Effect of Growth Hormone on Plasminogen Activator and Inhibitor Activity in Rat

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Abstract Growth retardation is one of the significant changes in chronic kidney disease (CKD) and is associated with increased morbidity and mortality. Disturbances in growth hormone (GH) are held responsible for several complications in CKD. GH is a protein based peptide hormone which directly or indirectly regulates renal functions to ensure homeostasis. We investigated the effects of growth hormone on plasminogen activators (PA) in rat kidney, PA and plasminogen activator inhibitor (PAI), glucose and fibrinogen in plasma and serum lipid profile. Rats were injected daily with 250 mU GH kg⁻¹ body weight subcutaneously for one week. Growth hormone treatment increased PA activity significantly in rat kidneys as compared to controls. No changes were seen in PA, PAI and fibrinogen levels in the plasma of two groups of rats. There was significant decrease in plasma glucose, total cholesterol and LDL-cholesterol levels in serum of treated group resulting in the decrease of HDL/LDL and total cholesterol/cholesterol ratios. However, triglycerides and VLDL showed significant higher activity in the serum of treated group as compared to controls. Our data suggests that GH administration might improve renal function by increasing PA activity in kidney as well as by reducing the cholesterol content in blood. GH may

be effective in improving growth failure as it helps to maintain the normal homeostatic balance.

Keywords Growth hormone · Kidney · Cholesterol · Plasminogen · Insulin · Fibrinogen

Introduction

GH stimulates growth/maturation and proliferation of cells. One of the major concerns is its effect on renal function. CKD is also widely accepted as a risk factor for cardiovascular diseases. Despite major advances in the understanding and management of uremic growth failure, 35 to 50 % of children with chronic kidney disease still grow up to become adults of small stature. The findings of the recent clinical trials on chronic renal failure patients indicated that short stature is positively correlated with the duration of renal failure [1]. It is also widely known that adult patients with GH deficiency due to hypopituitarism are at increased risk for atherosclerotic complications and have endothelial dysfunction. Markedly decreased number of GH receptors may also play an important role in disturbances of GH/IGF-1 axis activity [2]. GH has been reported to increase effective renal plasma flow (ERPF) and glomerular filtration rate (GFR) via IGF-1 [3]. GH therapy influences endothelial function in children with renal failure. However, there are no reports on the effect of GH on PA levels in kidney and plasma.

There are several other clinically significant metabolic effects of this hormone. A significant decrease in glucose oxidation as well as stimulation of lipid oxidation has been reported on infusion of GH in healthy postabsorptive subjects [4, 5]. Kidney is an important contributor to endogenous glucose production which accounts for close to 50 % during fasting. GH may increase gluconeogenesis and glucose

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cycling in liver and kidney. Dyslipidemia and impaired insulin sensitivity constitutes metabolic syndrome. These individual components as well as combinations have serious effects on the development of CKD and cardiovascular accidents. However the exact mechanism remains a matter of speculation. A number of therapeutic interventions such as GH that target individual components of metabolic syndrome and associated conditions may have direct benefits on the kidney and heart.

Endothelial cells produce and secrete PA. Tissue plasminogen activator (tPA) is the predominant PA within mouse glomeruli and is produced mainly by glomerular endothelial cells. The major site of uPA (urokinase plasminogen activator) production within the murine kidney is epithelium lining the straight parts of both proximal and distal tubules. uPA has been proposed to have a role in maintenance of tubular patency. Deficiency of plasminogen or combined deficiency of tPA and uPA is associated with severe functional and histological exacerbation of glomerular injury and fibrin deposition. Therapies aimed at enhancing PA production as well as secretion and increasing fibrinolytic activity may provide an efficient mechanism to prevent tubular obstruction. In the light of these observations, present investigation has been carried out to determine the effect of GH on PA activity in kidney as well as glucose and lipid metabolism.

Materials and Methods

Genotropin (recombinant growth hormone) was purchased from Kabi Pharmacia Sweden. Human tPA and bovine plasminogen were from American Diagnostica Inc., Greenwich, USA. Synthetic substrate S-2251 was from chromogenex, Sweden. Glucose, triglyceride and HDL-cholesterol kits were from J. Mitra and Co. Ltd., and Ranbaxy Laboratories Ltd., New Delhi, respectively. All other reagents used were of analytical grade.

Animals—male Wistar rats (100–150 g) were obtained from Central Animal House, Panjab University, Chandigarh and maintained under standard environmental conditions. The animals were housed in plastic cages with free access to pellet diet and water. Experiment protocols were approved from Institutional Animals Ethics Committee (IAEC). The rats of same weight and age were randomly divided into two groups (five animals in each group, $N = 5$). Group 1 served as control. Rats in group 2 were given daily subcutaneous injections of GH 250 mU/kg body weight for one week. Overnight fasted rats were sacrificed by cervical dislocation. Blood and kidney tissues were collected. All the experiments were replicated thrice.

Sodium citrate was used as anti-coagulant to collect the blood plasma and PA, PAI [6], glucose and fibrinogen [7]

estimations were carried out. Total cholesterol [8], triglycerides [9], HDL cholesterol [10] were estimated in serum. LDL and VLDL cholesterol were calculated according to Friedewald's formula. Kidney tissue homogenates were prepared in 0.25 M sucrose. PA activity was determined in diluted kidney homogenates and plasma euglobulin fractions.

Assay for plasminogen activator (PA) activity:

Assays were carried out in a 96 microwell plates [11] using plasmin substrate D-Val-Leu-Lys-*p*-nitroanilide (S-2251) at a final concentration of 37 μ g/ml. Plasminogen was present at 0.2 IU/ml. Heat inactivated liver microsomes (7 μ g protein) were used as activator. Blank incubations contained buffer in place of enzyme. Absorbance was read at 1 h intervals at 405 nm using a Titertek Multiscan plate reader. PA activity was expressed as international units of standard human tPA. Protein estimation in samples was also carried out.

Results were expressed as mean $\pm$ SD, significance was evaluated using Student's *t* test (software: GraphPad) and considered to be statistically significant at $P < 0.05$.

Results and Discussion

GH has a wide spectrum of physiological and renal effects. A number of observations suggest that hypothalamic–pituitary regulation of GH is perturbed in chronic kidney disease [12, 13]. Plasma GH levels falls off to low normal values after the institution of maintenance dialysis, an effect that may be mediated in part by acetate in the dialysis bath. Growth retardation may persist after renal transplantation due to multiple factors along with an abnormal GH axis. Adult GH deficiency condition occurs less frequently than in children but is associated with impaired contractility of heart and unfavorable changes in lipid profile. High levels of fibrinogen and abnormal lipid profile were also seen in the glomerular capillary tuft in the cells of endocapillary proliferative and exudative glomerulonephritis or in Bowman capsule in the form of proliferative crescentic glomerulonephritis [14]. The deposition of fibrin in the kidney may lead to endothelial injury. Fibrin endothelial interactions have been supported by in vivo and in vitro experiments. GH treatment given to the rats subcutaneously for a week increased PA activity significantly (Fig. 1). Increased PA activity might prevent inappropriate fibrin deposition and raises glomerular blood flow. Insulin like growth factor (IGF-1), a potent vasoactive hormone in vivo has been reported to be increased during GH treatment [15]. It has been shown to dilate renal afferent microvasculature capillaries.

Fig. 1 Effect of GH on PA activity in rat kidney

GH-mediated activation of PA activity may improve the endothelial function and prevent the deterioration of renal function. Endothelial cells express insulin growth factor (IGF-1) receptors which are indirectly regulated by GH. However, as we did not measure free IGF-1 levels in our study we cannot determine whether the observed effect on endothelial function is directly related to GH or mediated through increased free IGF-1 levels. Increase in PA activity in kidneys may encourage the removal of intraluminal fibrin or extracellular matrix components obstructing urinary flow.

Growth hormone (GH) treated group was compared with control group for plasma glucose, PA, PAI (Table 1) and serum lipid profile (Table 2). There was no significant change in PA and PAI levels in rat plasma after one week of GH treatment. These findings are consistent with the previous reports of circadian variations in the tPA and PAI levels [16] leading to fluctuations in the fibrinolytic activity. The fibrinogen content decreased in the treated group. Defibrinogen has been shown to decrease the number of glomerular fibrin crescents and severity of renal insufficiency. Blood glucose levels decreased significantly ($P < 0.02$) in the GH treated group indicating insulin like effects of GH. Insulin like effect of GH has been observed in diabetic rats [17]. GH has also been found to increase gluconeogenesis and glucose cycling in splanchnic tissues/

Table 1 Comparison of plasma PA, PAI, glucose and fibrinogen parameters between control and GH (250 mU/kg b.wt) treated rats (values are mean $\pm$ SD) of 5 animals in each group

Parameter	Control	Treated
Fibrinogen (mg/dl)	258.70 $\pm$ 14.28	242.35 $\pm$ 36.42
Glucose (mg/dl)	84.77 $\pm$ 4.86	66.95 $\pm$ 10.14*
PA (IU/ml)	1.34 $\pm$ 0.54	1.81 $\pm$ 0.57
PAI (AU/ml)	10.46 $\pm$ 4.19	09.26 $\pm$ 3.38

* $P < 0.02$

Table 2 Serum lipid profile in normal and treated (GH 250 mU/kg.b.wt) rats (values are mean $\pm$ SD) of 5 animals in each group)

Parameter	Control	Treated
Triglycerides (mg/dl)	37.23 $\pm$ 05.51	49.37 $\pm$ 04.56**
Total cholesterol (mg/dl)	108.8 $\pm$ 18.65	110.4 $\pm$ 07.83
HDL-cholesterol (mg/dl)	38.52 $\pm$ 07.47	48.16 $\pm$ 4.08*
VLDL-cholesterol (mg/dl)	7.44 $\pm$ 1.10	09.87 $\pm$ 0.91**
LDL-cholesterol (mg/dl)	62.83 $\pm$ 13.77	51.60 $\pm$ 10.64*
LDL/HDL ratio	1.63	1.07
Total cholesterol/HDL Ratio	2.82	2.29

* P value < 0.05

** P value < 0.01

liver or kidney. Kidney is an important contributor to endogenous glucose production and accounts for close to 50 % during fasting.

A lack of agreement exists regarding the role of GH in lipid metabolism. Triglyceride concentrations were found to be usually normal in GHD patients. In the present study triglyceride levels were found to be elevated in treated rats significantly ($P < 0.01$). Effects of GH on triglycerides could be due to an overproduction of VLDL-triglycerides by the liver, which in turn could be due to direct action of GH or a GH mediated increase in free fatty acids or insulin [18]. Lower activity of lipolytic enzymes may also be responsible for decreased triglyceride metabolism. GHD adults have an increased LDL to HDL ratio. In our study, GH treatment raised serum HDL-cholesterol levels ($P < 0.05$) and lowered LDL-cholesterol. LDL/HDL ratio decreased on GH treatment. GH treatment may reduce plasma levels of LDL primarily by speeding up the rate at which LDL is cleared from the blood by the liver by LDL receptors. LDL receptor mRNA levels in rats were found to increase 24 fold on GH treatment [19].

Present study suggests that GH treatment improves the haemostatic balance as it increases and PA activity in kidney lowers total and LDL-cholesterol levels and overall atherogenic index ratios. The small sample of our study limits the power of our data. However, to our knowledge this is the first study to demonstrate the effect of GH on PA activity in rat kidney. Our data may have important clinical implications for both pediatric and adult patients with renal failure and GHD.

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