**Pathophysiology of genetic deficiency in tissue kallikrein activity in mouse and man**

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**Summary**

Study of mice rendered deficient in tissue kallikrein (TK) by gene inactivation and human subjects partially deficient in TK activity as consequence of an active site mutation has allowed recognising the physiological role of TK and its peptide products kinins in arterial function and in vasodilatation, in both species. TK appears as the major kinin forming enzyme in arteries, heart and kidney. Non-kinin mediated actions of TK may occur in epithelial cells in the renal tubule. In basal condition, TK deficiency induces mild defective phenotypes in the cardiovascular system and the kidney. However, in pathological situations where TK synthesis is typically increased and kinins are produced, TK deficiency has major, deleterious consequences. This has been well documented experimentally for cardiac ischaemia, diabetes renal disease, peripheral ischaemia and aldosterone-salt induced hypertension. These conditions are all aggravated by TK deficiency. The beneficial effect of ACE/kininase II inhibitors or angiotensin II AT1 receptor antagonists in cardiac ischaemia is abolished in TK-deficient mice, suggesting a prominent role for TK and kinins in the cardioprotective action of these drugs. Based on findings made in TK-deficient mice and additional evidence obtained by pharmacological or genetic inactivation of kinin receptors, development of novel therapeutic approaches relying on kinin receptor agonism may be warranted.

**Keywords**

Kallikrein, kinins, ischaemia, diabetes mellitus, hypertension

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**Introduction**

Tissue kallikrein (Kallikrein 1, TK) was discovered through its blood pressure lowering effect, later ascribed to enzymatic activity and capacity to release the vasodilator peptides kinins from protein precursors, kininogens (1, 2). TK is a serine protease synthesised in several organs including kidney, exocrine glands, arteries and heart. It is the major kinin-forming enzyme in mammals (3) (Figure 1). Study of the role of TK in homeostasis and in disease has been hampered by lack of potent, specific and chronically administrable inhibitors, albeit use of kinin receptor antagonists has allowed raising, indirectly, hypotheses about TK’s role. Recently, study of the physiological role of TK and kinins has benefited from availability of murine and human genetic “models” of TK deficiency. The TK gene has been inactivated in the mouse, and in man a major loss of function polymorphism of the TK gene resulting in an inactivating mutation of the enzyme has been discovered (4-6).

**Tissue kallikrein is a major kinin-forming enzyme**

The gene coding for TK (the KLK1 gene) belongs to a large family of homologous genes clustered at a single locus. In man, the TK gene is on chromosome 19q13.2–13.4, and 15 kallikrein and kallikrein-related genes have been identified at this locus so far, with homology to the KLK1 gene ranging from 25% to 80% (7, 8). In the mouse, the species in which the kallikrein gene family was originally described, over 30 structurally related genes have been identified at the same locus on chromosome 7 (9, 10). While most of these genes code for proteins containing the serine protease catalytic triad, histidine, aspartic acid, and serine, and are catalytically active, only TK has an extended active site that allows it to accommodate kininogen, cleave it and thereby to release kinins (5, 11).

The KLK1 gene encoding TK spans roughly 5.2 Kb in man and comprises five exons (5, 12). This gene codes for a 262 amino acid,
catalytically inactive precursor, prokallikrein. After removal of a signal peptide and cleavage of an N terminal 17 amino acid pro-fragment coded for by exon 1, TK becomes active. The enzyme(s) activating prokallikrein in vivo remain unknown. However, the prokallikrein activating enzyme(s) seem to be ubiquitous, as active kallikreins have been found in all tissues where expression of the KLK1 gene occurs, and in fluids derived from these tissues, together with kinins. The mature human TK has 238 amino acids. The active site is coded for by exons 2, 3, and 5 (5, 12). Three-dimensional models of TK derived from crystallographic data indicate that kallikrein has active site features common to serine proteases but an extended substrate binding site, consistent with its capacity to bind the large protein substrates, kininogens (5, 13).

Kininogens are the main physiological substrates of TK. However, several non-kinin-mediated effects of TK have been described, suggesting that TK has other protein substrates and enzymatic specificities. Thus, TK has been shown to activate the kinin B2 receptor by direct proteolytic interaction, in addition to its indirect effect via kinin formation (14). TK has also been reported to be involved in proteolytic activation of the epithelial sodium channel in the kidney, like several other proteases, in inactivation of the colonic H+,K+-ATPase and may participate in regulation of potassium or calcium handling by the renal tubule (15-18).

TK is structurally and physiologically very different from pre-kallikrein, or kallikrein B, often called “plasma kallikrein” (PK), which is the second enzyme known to produce kinins in vivo. The PK gene (KLKB1) is located on chromosome 4 in man and is structurally unrelated to the KLK1 gene and the kallikrein gene family (19). PK is synthesised by the liver and secreted in plasma. PK circulates as a catalytically inactive protein activated during the so-called contact phase of coagulation or fibrinolysis (20). During activation of the PK- high-molecular-weight kininogen-complex in plasma and on endothelial cell surface, kinin is locally released. PK probably does not contribute importantly to kinin formation in healthy individuals outside this circumstance. Reduced plasma bradykinin concentration has been reported in factor (F)XII-deficient mice, but artefactual post-sampling kinin generation in non-deficient mice was not excluded in that study (21). PK may, however, play a pathogenic role through kinin release in pathological situations where the contact system is activated (22). Moreover, activation of the PK-kininogen complex with release of kinins may also occur independently of the contact system (23-25).

Kinins are potent endothelial activators

The pharmacological effects of kinins have been well described at the systemic and cellular levels, and target organs have been identified (26). Kinins are especially active on the vascular endothelium where they trigger release of several paracrine and autocrine mediators, like nitric oxide (NO), prostanoioids and leukotrienes and so-called hyperpolarising factors, which promote smooth muscle cell relaxation, and inhibit platelet adhesion and aggregation (27). Through action of these mediators, kinins are potent vasodilators and inhibitors of blood coagulation. In addition, kinins promote fibrinolysis by stimulating release of tissue plasminogen activator from the endothelium (28) (Figure 1).

Kinins are produced in low amount in the circulation. The physiological role of endogenously produced kinins has been probed indirectly by using angiotensin converting enzyme (ACE, which is also a major kinin-inactivating enzyme, kininase II) inhibitors, and directly by using kinin receptor antagonists or more recently kinin B2 receptor-deficient mice (29, 30). These pharmacological and genetic tools have provided valuable information especially in pathological situations. However, kinins act on two types of receptors, B1 and B2. The B2 receptor is constitutively synthesised in tissues, contrary to the B1 receptor, and is generally considered as being the receptor involved in the main effects of kinins in healthy animals, including the vascular effects (27, 31). The synthesis of the B1 receptor is induced in several pathological situations that includes ischaemia, diabetes and bacterial infections (32-35). There is also evidence that, in absence of functional B2 receptor, in arteries and heart the B1 receptor is induced and takes over functions of the B2 receptor (32, 36). The role of kinins in homeostasis and in disease can therefore not be fully assessed by using B2 receptor deficient mice. Recently, mice deficient in both B1 and B2 receptors have been engineered and these animals may provide, like the TK-deficient mice, further information on the physiological role of kinins (33, 37). However, mechanisms of kinin formation in tissues and regulation of this formation cannot be followed at the receptor level.

Inactivation of the tissue kallikrein gene in the mouse

Disruption of the TK gene in the mouse results in a 95% or more reduction in kinin-forming activity on kininogen in kidney, colon, pancreas, and salivary glands of homozygous TK-deficient mice.
(4). For the salivary glands, which have 100 to 1,000 times more kinin-forming activity than the other organs, 99% suppression in this activity is observed in TK-deficient mice, despite synthesis in these organs of several other protein products of the kallikrein gene family structurally related to TK (9). Kinin forming activity is 30-40% of wild-type mice in heterozygote animals. Kinin levels are reduced by roughly 80% in kidney of TK-deficient mice (4). These observations confirm that, in healthy animals, TK is the main kinin-forming enzyme in most if not all organs, and probably the only one having physiological relevance.

The TK gene is expressed in arteries of wild-type mice. When the carotid artery of these mice is perfused with kininogen, vasodilatation occurs and this vasodilatation is abolished by a kinin B2 receptor antagonist. In contrast, in TK-deficient mice, kininogen does not dilate the artery (38). These observations document the presence and functionality of TK in arteries. They also suggest that TK is the sole kinin-forming enzyme synthesised by arteries.

Genetic defect in tissue kallikrein activity in humans

Early studies suggested that the large among-subject variability in urinary TK activity level in man reflected, in a large part, a genetic polymorphism of this activity. Family studies have indeed documented familial aggregation of urinary TK activity and proposed a transmission model for this activity, with a major gene effect accounting for one half of the variance of the trait (39, 40). When the TK gene was studied later, it was found that this gene was highly polymorphic. Several single nucleotide polymorphisms (SNP) were identified in the coding sequence, promoter region and first intron of the TK gene (5). Two of these sequence variations are non-synonymous substitutions in exon 3 and change an amino acid in TK, either in position 53 (the minor allele coding for Histidine instead of Arginine) or in position 121 (Glutamate is coded instead of Glutamine). Interestingly, the R53H polymorphism was associated with a reduction of roughly 50% in urinary TK activity in populations, where only heterozygote subjects were identified. Studies with recombinant kallikrein variants established that the R53H mutation altered enzyme activity. The enzymatic defect induced by the R53H mutation is substrate dependent. It is especially marked for hydrolysis of kininogen, with a residual kinin-forming activity of the R53H variant of less than 1% of the wild-type enzyme (5). Study of a molecular model of TK based on crystallographic data suggests that Arg53 is located in a putative kininogen binding subsite in the active site. On the other hand the Q121E mutation was not associated with any significant alteration in TK activity. The glutamine in position 121 is located far away from the active site (5).

Allelic frequency of the R53H mutation is 0.03 in European and 0.07 in African-Caribbean populations. Thus, in European and North American white populations, 6-7% of subjects are heterozygote for this mutation (5). Homozygote 53H subjects should account for roughly one in 2,000 individuals, and none have been available for study so far. Because of its low frequency, the R53H mutation is unlikely to account for the entire hereditary component of urinary TK activity, which was estimated to account for more than 50% of the variance of the trait in a familial transmission study (40). Other genetic variations, at the same locus or in other, still unidentified genes, may also be involved. However, the other polymorphisms identified in the TK gene were not associated with urinary TK activity, except for those that were in linkage disequilibrium with the R53H polymorphism (5, 41).

Tissue kallikrein deficiency induces arterial dysfunction and renal tubular defects in mouse and man

TK-deficient mice have unaltered blood pressure in normal conditions but display a defect in arterial function with abnormal vaso-motor response to blood flow. Flow-dependent vasodilatation, a fundamental feature of arterial physiology ensuring proper delivery of blood to tissues, is severely impaired in these mice (4, 38). This defect is observed in both capacitance and resistance arteries (42, 43). Flow-mediated vasodilatation is an endothelium-dependent process, and these observations in TK-deficient mice are indicative of endothelial dysfunction (38, 44). Interestingly, heterozygote mice with only partial deficiency in TK also display a defect in flow-dependent vasodilatation, an observation consistent with findings made in partially TK-deficient human subjects (see below) suggesting that TK level is a critical determinant of arterial function (38) (Figure 2).

Arterial dysfunction was also observed in human subjects carrying R53H mutation and partially deficient in TK activity (45). Flow-dependent dilatation was not grossly impaired in these subjects, but shear stress was increased. Moreover, although elevated shear stress should induce vasodilatation, a paradoxical narrowing of the artery lumen was observed (45). These functional abnormalities are suggestive of endothelial dysfunction, even if, as in the mouse, the vasodilator response to exogenous acetylcholine was
found unaltered (45, 46). These observations document a new, mild form of arterial dysfunction occurring in subjects carrying, as heterozygote a defective TK mutation (Figure 3).

Studies in TK-deficient mouse or man therefore suggest that TK is involved in arterial function, in both species. TK activity had previously been described in arteries (47), but the physiological role of vascular TK remained undocumented. TK activity level is low in artery compared to other organs like kidney or exocrine glands (4, 38), but nevertheless TK level plays a critical role in arterial physiology. This role is most likely kinin-mediated, as a defect in flow-induced vasodilatation is also observed in kinin B2 receptor-deficient mice (38). TK is also involved in angiotensin II AT2 receptor-dependent vasodilatation, which is impaired in TK-deficient mice (44). This physiological coupling between AT2 and TK, which is also observed in the heart, will be discussed below.

TK is synthesised in abundance in the renal distal tubule and has a high turnover rate due to urinary and plasma secretion (48). The physiological role of renal TK is, however, not well understood. TK-deficient mice display a defect in tubular calcium absorption with exaggerated urinary calcium excretion (15). Interestingly, the partially TK-deficient R53H human subjects have also been found to have abnormal renal calcium handling. The defect is, however, mild, only evidenced after furosemide administration (49). TK seems to be involved in control of distal tubular functions, especially calcium absorption and potassium secretion. These actions may not be kinin-mediated. As discussed above, TK, like other tubular proteases, may influence electrolyte channel or pump activity though partial proteolysis (15-18).

A circulating form of TK is found in plasma. In man, plasma TK level is influenced by dietary sodium and potassium intake, an observation suggesting that plasma TK originates mainly from the kidney (50, 51). Plasma TK level has a dual genetic and dietary determinism and is influenced by the R53H mutation (51). In the rat and mouse, however, plasma TK originates mainly from the salivary glands that are well developed in these species (52, 53).

**Tissue kallikrein deficiency and blood pressure**

TK was repeatedly suggested to be involved in blood pressure regulation and in protection against hypertension. Early observations documented reduction in urinary TK activity in human essential hypertension and in hypertensive rats (54, 55). Causality could, however, not be established by these observational studies. However, causality between low urinary kallikrein activity and high blood pressure or risk of hypertension was suggested by family transmission studies (39, 40).
Studies in TK-deficient mice only partially support the hypothesis of a role for TK in blood pressure regulation. TK deficiency does not induce hypertension in mice studied in basal condition and on a regular sodium diet (4, 56). However, when aldosterone is administered chronically to these mice, together with high salt diet, blood pressure raises and remains higher than in wild-type littermates (57). Moderate cardiac septal hypertrophy is observed in hypertensive TK-deficient mice. Interestingly, aldosterone stimulates renal TK synthesis in wild-type animals. The antihypertensive effect of TK is probably due to a tubular action limiting sodium retention (57). These observations show that TK has antihypertensive effect in the setting of primary hyperaldosteronism and suggest that TK may also limit hypertension development in other physiological situations where sodium retention can trigger blood pressure elevation.

TK deficiency has no detectable effect on blood pressure in experimental models of hypertension where blood pressure elevation is renin rather than volume dependent, like renovascular hypertension induced by unilateral renal artery clipping (58), or incipient diabetic hypertension (34).

**Tissue kallikrein deficiency aggravates experimental cardiac ischaemic diseases**

Arterial dysfunction in R53H subjects may increase risk in these subjects of developing cardiovascular diseases with aging, or in pathological settings like diabetes. This hypothesis needs to be tested in population studies. Study of the TK-deficient mouse may also provide information concerning TK's role in progression of cardiovascular disease and document causality between TK and kinin deficiency and severity of these diseases. This is well illustrated by ischaemic heart disease.

The heart of TK-deficient mice raised in C57BL6 background is morphologically and functionally indistinguishable from the heart of wild-type littermates, and blood pressure is also not altered in these mice (56). This allows straightforward interpretation of the effect of the TK gene inactivating mutation on disease related phenotypes in experimental cardiac diseases.

In acute cardiac ischaemia and ischaemia-reperfusion injury induced by temporary coronary occlusion TK-deficient mice display a defect in cardioprotective mechanisms limiting necrosis. The infarct size reducing effect of ischaemic preconditioning is reduced by 40% in TK-deficient mice (32) (Figure 4). Ischaemic preconditioning is an experimental manoeuvre in which repeated short cycles of ischaemia reperfusion are performed before inducing prolonged ischaemia. This manoeuvre activates a number of cardiac protective mechanisms, which are only partly understood, resulting in infarct size reduction after sustained occlusion. Although the clinical relevance of ischaemic preconditioning cannot be established easily, this phenomenon is believed to occur in man in coronary insufficiently and play a role in disease outcome, protecting against myocardial infarction (59).

In addition, in the setting of acute cardiac ischaemia and ischaemia-reperfusion injury, the well documented infarct size limiting effect of ACE inhibitors, or of angiotensin II AT1 receptor antagonists is abolished in TK-deficient mice (32, 60) (Figure 4). The cardioprotective effect of these drugs in acute cardiac ischaemia requires TK and is mainly, if not entirely, mediated by kinins in the mouse. The hypothesis of a prominent role of kinins in the cardioprotective effects of ACE inhibitors relies on a well established physiological pathway based on ACE's prominent role in kinin metabolism and has been previously documented by using B2 receptor antagonists and B2 receptor-deficient mice (30, 61). Moreover, the effect of TK deficiency in ischaemia reperfusion can be mimicked by ACE gene duplication resulting in kinin depletion (59). The role of TK in the effect of angiotensin II AT1 receptor antagonists may appear more surprising. However, AT1 receptor blockade stimulates renin secretion and increases angiotensin II production with subsequent activation of the AT2 receptor (62). AT2 receptor activation triggers kinin release in heart and arteries and subsequent B2 receptor activation. The effect of an AT1 receptor blocker in the ischaemic heart can also be abolished by pharmacological blockade of the B2 receptor (63). Studies in TK-deficient mice indicate that TK is involved in the physiological coupling between AT2 and B2 receptor activation (44, 60). Heterodimerisation of the AT2 and B2 receptors may also play a role in this coupling (64) (Figure 1). In any case, consistent...
studies show that kinins play a major role in the cardioprotective effect of AT1 receptor blockers, at least in rodents.

In post-ischaemic heart failure induced by irreversible coronary ligature and myocardial infarction, TK deficiency is also detrimental. TK deficiency aggravates cardiac remodelling and decreases survival rate, especially in the long term, after several months of evolution (65).

Overall these studies point toward a major role of TK in cardioprotection in cardiac ischaemia and post ischaemic heart failure. This role of TK is kinin mediated.

**Tissue kallikrein deficiency aggravates diabetic renal disease**

Chronic hyperglycaemia induces kidney dysfunction that leads to diabetic renal disease. Diabetic nephropathy is currently a major cause of endstage renal failure and is also associated, already at its early stage, with increased incidence of major cardiovascular events, especially myocardial infarction. Diabetic nephropathy and associated cardiovascular events are now the main causes of mortality among diabetic patients (66). Incipient diabetic nephropathy is marked, clinically, by increased urinary albumin excretion.

Experimentally, diabetic nephropathy progression is accelerated in mice carrying a duplication of the ACE/kininase II gene and depleted in kinins and also in mice deficient in kinin B2 receptor (67, 68). Clinically, a well documented genetic variation in ACE/kininase II level is an established susceptibility and prognosis factor for diabetic nephropathy (69, 70). These observations suggest a protective effect of kinins in the kidney in the setting of hyperglycaemia.

Consistent with this hypothesis TK-deficient mice, when rendered diabetic by chemical pancreatic destruction, display enhanced urinary albumin excretion (increased 2.4 times at one month) and renal dysfunction (34) (Figure 5). The synthesis of TK and also kininogen and kinin receptors is enhanced in the kidney at early stage of diabetes (34). Activation of the kallikrein-kinin system contributes to protection against kidney damage. Interestingly, when Akita mice, which are diabetic, are rendered deficient in kinin B2 receptor they suffer from enhanced diabetic organ damage, not limited to the kidney, and display a pro-senes-

cence phenotype (71). These different mouse models and experimental approaches all point toward a major role for TK and kinins in organ protection in the setting of hyperglycaemia.

**Tissue kallikrein deficiency aggravates peripheral ischaemic disease**

Kinins and progenitor cells

Hindlimb ischaemia can be induced in the mouse by ligation of the femoral artery. Recovery occurs in a few weeks through development of neovascularisation unless animals are also rendered chronically hyperglycaemic. Hindlimb ischaemia is clearly aggravated in TK-deficient mice which display a major defect in post-ischaemic angiogenesis (72). The proangiogenic effect of TK in this setting is kinin-mediated and may involve in part progenitor cells. A subset of circulating progenitor cells has been reported to be kinin sensitive and respond to kinins by mobilisation and differentiation into proangiogenic phenotype (73, 74). Interestingly, the beneficial effect of ACE inhibitors in experimental post-ischaemic angiogenesis in the rat is for a large part kinin-mediated (75).

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**Summarisation and perspective**

Study of mutated mice and human subjects genetically partially deficient in TK activity has allowed recognising the role of TK and its peptide products kinins in arterial function in both species. TK appears as the major kinin forming enzyme in arteries, heart and kidney. In healthy condition, TK deficiency induces only mild defective phenotypes in the cardiovascular system and the kidney. However, in experimental pathological situations where TK synthesis and kinin production increase, TK deficiency has major consequences. This has well documented for cardiac and peripheral ischaemic disease, diabetic nephropathy and aldosterone-salt hypertension. These diseases are aggravated by TK deficiency.

Clinical relevance of observations made in TK-deficient mice may be tested by studying cardiac and renal diseases in partially TK-deficient human subjects. Information on the putative protective role of the kallikrein-kinin system in these diseases in man has already been gained by studying subjects with genetically high ACE level and, presumably, reduced kinin bioavailability (69, 76).

Data obtained in mouse and man suggests that kallikrein and kinins are putative therapeutic targets. ACE inhibitors or angiotensin II AT1 receptor antagonists potentiate kinin action and have proven to be efficient in a number of cardiovascular and renal diseases (77).
However, the therapeutic efficacy of ACE inhibitors and AT1 receptor blockers remains partial and may be limited by kinin bioavailability. Design and experimental evaluation of potent pharmacological intervention based on kinin receptor agonism is warranted (78).

Conflicts of interest
None declared.

References


