorphism was recently shown to correlate with a decreased risk of developing acute kidney injury during septic shock [51]. Kallistatin levels are markedly reduced in both humans and mice with sepsis syndrome [24,25]. However, kallistatin administration protects against lethality and organ injury in animal models of toxic septic shock. For example, transgenic mice expressing rat kallistatin are highly resistant to mortality induced by LPS [52]. Mice receiving kallistatin protein before polymicrobial sepsis exhibited attenuated lethality, peritoneal bacterial counts, splenic cell apoptosis, and renal injury and inflammation [17]. Furthermore, postponed kallistatin treatment after the onset of sepsis improved survival and prevented multi-organ injury in mouse models of established polymicrobial sepsis and endotoxemia [53]. Systemic inflammation was reduced by kallistatin as demonstrated by lower circulatory levels of TNF- α and HMGB1 [17,53]. Kallistatin up-regulated Suppressor of Cytokine Signaling 3 (SOCS3) expression in the kidney and lung, and decreased liver injury and hepatic TNF- α and Toll-Like Receptor 4 (TLR4) expression in septic mice [53]. These findings indicate that kallistatin is depleted during septic shock, yet exerts protective effects against multi-organ damage, inflammation and mortality in mice with sepsis syndrome.

Kallistatin levels are reduced in diabetes and obesity

Chronic inflammation of adipose tissue is related to the development of type 2 diabetes and obesity. Kallistatin levels are markedly reduced in the circulation and retinas of STZ-induced

diabetic rats [54]. In addition, kallistatin levels are decreased in the vitreous fluid of patients with diabetic retinopathy; however, circulating kallistatin levels were shown to be elevated in patients with type 1 diabetes with vascular complications [55,56]. Moreover, kallistatin appears to play a potential role in cardio metabolic disorders, and perhaps the development of obesity, as plasma kallistatin levels are negatively correlated with waist circumference, low-density lipoprotein and total cholesterol, but positively associated with high-density lipoprotein in healthy, normotensive African American youths [57]. These findings indicate that reduced kallistatin levels are linked to diabetes and adiposity. It is known that TNF- α expression in infiltrating macrophages is elevated within adipose tissue of obese subjects, and thereby exerts autocrine and/or paracrine effects [58]. TNF- α plays a key role in adipogenesis by maintaining active Wnt-signaling and inhibiting insulin-induced Akt-eNOS activation and NO formation, collectively leading to inflammation and insulin resistance [59]. Thus, kallistatin inhibits inflammation through multiple mechanisms: 1) antagonizing TNF- α -mediated cell signaling pathways [16]; 2) blocking HMGB1-mediated inflammatory gene expression [17]; and 3) stimulating eNOS expression and activation and NO formation [30,32,33]. As a unique inhibitor of inflammation, kallistatin may play an important role in protection against the development of obesity and diabetes.

Kallistatin as a new biomarker for human diseases

Kallistatin levels in the circulation, body fluids or tissues are reduced in animal models of hypertension, cardiovascular, renal and cerebral injury, septic shock, diabetes, and hepatocellular carcinoma [5,24,29,42,46,52,54,60,61]. Moreover, kallistatin levels are decreased in patients with liver disease, septic shock, inflammatory bowel disease, severe pneumonia and acute respiratory distress syndrome [25,62,63]. Serum kallistatin levels are also significantly lower in patients with colon and prostate cancer (Figure 3). Likewise, plasma kallistatin levels are reduced in healthy African American youths with adiposity and cardio metabolic risk factors [57]. In addition, vitreal kallistatin levels are decreased in diabetic patients [55]. Therefore, kallistatin has the potential to be a molecular biomarker for patients with sepsis, cardiovascular and metabolic disorders, and cancer (Table 1).

Conclusion

Kallistatin is an endogenous protein that exerts a wide spectrum of biological activities that lead to protection against multi-organ damage and mortality. Kallistatin stimulates multiple pathways to inhibit angiogenesis, inflammation, apoptosis, oxidative stress, fibrosis and cancer. Animal studies have shown that circulating

Table 1: Kallistatin levels are reduced in human diseases.

Liver disease
Septic shock
Diabetic retinopathy
Inflammatory bowel disease
Obesity
Severe pneumonia
Acute respiratory distress syndrome
Colon and prostate cancer

or tissue kallistatin levels are markedly reduced in hypertension, diabetes, cardiovascular and renal injury, whereas kallistatin administration reduces the pathogenesis of hypertension, organ dysfunction, inflammatory arthritis, sepsis, and cancer progression. Importantly, kallistatin levels are severely diminished in patients with liver disease, septic syndrome, diabetes, pneumonia, inflammatory bowel disease, and cancer, as well as African American adolescents with adiposity. Therefore, kallistatin may serve as a new biomarker for the prediction of patient outcomes.

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