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The Effect of Antifibrinolysis on Renal Stone Formation

Haruo Hisazumi[1], Lennart Andersson[2] and Arne Ljungqvist[3]

[1]Department of Urology, School of Medicine, Kanazawa University, Kanazawa, Japan

[2]Department of Urology and [3]Department of Pathology, Karolinska Hospital, Stockholm, Sweden

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Summary. The influence of decreased urinary fibrinolysis on renal stone formation was studied in rats fed on a calculus producing diet. The alteration in the isolated glomerular fibrinolytic activity indicating the amount of urokinase produced has also been investigated using a histochemical fibrin slide technique. The glomerular fibrinolytic activity was significantly enhanced by both tranexamic acid and the dietary treatment. The induced antifibrinolysis resulted in a significantly higher incidence of grossly visible calculi in the renal pelvis and in calcified deposits along the cortico-medullary border of the kidney. The results support the concept that a decline in urinary fibrinolytic activity can lead to the formation of renal stones.

Key words: Urolithiasis, Tranexamic acid, Fibrinolysis.

INTRODUCTION

It is well known that high molecular weight substances form the matrix of urinary calculi (4, 5, 16). Such macromolecules constitute part of the total non-dialyzable solids (TNDS) in the urine and some of these are believed to originate from the ground substance of the kidney. Urinary fibrinolytic activity is a possible factor in controlling the amount of TNDS in the urine (6, 7), and it has been suggested that a fall in the urinary fibrinolytic activity results in an increase in the amount of high molecular weight substances in the urine (7).

As early as 1954, Boyce et al. (3) found an increased amount of TNDS in the urine of patients with recurrent urolithiasis. In a previous study, a significant increase in TNDS and a considerable decrease in fibrinolytic activity was found in the urine of patients with urolithiasis when compared with a control group (17). There is experimental evidence that the urinary macromolecules constitute the substrate of the proteolytic enzyme plasmin (6, 7, 16).

The aim of the present study was to investigate the influence of antifibrinolysis on the formation of renal stones induced in the rat by the administration of a semipurified diet. In a preliminary experiment, such a diet was shown to produce a patchy increase in mucopolysaccharides in proximal tubular cells at the cortico-medullary border in the kidney followed by calcification. The fibrinolytic activity in the kidney tissue during renal stone formation was also studied in the present experiments.

MATERIALS AND METHODS

Female Sprague-Dawley rats weighing approximately 180 g were used. They were divided into 4 groups, each consisting of 20 rats.

Group 1. These rats received tap water and a semipurified diet known to be calculus-producing (8).

Group 2. These rats received the semipurified diet and tap water containing 0.07 M tranexamic acid. This concentration is sufficient to inhibit urinary fibrinolytic activity when given to rats ad libitum (13).

Group 3. These rats received standard pellets ("Muspellets", Harald Fors, Sweden) and tap water containing 0.07 M tranexamic acid.

Group 4. These rats served as controls and received the standard diet and tap water.

Food and drinking fluid were given ad libitum to all rats. The rats were weighed once a week and the total amount of drinking water and ration consumed per cage of rats was measured.

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After 1, 3, 6 and 8 weeks, 5 rats of each group were anaesthetized with ether and the kidneys and urinary tracts explored for the presence of calculi. The urine was collected by means of bladder puncture using a one ml syringe with a hypodermic needle (0.6 x 25 m/m). The kidneys were removed. One kidney (right) in each animal was subjected to histological examination and the other kidney was used for quantitative assessment of glomerular fibrinolysis.

Urinary fibrinolytic activity was estimated by the fibrin plate method of Astrup and Müllertz (1) with some modifications (11), and expressed in Ploug units of urokinase per one ml of urine (Urokinase reference standard, Calbiochem, San Diego, Calif., USA). Bovine plasminogen-rich fibrinogen (97% clotable; IMCO, Co. Ltd, Stockholm, Sweden) was dissolved in saline barbital buffer (pH 7.75, total ionic strength 0.15 M) at a concentration of 0.2%. Bovine thrombin (Thrombin, Sigma Chemical Co., St. Louis, USA) was dissolved in 0.9% saline at a concentration of 25 NIH units/ml. Both solutions were used for making fibrin plates.

The outer cortex of the left kidneys was used for quantitative assessment of the glomerular plasminogen activator activity (12, 15, 18). Isolated glomeruli were obtained according to the procedure described by Fong and Drummond (10). After the last centrifugation the pellet which consisted of isolated glomeruli, was suspended in a 1% solution of human plasminogen-rich fibrinogen, (90% clotable; Kabi, Co, Stockholm, Sweden) in 0.15 M phosphate buffer at pH 7.6. Ten µl of human thrombin (Topostasin, Roche, Basel, Switzerland, 25 NIH units/ml) were placed on a demarcated area (3 x 2.5 cm) of a glass slide and 100 µl of the fibrinogen solution containing the glomerular suspension were spread over the thrombin to form a fibrin film approximately 0.06 mm thick. The preparations were incubated in a moist chamber at 37°C for 60, and 120 min. After incubation, the fibrin slides were fixed in 4% formaldehyde (pH 7.0) for one hour at room temperature, stained with Harris' alum-hematoxylin and mounted.

The measurement of glomerular fibrinolysis was performed according to the method described by Sraer and Boelaert (18). This included the determination of the ratio of lytic area diameter/glomerular diameter in the microscope on at least 60 well isolated glomeruli from each kidney and the mean ± SD of the ratio taken as the fibrinolytic index for the kidney (Fig. 1). For control preparations, plasminogen-free fibrinogen obtained by the affinity chromatography on L-lysine substituted Sepharose (Daiichi pure chemicals Co. Ltd, Tokyo, Japan) was employed.

For histological examinations a large number of transverse sections were taken from each kidney. Some of the sections were stained with hematoxylin-eosin and other sections with van Kossa's stain for calcium.

RESULTS

It can be seen in Table 1 that the combined adminstration of the semipurified diet and tranexamic acid resulted in an initial decrease in body weight followed by a smaller increase than in the other groups of rats.

One of the 20 rats given the semipurified diet and tranexamic acid died after 4 weeks. The

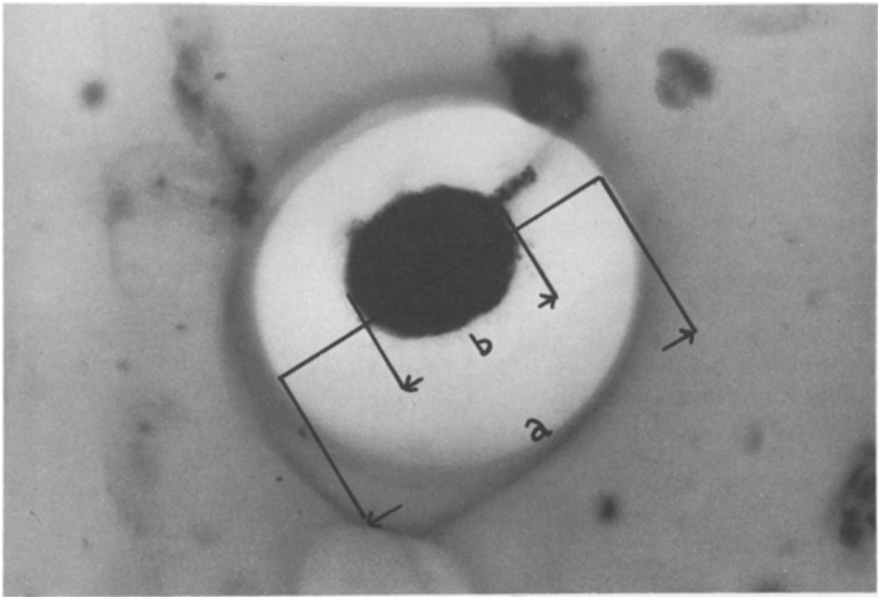

Fig. 1. Fibrin slide of kidney showing a zone of fibrinolysis (diameter of zone = a) surrounding a glomerulus (diameter = b). Fibrinolytic index = a/b. Magnification x 100

cause of death could not be assessed since the body was severely damaged by other rats in the same cage.

The range of tranexamic acid intake was 220-370 mg per 100 g body weight per day.

Urinary Fibrinolytic Activity

It proved impossible to collect the urine in every rat since in many the bladder was almost empty at the time of sacrifice. Urine samples were obtained in 8, 6 and 1 rats after 1, 6 and 8 weeks on the diet and/or tranexamic acid. Among them, rats given tranexamic acid showed no fibrinolytic activity on the fibrin plate assay whether they had been given the semipurified diet or not. The urinary fibrinolytic activity in the rats not given tranexamic acid ranged from 0.5 to 2.0 Ploug units per ml of urine. The effect of the semipurified diet alone on the urinary fibrinolytic activity could not be assessed since the number of samples were too small to permit any statistical analysis.

Glomerular Fibrinolysis

The fibrinolytic indices for the various groups of rats are shown in Table 2. The rats given the semipurified diet and/or tranexamic acid showed a significantly higher index than the control rats after 3, 6 and 8 weeks ($p<0.01$ or $p<0.001$), the highest index values being recorded in the rats given both the semipurified diet and tranexamic acid. In all experimental groups, the index was highest after 3 weeks after which it gradually declined although it did not return to the week 1 level. These results indicate that both the tranexamic acid and the semipurified diet treatments resulted in a significantly enhanced local fibrinolytic activity in the kidney. It is impossible, however, to say whether these increases in local fibrinolytic activity resulted from a primary action on fibrinolytic properties in the kidney by the treatment or was a secondary event to other biological or histological alterations induced by the treatments.

Gross Kidney Alterations

The number of rats which on gross examination showed calculi in the renal pelvis, ureters and bladders are shown in Table 3. Urolithiasis developed in 3 of 20 rats receiving the semipurified diet and tap water (Fig. 2).

Of the 19 rats that received the semipurified diet and tranexamic acid 13 developed urolithiasis (Fig. 3), and of the 20 rats given the standard diet and tranexamic acid 4 developed urolithiasis (Fig. 4).

Table 1. The effect on weight gain in growing rats given different diets with and without the simultaneous administration of tranexamic acid

Diet	Average initial body weight (g)	Average body weight gain (g)			
		1	3	6	8
		(Periods of feeding : weeks)			
Semipurified diet and tap water	182	+14			
	191		+18		
	189			+39	
	182				+50
Semipurified diet and tranexamic acid	188	-30			
	185		-22		
	184			+7	
	186				+4
Standard diet and tranexamic acid	187	-4			
	193		+19		
	181			+36	
	190				+46
Standard diet and tap water	188	+23			
	186		+33		
	186			+46	
	195				+60

Table 2. The effect of different diets and tranexamic acid on glomerular fibrinolysis

Diet	Fibrinolytic index (MV ± SD)			
	1	3	6	8
	(Periods of feeding : weeks)			
Semipurified diet and tap water	2.32 ±0.22	3.07[b] ±0.11	2.68[b] ±0.15	2.58[b] ±0.17
Semipurified diet and tranexamic acid	2.78[b] ±0.03	3.12[b] ±0.15	2.71[a] ±0.28	3.17[b] ±0.26
Standard diet and tranexamic acid	2.26 ±0.17	2.87[b] ±0.23	2.58[a] ±0.22	2.68[b] ±0.11
Standard diet and tap water	2.13 ±0.08	2.16 ±0.10	2.13 ±0.10	2.05 ±0.15

[a] $p < 0.01$

[b] $p < 0.001$

Table 3. The effect of different diets and tranexamic acid on the incidence of urinary calculi after various periods of time

No. of rats with calculi				
Periods of feeding (weeks)	Semipurified diet and tap water	Semipurified diet and tranexamic acid	Standard diet and tranexamic acid	Standard diet and tap water
1	0	0	0	0
3	0	4	0	0
6	0	4[a]	1	0
8	3	5	3	0

Each subgroup consists of 5 rats

[a] One rat died spontaneously

No urolithiasis developed in the control group.

As can be seen in Table 3, the administration of tranexamic acid remarkably enhanced the incidence of urolithiasis which was produced by the semipurified diet. Furthermore, the incidence of stone formation in the two groups receiving tranexamic acid was significantly higher than in the control group ($p < 0.05$ or $p < 0.001$). The administration of tranexamic acid and the standard diet produced urolithiasis in 4 of 20 rats and 3 of these belonged to the group of 5 rats that had been fed for 8 weeks.

Microscopic Kidney Changes

The kidneys from the rats of groups 3 (standard food and tranexamic acid) and 4 (standard food and tap water) were all completely normal with the exception of occasional small calcified deposits in the cortico-medullary zone in 2 kidneys of group 4 and some calcified material in the renal pelvis in three rats from group 3. The latter kidneys also revealed some degree of pyelonephritis and necrosis of the papillae.

In the rats of group 1 (semipurified diet and tap water), all kidneys were completely normal after 1-3 weeks. In 3 of the 5 rats killed after 6 weeks calcium deposits were seen along the cortico-medullary border and in 2 of them calcified material was found in the renal pelvis. In these 2 kidneys calcium deposits were also found underneath the epithelium of the renal pelvis.

In the rats of group 2 (semipurified diet and tranexamic acid) the 5 kidneys examined after 1 week were all normal. The 5 rats killed after 3 weeks all showed calcified deposits along the cortico-medullary border and they all contained

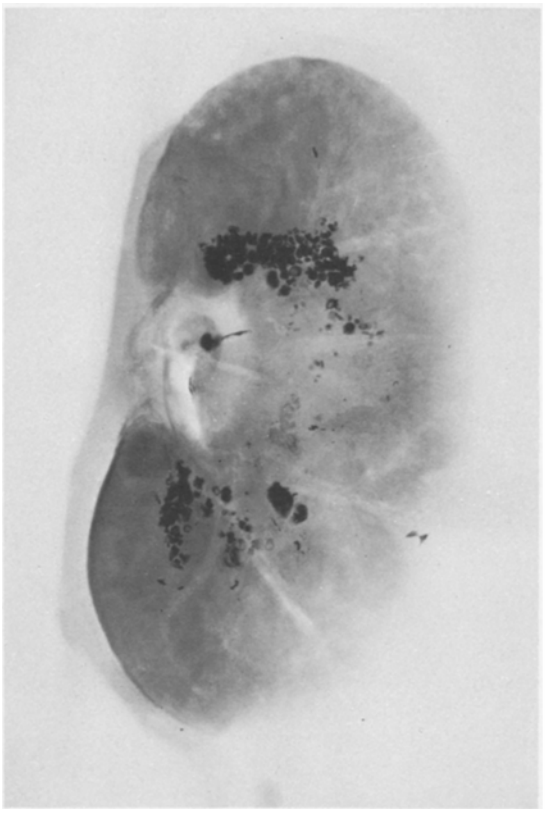

Fig. 2. Radiographic demonstration of renal calculi in a rat fed on the semipurified diet and tap water for 8 weeks

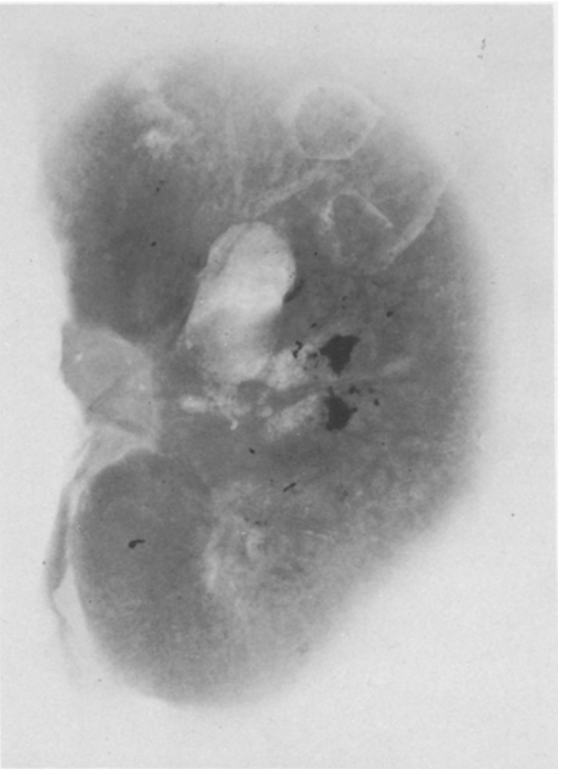

Fig. 4. Radiographic demonstration of renal calculus in a rat fed on the standard diet and tranexamic acid for 8 weeks

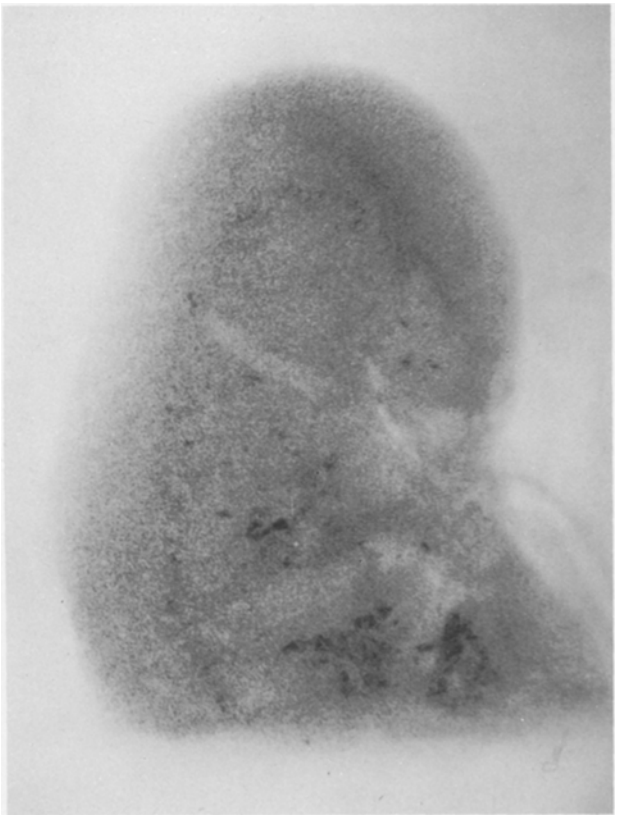

Fig. 3. Radiographic demonstration of renal calculi in a rat fed on the semipurified diet and tranexamic acid for 8 weeks. Note the presence of fine calcified deposits along the cortico-medullary border

grossly visible stones in the renal pelvis. After 6 weeks the remaining four rats showed some deposition of calcified material along the cortico-medullary border and in 3 of them calcified material was visible microscopically in the pelvis; these 3 kidneys all had grossly visible stones in the pelvis.

All 5 kidneys examined after 8 weeks had formed renal pelvic stones and calcium deposits were seen both along the cortico-medullary border and subepithelially in the renal pelvis. In 3 of these kidneys the deposition along the cortico-medullary border was, however, of a minor degree.

The calcified deposits along the cortico-medullary zone reported above either consisted of calcium deposits at the site of individual tubular epithelial cells or of complete calcification of a segment of the tubule. The latter and more severe calcification was mainly found in the kidneys from the rats of groups 1 and 2. The tubu-

lar segments involved were the loops of Henle and/or distal tubules. The deposits underneath the pelvic epithelium found in some kidneys of group 1 and in numerous kidneys of group 2 could sometimes be seen to involve the most distal portion of the collecting ducts.

DISCUSSION

The semipurified diet which produces a high incidence of oxalate and phosphate urolithiasis in lambs and rats was introduced by Chow et al. (8) and has been used in studies of renal stone prevention by dimethylsulfoxide and alanine (8, 9). In our pilot study in rats fed on such a semipurified diet the kidney tissue was studied for mucopolysaccharides and calcium by histochemical methods. The semipurified diet produced a patchy increase in tubular mucopolysaccharide at the cortico-medullary border. Calcium was subsequently deposited in these areas but not in other areas. From these findings it would appear that the alterations produced in the ground substance of the renal tubules form the basis for subsequent calcification. Such a conclusion can also be drawn from animal experiments of renal calcification produced by parathormone, oxamide and uric acid (2).

It is believed that the physiological importance of urinary fibrinolytic activity provoked by urokinase and tissue activators of plasminogen is to maintain the patency of the urinary tract including the renal tubules; this is achieved by digestion of obstructive organic material. Thus intratubular blood clots or fibrin deposits, casts of urinary macromolecules and accumulations of mucopolysaccharide would be subjected to fibrinolytic activity as a substrate of plasmin. The liquefaction and partial degradation of these materials would reduce the formation of stone matrix. In contrast, a decrease in fibrinolytic activity would result in an increase in the amount of stone matrix. Charlton (7) showed that TNDS were subjected to the action of plasmin and that a decrease in the urinary fibrinolytic activity in dogs given predonisone was accompanied by a highly significant increase in TNDS in the urine. An increase in TNDS was also found in the urine of stone formers. The data in our previous study (16) confirmed Charlton's observation in patients with urolithiasis.

In the present study, the administration of tranexamic acid or semipurified diet produced grossly visible urinary calculi in 3 of 5 rats after 8 weeks and the combination of tranexamic acid and the semipurified diet resulted in a high incidence of calculus formation after 3, 6 and 8 weeks. The microscopic study showed that the stone formation was parallelled or preceeded by the accumulation of calcium deposits in the tubules along the cortico-medullary border, except for the rats given tranexamic acid alone in which no such deposits were found.

On the basis of the present results it is reasonable to assume that a lowering of urinary fibrinolytic activity enhances the accumulation of stone matrix in the urinary tract followed by the production of urolithiasis. It is possible that one source of stone formation is the accumulation of mucopolysaccharides along the cortico-medullary border followed by calcification.

Urokinase is present in the urine of man and animals. An identity between human urokinase and human kidney tissue culture activator suggests the kidney as the source. A number of renal structures have been considered the site of urokinase production; tubular epithelia, endothelial cells of intertubular capillaries, vasa recta, collecting ducts, venous endothelia, calyceal epithelia and glomeruli. Using an immunofluorescent technique, Kawai et al. (14) suggested that glomeruli are the most likely site. In addition, using an immunological and histochemical fibrinolysis autography method and human isolated glomeruli, Misaki (15) demonstrated that the glomerulus is a potent source of urokinase production and the fibrinolytic index is a useful quantitative measure. To our knowledge, there is no substantial study concerning the effect of tranexamic acid or semipurified diet treatments on local fibrinolysis. It is therefore difficult to draw any certain conclusion on the cause of the enhanced glomerular fibrinolysis found after these treatments, as evidenced by a significantly higher level of the fibrinolytic index. It would appear conceivable that the strong inhibition of urokinase activity, exhibited by tranexamic acid, elicits an enhanced production of urokinase by the mechanism of homeostasis.

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Assistant Professor Haruo Hisazumi
Department of Urology
School of Medicine
Kanazawa University
Kanazawa 920
Japan