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Calcium Oxalate Crystal Growth in Normal Urine: Role of Contraceptive Hormones

R. Tawashi, M. Cousineau and G. Denis

Faculté de Pharmacie et Département de Physiologie, Université de Montréal, and Service de Néphrologie, Hôpital Sacré-Coeur, Québec Canada

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Summary. Oral contraceptives inhibited the growth of calcium oxalate crystals in female urine, and the growth rate inhibition depended on the dose of ethinyl-oestradiol in the oral contraceptive agent. The results suggest that the crystallization kinetics of calcium oxalate in urine could be under the control of oestrogenic hormones by an unidentified mechanism.

Key words: Oral contraceptives, Calcium oxalate crystals, Growth inhibition.

One of the most striking characteristics of stone disease is the pronounced difference between the sexes. Epidemiological studies indicated that the male : female ratio is 2 : 1 and the recurrence rate ist higher in males (80%) than in females (60%) [3, 9]. It is also documented that both men and women lose bone after reaching the age of 40. The average rate of loss is three times greater in women than in men [5]. It is thought that oestrogenic hormones might be involved in the formation of insoluble calcium salts. The possible association between oral contraceptives and urinary stone formation in women was reported by Von Klinger and coworkers who found that the frequency of urolithiasis was markedly lower in women on oral contraceptives [2]. Furuhjelm and Zador suggested that there might be a biochemical basis for the association between the calcification process and oestrogenic activity [1], but the exact role of oestrogens is not clearly understood.

The purpose of this work was to study the crystallization kinetics of calcium oxalate in the urine of males, females and females on oral contraceptives. The determination of the crystal growth-rate under defined experimental conditions could offer possible clues as to the role of oestrogenic hormones in calcification.

Material and Methods

The urine of normal healthy individuals, males, females and females taking oral contraceptives (age 20–35), with no history of stone formation, was used. 49 subjects on an uncontrolled diet participated in this study. 12 female subjects received oral contraceptive agents as combination tablets containing 0.03 mg or 0.05 mg ethinyl oestradiol with 1 mg progestin.

The urine sample was taken before lunch. Total calcium concentration (Corning calcium analyser Model 940) and pH were determined immediately for each urine sample. All samples were within the normal physiological limits and in accordance with the recently reported circadian rhythm for normal subjects at this period of the day [8].

Each sample was filtered through a Millipore filter 0.22 μm. The filtered urine sample (50 ml) was used for each growth experiment. Calcium chloride 0.25 ml (1 M) in bidistilled water was added to 50 ml of the filtered urine (increasing calcium concentration by 5 mmol/l). The pH was adjusted to 5.7 and 2.5 ml of sodium oxalate (0.005 M) was added to the system which was maintained at 37 °C, (increasing the oxalate concentration by 0.25 mmol/l). The system was kept at 37 °C for 3 h without any agitation.

The crystals obtained were separated and characterized as calcium oxalate dihydrate (Weddellite) by scanning electron microscopy and x-ray diffraction as described previously [4–7]. The size distribution of the calcium oxalate crystals obtained in each urine specimen was determined using a particle counting technique (Coulter counter Model TA).

In kinetic studies growth was followed up to the moment of agglomeration. We monitored the size number data in a fixed volume (0.5 ml) of the system as a function of time. Calcium chloride 0.75 ml (1 M) in bidistilled water was added to 150 ml of the filtered urine. The pH was adjusted to 5.7 and 7.5 ml of sodium oxalate (0.005 M) was added. The system was kept at room temperature and agitation was maintained throughout the experiment at 200 rpm.

The change in particle radius in a given period of time was used as a basis for the calculation of the growth rate in cm/s. The details have been described by the authors in a previous communication [6, 7].

Results and Discussion

As shown in Table 1 there is no significant difference between the median volume diameter D_{50} of calcium oxalate

Table 1. Size characteristics of calcium oxalate dihydrate crystals grown in the urine of males, females and females on oral contraceptives

Subjects	Number of subjects	pH ± S.E.	Mean diameter D_{50} (μm) ± S.E.	Uniformity factor ± S.E.
Males	22	6.03 ± 0.12	16.54 ± 0.91	2.66 ± 0.09
Females	10	5.91 ± 0.22	17.08 ± 1.08	2.40 ± 0.25
Females on oral contraceptives	13	5.73 ± 0.16	12.70[a] ± 0.52	2.90 ± 0.18

[a] Significant at $P < 0.05$

Table 2. Growth rates of calcium oxalate crystals in the urine of males, females and females on oral contraceptives

Subject	Number of subjects	Growth rate of calcium oxalate crystals (cm/s ± S.E.)	Significance
Male	18	$14.1 \times 10^{-8} \pm 1.4 \times 10^{-8}$	N.S.
Female	19	$9.5 \times 10^{-8} \pm 1.5 \times 10^{-8}$	
Female on oral contraceptives	12	$1.8 \times 10^{-8} \pm 0.8 \times 10^{-8}$	$P < 0.05$

Table 3. Effect of the dose of estrogen in the oral contraceptive agent on the growth rate of calcium oxalate crystals in urine

Dose of oestrogen in the oral contraceptive agent	Number of subjects	Growth rate of calcium oxalate crystals (cm/s ± S.E.)	Significance
Control	19	$9.5 \times 10^{-8} \pm 1.5 \times 10^{-8}$	
0.03 mg ethinyl estradiol	9	$2.2 \times 10^{-8} \pm 1.0 \times 10^{-8}$	$P < 0.05$
0.05 mg ethinyl estradiol	3	$0.6 \times 10^{-8} \pm 0.1 \times 10^{-8}$	$P < 0.05$
0.05 mg mestranol	5	$1.4 \times 10^{-8} \pm 0.6 \times 10^{-8}$	$P < 0.05$

crystals grown in the urine of males and in the urine of females. There is, however, a significant difference ($P < 0.05$) between the median diameter of the crystals grown in the urine of female on oral contraceptives and controls (12.70 ± 0.52 μm vs 17.08 ± 1.08 μm). The determination of the uniformity factor – which provides a measure of the uniformity of distribution – indicates that the size distribution of the crystals has not been affected by oestrogenic hormones [6].

Table 2 summarizes the results of the growth kinetic study. The calcium oxalate crystal growth rate in the urine of females is not significantly different from that obtained in the urine of the male. The relatively higher co-efficient of variation for the female group (68.8%) vs. the male group (42%) could be attributed to the variations in the concentration of urinary excreted oestrogens during the different phases of the menstruation cycle. However, the intake of oral contraceptives produced a remarkable retardation of the growth rate of calcium oxalate crystals in the urine of female subjects. The growth rate decreased $9.5 \times 10^{-8} \pm 1.5 \times 10^{-8}$ cm/s to $2.2 \times 10^{-8} \pm 1.0 \times 10^{-8}$ cm/s ($P < 0.05$).

Table 3 shows the effect of the dose of ethinyl oestradiol on the growth rate of calcium oxalate crystals in female urine. It is interesting to notice that growth inhibition increased with an increase of the dose of the oestrogenic hormone in the oral contraceptive tablet. The 0.05 mg ethinyl oestradiol tablet exerted a higher inhibitory effect than the 0.03 mg dose. It was also found that in 5 subjects receiving 0.05 mg of mestranol the growth rate was inhibited from $9.5 \times 10^{-8} \pm 1.5 \times 10^{-8}$ cm/s to $1.4 \times 10^{-8} \pm 0.6 \times 10^{-8}$ cm/s ($P < 0.05$).

The authors are aware of the fact that the ionic concentration of calcium and oxalate and the presence of other urinary constituents – e.g. magnesium, citrate, uromucoids etc. – could modify the growth kinetics of calcium oxalate crystals. These variables were reduced but by no means eliminated by performing the growth experiments under controled conditions of temperature, pH and agitation intensity. In addition, a fixed concentration of calcium and oxalate was added to the filtered growth media, which is a selected urine sample at a specific circadian time (before lunch). Although a significant decrease in the growth rate of calcium oxalate crystals in the urine of females on oral contraceptives has been demonstrated to be due to the presence of oestrogens, directly or indirectly, the precise mechanism has not been identified.

On the basis of these preliminary results, one can speculate that oestrogen hormones and/or their metabolites may play a key role in the formation of calcium oxalate crystals in the urinary tract.

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R. Tawashi, Ph.D.
Faculty of Pharmacy
University of Montreal
C.P. 6128, Succ. "A"
Montréal, Québec
H3C 3J7
Canada