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Pyelonephritis: renal urokinase activity in rats on essential fatty acid diets

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Abstract This study was undertaken to assess whether additions of different oils to the diets of male rats would affect the renal urokinase (UK) activity of healthy and pyelonephritic kidneys. Four groups of fatty acid diets were studied: fat-free, coconut oil, fish oil and evening primrose oil (EPO). Pyelonephritis was obtained by unilateral extrarenal urinary obstruction and subcutaneous injection of *Escherichia coli*. The UK activity of the non-obstructed kidneys did not differ statistically between rats infected and not infected with bacteria ($P > 0.056$), except within the coconut oil group. A statistically decreased UK activity was obtained with bacteria injected animals on a coconut oil diet ($P < 0.0001$). This phenomenon, namely a decrease in UK activity, was also seen with pyelonephritic kidneys of rats on fat-free, coconut and fish oil diets ($P < 0.0065$). However, the UK activity of the obstructed kidneys with and without infection in the EPO group remained similar ($P = 0.8477$). These results suggest that the UK activity in infection-induced renal stones may be restored by EPO containing diets and may be of high relevance in the prevention and treatment of infection-induced renal stones. This revelation now needs to be more fully investigated.

Key words Coconut oil · Essential fatty acids · Fish oil · Kidneys · Evening primrose oil · Rats · Sialidase (Neuraminidase) · Urokinase

The activity of the renal serine protease, urokinase, may play an important role in urolithiasis. It has been found that the average inhibition of urokinase (UK) activity by urine from persons with and without renal stones was 77.06% ± 12.72 and 47.12% ± 11.72 respectively ($P < 0.001$) [1]. In recent studies it has been demonstrated that risk factors such as uric acid, calcium, age and sex affect the UK activity [1–3]. Therefore, it has been theorized that a decrease in UK activity could lead to an increase in renal uromucoid concentration, which then would favour renal stone formation, according to the matrix theory on urolithiasis.

Recent findings suggest that microorganisms decrease the UK activity in vitro [11] and in vivo [unpublished data]. These conditions would further favour stone formation. In support of this theory an increase in total human urinary protein is found with pyelonephritis and renal stone formers [4, 9]. However, if the decrease in UK activity could be eliminated, less favourable conditions for stone formation would prevail as a result of increased UK activity and decreased uromucoid concentrations. Since certain essential fatty acids (EFA) have been studied in connection with renal stone formation [5] and glomeruli macrophage production [10], it was decided to compare the renal UK activity of rats with and without pyelonephritis on diets supplemented with different oils. Our results showed that, in contrast to evening primrose oil (EPO) the UK activity was significantly lower in the pyelonephritic kidneys of the other three groups than in the obstructed kidneys that were not infected and which served as controls.

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Materials and methods

Reagents and chemicals

All reagents were of "Analar" grade. E. Merck (Darmstadt, FRG) and BDH (Poole, Dorset, England) supplied sodium phosphate, EDTA and Triton X-100. The substrates plasminogen (human plasma) and D-valyl-L-leucyl-L-lysine p-nitroanalide, as well as lyophilized UK powder from human kidney cells, were obtained from Sigma Chemical Co (St Louis, Mo). Nutrient broth no 2 was produced by Oxoid (Basingstoke, Hants., England). Substances of the semi-chemically defined diet were: fibrous cellulose powder CF I (Whatman BioSystems Ltd, Maidstone, Kent, England), Casein QN

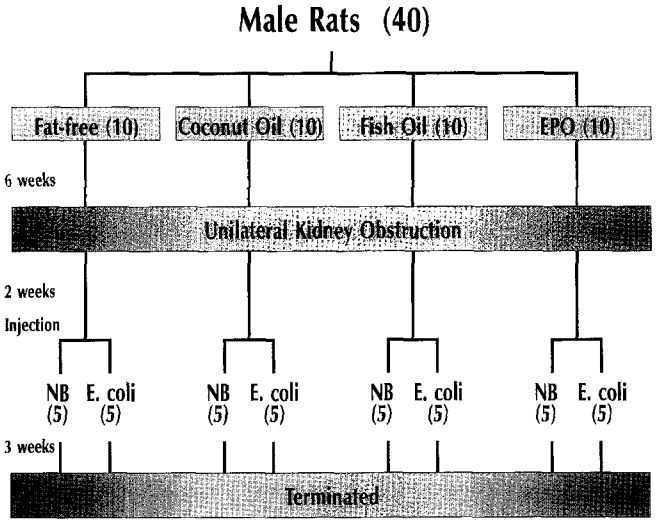

Fig. 1 Schematic presentation of experimental procedures with male rats. ( ), Number of rats in various groups

Table 1 Fatty acid composition of oils (% mol/mol)

Fatty acid	Coconut oil	Fish oil	Efamed oil
C12:0	50.18	–	–
C14:0	23.94	5.8	0.1
C16:0	11.93	13.0	5.6
C16:1	0.21	7.4	–
C18:0	3.32	2.6	1.9
C18:1	8.36	11.7	8.3
C18:2	2.05	1.3	73.0
C18:3(6)	0.1	0.2	8.0
C18:3(3)	ND	0.7	–
C20:3	ND	–	–
C20:4	ND	–	–
C20:5	ND	16.7	
C22:6	ND	11.2	–

ND, not detected

(Meffle, Wasserburg 2, FRG), Mineral mixture (Kyron Laboratories, Johannesburg, SA) and Vitamin mixture (Truka, Germiston, SA). Pure coconut oil, fish oil and EPO were supplied by Scotia Pharmaceuticals (Guildford, England).

Semi-chemically defined diet

The basic defined diet consisted of the following (grams): Casein 150, Mineral mixture 50, Vitamin mixture 10, cellulose 10, water 50, sugar 550, maize starch 100.

Microorganisms

Nutrient broth medium (ten times diluted with water) was used as the growth medium for *E coli*. The inoculated flasks were incubated overnight at 37°C, centrifuged (1700 × g, 30 min, 5°C), suspended in nutrient broth and counted in a haemocytometer (Neubauer).

Animals

Male Sprague-Dawley rats were housed in a temperature-controlled room with a constant 12-hour light, 12-hour dark cycle. The defined diet of 200 g per kg body mass was given daily and water ad libitum. The treatment of rats can be summarized as follow (Fig. 1): 6-week old animals (40) were put an special diets. Each group consisted of 10 animals. Group I was fed a fat-free diet; whilst either coconut oil, fish oil or EPO was added to the diet of the other 3 groups. Table 1 shows the fatty acid composition of the oils which were daily administered by gavage in a dose of 1 ml per kg body mass. Six weeks later unilateral ureteric obstrucion was performed through a midline transperiotoneal approach. The colon was mobilized and the upper thiad of the ureter isolated and ligated with 5/0 Dexon distal to the pelvi ureteral junction. The abdomen was anatomically repaired. The success of the obstruction was monitored with an intravenous pyelogram. After 2 weeks of recovery, 5 rats from each group of 10 were subcutaneously injected with *E coli* (200 µl containing 1.9×10^8 bacteria). The remaining 5 rats of each group then served as controls and were injected with nutrient broth (200 µl) only. Rats were terminated using carbon dioxide after a further 3 weeks.

Preparation of kidney cytosol

The kidneys were removed and placed in 5 ml ice cold 0.1 M sodium phosphate buffer, pH 7.5, containing 10 mmol/l EDTA and 0.1 g/l Triton X-100. The tissue was homogenized for 15 s at 9500 rpm with an Ultra Torrax T25 (Janke & Kunkel, IKA-Labortechnik, Staufeni). The homogenate was centrifuged at 1700 × g, for 30 min at 4°C, and the supernatant stored in ice. Protein concentration was determined with the Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, Calif.). Bovine albumin was used as standard. Absorbance was measured in a Hitachi 150-20 spectrophotometer (Tokyo, Japan) at 595 nm.

UK activity determination

UK activity was assayed according to a modified method of Wiman et al. [12]. Briefly, 5 µl cytosol was added to 400 µl activator reagent and 423 µl of 0.1 mol/l sodium phosphate buffer (pH 7.5) containing 10 mmol/l EDTA and 0.1 g/l Triton X-100. The activator reagent was composed of 1.0 µmol/l plasminogen and 0.6 mmol/l D-valyl-L-leucyl-L-lysine p-nitroanalide dissolved in 0.1 mol/l sodium phosphate buffer. All the additions were performed in ice. The total volume was 828 µl. The tubes were then placed in a waterbath for 90 min at 37°C. After the desired incubation period the reaction was stopped by placing the tubes in ice and the addition of 0.1 ml 50% acetic acid to each tube. The difference in absorbance between the blank and control (containing cytosol) was measured with a Hitachi spectrophotometer at 405 nm. The molar extinction coefficient for p-nitrophenol was taken as 9620 $mol/l^{-1}cm^{-1}$ [1].

Statistical analysis

Groups were compared using the appropriate *t*-test after first testing for equal variance between the groups using Levene's test for equal variance. The variation in the data was small. Because of the small sample sizes, the result of the *t*-test was further confirmed using the Mann-Whitney test.

Results

The renal UK activity obtained within each diet group is illustrated in Fig. 2. The UK activity of the non-obstructed kidneys did not differ significantly between the animals either infected or not infected with bacteria ($P > 0.056$) except within the coconut oil group. A statistically decreased UK activity was obtained for bacteria-infected rats

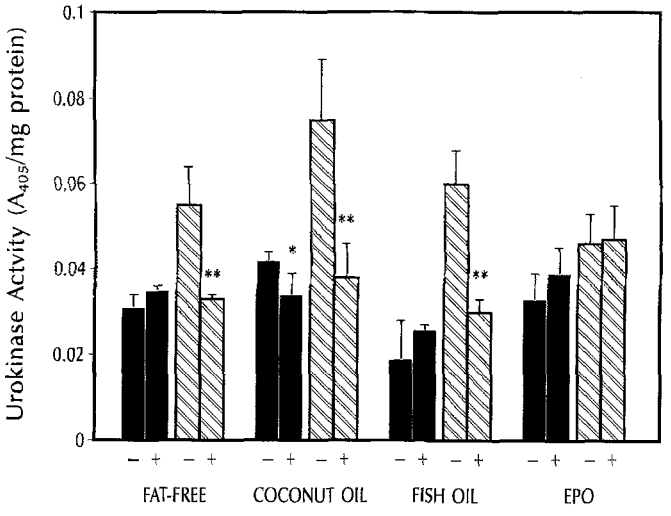

Fig. 2 Renal urokinase activity of rats kept on different fatty acid diets. (−), Injected with nutrient broth without bacteria; (+), injected with nutrient broth with *E coli*. Data represent the mean ± SD of 5 kidneys. ■ Non-obstructed; ▨ obstructed

on a coconut oil diet ($P < 0.0001$). A decrease in UK activity was also obtained with obstructed kidney rats that were infected with bacteria in contrast to obstructed kidney rats not infected with bacteria. This phenomenon was present in the fat-free ($P = 0.0065$), coconut ($P = 0.001$) and fish oil ($P < 0.0001$) diet groups, but was not apparent in the EPO diet group that had been similarly treated. UK activities obtained using EPO were equivalent ($P = 0.8477$). That is, with the exception of the coconut oil group ($P = 0.03178$), the UK activities of the obstructed non-infected rats were statistically similar for the fat-free, fish oil and EPO groups ($P > 0.1400$). For the obstructed, infected rats, the UK activity of both the fat-free and coconut oil groups was the same ($P = 0.2189$), whilst the UK activity significantly decreased ($P = 0.0344$) and increased ($P = 0.0057$) for the fish oil and EPO groups respectively.

Discussion

Urokinase activity may play an important role in renal stone formation. Decrease or increase in this urinary enzyme activity may respectively promote or inhibit renal stone formation. It has been demonstrated in vitro that microorganisms associated with infection-induced renal stone decrease UK activity. This phenomenon was then investigated in rats on a standard laboratory diet. The renal UK activities did not differ significantly between kidneys of the same rat. In contrast, when drainage from one kidney of a rat was externally obstructed, the UK activity increased significantly which might have been caused by an increased fibrinolytic effect. Therefore, the non-infected kidneys of the non-obstructed and obstructed groups were used as controls. Infection with *E coli* showed that the UK activity of the pyelonephritic kidney was significantly lower than the activity of non-infected obstructed kidneys. The question arises as to whether this phenomenon would prevail if the diet were supplemented with various EFA.

The UK activity of non-obstructed kidneys remained the same irrespective of whether they were infected or not infected with bacteria in the fat-free, fish oil and EPO groups. However, a decrease in UK activity was observed with infected rats on coconut oil. In the case of the obstructed kidneys the diets affected the UK activity differently. Although similar UK activities were obtained with the non-infected obstructed kidneys of all diet groups (except for coconut oil) the UK activities of the infected obstructed kidneys were all significantly decreased in comparison to the non-infected obstructed kidneys which served as controls. The only exception observed was with the EPO group. This phenomenon was confirmed by statistical analysis of the UK activities of the infected obstructed kidneys of rats on various diets. The only diet group which showed a significant increase in UK activity was obtained with the EPO diet.

This phenomenon can possibly be explained as follows: it is known that bacteria inhibit UK activity [11] and that linoleic acid enhances the number of macrophages in the affected area [10], therefore kidneys with pyelonephritis from rats on the fat-free, coconut and fish oil diets – which were deficient in linoleic acid – should have fewer macrophages to phagocytize bacteria. This could have led to the observed significant decrease in UK activity. In the case of the EPO group whose diet was rich in linoleic acid, no decrease in activity was observed and UK activity remained within normal range for the obstructed kidneys. Another possible explanation would be that macrophages could be stimulated by EPO to produce more UK under certain conditions, since it is known that macrophages synthesize UK [6–8]. Whatever the reason, these results indicate that the UK activity of kidneys with pyelonephritis is increased by administering EPO and that EPO could thus be useful in both prevention of the stones and in the treatment of patients with infection-induced renal stones. These aspects remain to be investigated.

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