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ORIGINAL PAPER

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An approximate estimate of the ion-activity product of calcium oxalate in rat urine

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Abstract The objective of this report was to derive a simplified approximate estimate of the ion-activity product of calcium oxalate (AP_{CaOx}) in rat urine. The relative effect of each urine variable was assessed by means of iterative computerised approximation with the EQUIL2 program. A basic urine composition was chosen from literature and experimental data. The most pronounced influence on AP_{CaOx} was recorded for urinary calcium, oxalate, citrate, magnesium and volume. Based on these calculations, an AP(CaOx) $index_{RAT}$ was formulated: $\frac{A \cdot Calcium^{0.93} \cdot Oxalate^{0.96}}{(Citrate+F)^{0.60} \cdot Magnesium^{0.55} \cdot Volume^{0.99}}$. For a 24-h urine sample, factor A takes the value 4067 and factor F should be set to 0.015. Conclusion. A simplified approximate estimate of AP_{CaOx} was derived for rat urine. There was a reasonably good correspondence between AP(CaOx) $index_{RAT}$ and AP_{CaOx}, as derived from EQUIL2 ($r = 0.890$), provided the other urine variables do not deviate very much from that in the basic composition.

Keywords Calcium oxalate · Rat · Urine · Ion-activity product

Introduction

A better understanding of the mechanisms of calcium oxalate stone formation and elucidation of factors responsible for the crystallisation process require animal studies. In all such experimental settings, it is essential to get information on the levels of supersaturation in the urine samples.

The ion-activity product of calcium oxalate can be calculated by computerised iterative approximation [1, 2, 3]. In order to succeed with these calculations, it is necessary to analyse urine composition for a large number of variables. An extensive analytical programme of this kind is both expensive and complicated and might, moreover, be technically demanding when the urine volumes are small.

Simplified estimates of the ion-activity products of calcium oxalate (AP(CaOx) index) and calcium phosphate (AP(CaP) index) have previously been derived for human urine [3, 4, 5]. A prerequisite for the use of these indices is, however, that the composition of a particular urine sample corresponds to that of human urine, and that the urine variables that are not included in the index formula do not deviate too much from that seen normally. Urine from rats has a composition that in several respects differs from that in human urine. For that reason it is not possible to make a simple adaptation of the AP(CaOx) index to make it useful for rat urine.

We describe below a simplified estimate of the ion-activity product of calcium oxalate in rat urine, based on the urinary excretion of calcium, oxalate, citrate, magnesium and the urine volume.

Methods

Ion-activity products were derived by means of computerised iterative approximation with the EQUIL2 program [1].

An average basic urine composition was chosen from literature data [6] as well as from our analytical findings in rat urine. When the latter measurements differed from literature data, an approximate mean was used for some of the variables. According to our own measurements, the following average 24-h excretion levels were recorded: calcium, 0.0095 mmol; oxalate, 0.0058 mmol; citrate, 0.0744 mmol; magnesium, 0.0840 mmol; sodium, 0.62 mmol; potassium, 0.88 mmol. The volume was 10.9 ml and the pH 6.7. According to the results by Bushinsky et al. (6) the 24-h values were as follows: calium, 0.0095 mmol; oxalate, 0.0060 mmol; citrate, 0.1071 mmol; magnesium, 0.2870 mmol; sodium, 1.68 mmol;

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Table 1 Basic composition of rat urine used in the calculations

Urine variable	mmol/24 h
Calcium	0.0095
Oxalate	0.0058
Sodium	1.15
Potassium	1.00
Sulfate	0.0032
Magnesium	0.185
Citrate	0.09
Ammonium	0.400
Phosphate	0.65
Carbonate	0
pH	6.10
Volume	13.2 ml

potassium, 1.08 mmol; phosphate, 0.6488 mmol; ammonium, 0.400 mmol; sulphate, 0.0032 mmol; the volume 13.2 ml and pH 6.1. The basic urine composition that thus was used in the calculations is given in Table 1.

For each urine variable an exponent y was derived according to the general formula:

$AP_{calciumoxalate} = k.\text{variable}^{y}$

in which the factor k represents the impact of all other urine constituents than that currently of interest.

Urinary calcium, oxalate, citrate and magnesium were assessed in 24-h urine samples collected from rats. The average weight of the animals was 260 ± 20 g. The rats were kept in metabolic cages and each sample was acidified with hydrochloric acid before analysis. Calcium was determined by atomic absorption spectrophotometry (Perkin Elmer Atomic Spectrophotometer 290-B, Norwalk, CT, USA), magnesium following combination with calmagite (Labtest Diagnostics Kit, Minas Gerais, Brazil), oxalate by an enzymatic method [7] using the Sigma Oxalate Diagnostic Kit (Sigma Chemical Co., St Louis, MO, USA) and citrate by an enzymatic assay using citrate lyase [8].

Results

Variations of the individual urine variables disclosed relationships with an ion-activity product of calcium oxalate (AP_{CaOx}), as shown in Fig. 1. As expected, oxalate, calcium, volume, citrate and magnesium had the most pronounced influence on AP_{CaOx}. This results allowed us to formulate an ion-activity product of calcium oxalate in rat urine ($AP(CaOx)index_{RAT}$) with the following general form:

$$AP(CaOx)index_{RAT} = \frac{A \cdot Calcium^{a} \cdot Oxalate^{b}}{(Citrate + F)^{c} \cdot Magnesium^{d} \cdot Volume^{e}}$$

The values of the exponent y that gave the best fit are shown in the relationships below:

$10^{8}.AP_{CaOx} = k_1.\text{calcium}^{0.93}$

$10^{8}.AP_{CaOx} = k_2.\text{calcium}^{0.96}$

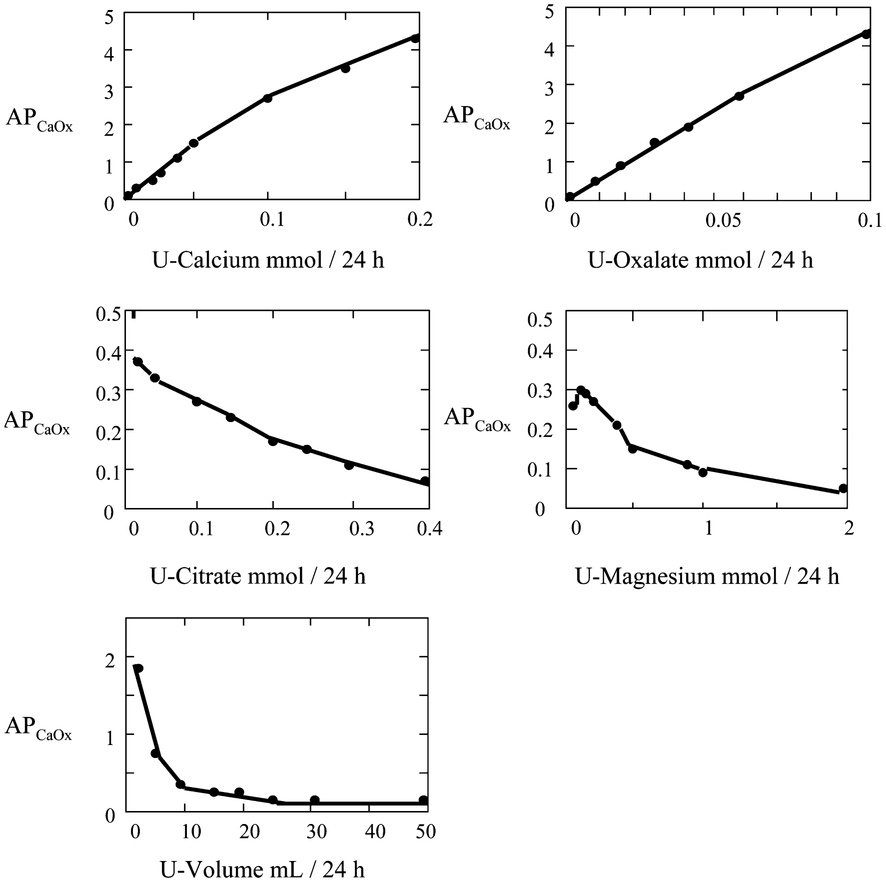

Fig. 1 These curves show the effect on AP_{CaOx} of changes in the urinary excretion of calcium, oxalate, magnesium, citrate and volume. AP_{CaOx} was derived from calculations with the EQUIL2 program by changing one variable at a time. The basic urine composition was that shown in Table 1

$$10^8.\mathrm{AP}_{\mathrm{CaOx}}=k_3.\mathrm{calcium}^{-0.99}$$

$$10^8.\mathrm{AP}_{\mathrm{CaOx}}=k_4.(\mathrm{citrate}+\mathrm{F})^{-0.60}$$

$$10^8.\mathrm{AP}_{\mathrm{CaOx}}=k_5.\mathrm{magnesium}^{-0.55}$$

For a 24-hour collection period, the complete relationship accordingly is as follows:

$$AP(CaOx)index_{RAT} = \frac{4067 \cdot Calcium^{0.93} \cdot Oxalate^{0.96}}{(Citrate + 0.015)^{0.60} \cdot Magnesium^{0.55} \cdot Volume^{0.99}}$$

In this expression, the total excretion of calcium, oxalate, citrate and magnesium should be expressed in millimoles and the volume in millilitres. The factor A (=4067) is dependent on the duration of the collection period, which in this case was 24 h. For other collection periods, the factors A and F take the values given in Table 2.

In order to investigate the accuracy of the index AP(CaOx)index$_{\mathrm{RAT}}$, a comparison with $\mathrm{AP}_{\mathrm{CaOx}}$ as derived from EQUIL2 was carried out with different combinations of the urine variables. The correlation coefficient for these calculations was 0.89 (Fig. 2). When the urine composition deviates very much from that seen normally, the difference between $\mathrm{AP}_{\mathrm{CaOx}}$ and AP(CaOx)index$_{\mathrm{RAT}}$ expectedly increases. Although an exact correspondence between $\mathrm{AP}_{\mathrm{CaOx}}$ and AP(CaOx)-index$_{\mathrm{RAT}}$ was not obtained, the correlation was reasonably good and there was very good agreement between the two parameters when the urine composition was within a physiological range.

In order to illustrate the effects of changes in individual urine constituents, $\mathrm{AP}_{\mathrm{CaOx}}$ values were calculated for a 50% reduced and a 50% increased excretion of each urine variable. The calculations were carried out at the two basic $\mathrm{AP}_{\mathrm{CaOx}}$ levels: 0.29×10^{-8} and 1.89×10^{-8} (mol/l)^2. The first $\mathrm{AP}_{\mathrm{CaOx}}$ was obtained with a urine composition, as shown in Table 1, and the second one by setting the 24-h oxalate excretion to 0.04 mmol. These data show that caution in interpretation of the AP(CaOx) index $_{\mathrm{RAT}}$ is necessary when large variations in phosphate excretion can be expected. The phosphate excretion of 0.65 mmol per 24 h corresponds to a phosphate concentration of 50 mmol/l.

When the AP(CaOx)index$_{\mathrm{RAT}}$ was calculated for urine collected from 46 rats, values between 0.24 and 2.73 were recorded. The mean (SD) AP(CaOx)index$_{\mathrm{RAT}}$ was 0.76 (0.54). The median index was 0.58. The cumulative frequency distribution of the index is shown in Fig. 3.

Table 2 Values of A for different collection periods

Hours	Factor A	Factor F
24	4,067	0.015
18	3,900	0.011
16	3,749	0.010
12	3,513	0.008
8	3,121	0.005
6	2,975	0.004
4	2,598	0.003
2	2,146	0.003
1	1,844	0.0006

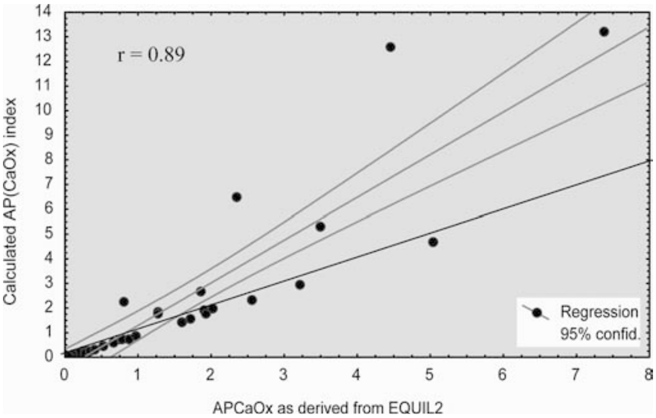

Fig. 2 Relationship between $\mathrm{AP}_{\mathrm{CaOx}}$ and AP(CaOx) index $_{\mathrm{RAT}}$ at various theoretical combinations of urine variables in a 24-h sample. The $\mathrm{AP}_{\mathrm{CaOx}}$ was derived from calculations with the EQUIL2 program and the AP(CaOx) index $_{\mathrm{RAT}}$ according to the formula described in this paper. The regression line with a 95% confidence interval is indicated as well as the line of identity

Table 3 The relative effect on $\mathrm{AP}_{\mathrm{CaOx}}$ (percent change) caused by a 50% reduction or increment of each urine variable

Urine variable	Basic $\mathrm{AP}_{\mathrm{CaOx}} = 0.29\times10^{-8}$(mol/l)^2		Basic $\mathrm{AP}_{\mathrm{CaOx}} = 1.89\times10^{-8}$(mol/l)^2	
	Reduction 50%	Increase 50%	Reduction 50%	Increase 50%
Calcium	−48	***+57***	−50	***+49***
Oxalate	−48	***+43***	−48	***+45***
Citrate	***+14***	−16	***+15***	−16
Magnesium	0	***−16***	−2	***−12***
Phosphate	+10	−7	+9	−8
Volume	***+96***	−34	***+96***	−33
pH	+3	−3	+3	−5
Sodium	+7	−7	+7	−6
Potassium	+7	−7	+6	−5
Sulphate	0	0	0	0
Ammonium	+3	−3	+2	−2

The calculations were carried out with the EQUIL2 program at two basic levels of $\mathrm{AP}_{\mathrm{CaOx}}$: 0.29×10^{-8} and 1.89×10^{-8} (mol/l)^2

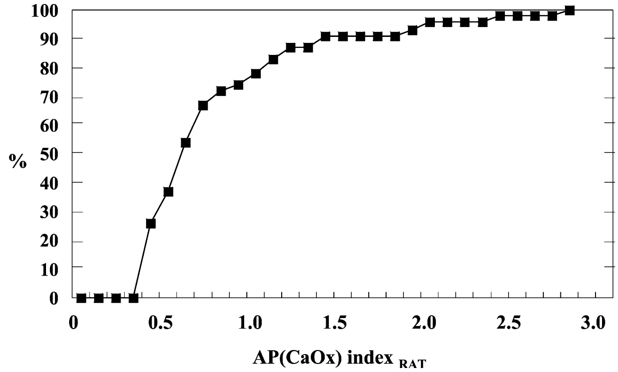

Fig. 3 Cumulative frequency distribution of AP(CaOx) index $_{\mathrm{RAT}}$ values calculated from recordings in urine of 46 rats

Table 4 AP_{CaOx} and AP(CaOx) index RAT at different levels of oxalate excretion

Urinary oxalate mmol per 24 h	$10^8 \cdot AP_{CaOx}$ $(mol/l)^2$	AP(CaOx) index $_{\mathrm{RAT}}$
0.006	0.29	0.27
0.05	2.32	2.13
0.1	4.26	4.14
0.2	7.12	8.05
0.3	10.2	11.9
0.4	10.9	15.7

For experimental conditions in which a supernormal excretion of oxalate is induced, for instance by administration of ethylene glycol, it is of interest to see how well the AP(CaOx) index $_{\mathrm{RAT}}$ corresponds to AP_{CaOx}. Such a comparison is shown in Table 4 from which it is evident that with up to at least a 24-h oxalate excretion of 0.3–0.4 mmol there is an acceptable correspondence between the two parameters. It is of note that with the urine composition used for the calculations, the oxalate concentrations at these levels of oxalate excretion is 22.7–30.3 mmol/l, which indeed is a very high concentration even in oxalate loading experiments.

Discussion

The AP(CaOx) $index_{RAT}$ described in this paper might be useful to approximately express the ion-activity product of calcium oxalate in rat urine.

The exponents for calcium, citrate and magnesium in this index differ from those in the corresponding index for human urine [4]. This is explained by the low saturation that is recorded in rat urine with the basic composition shown in Table 1. Moreover, the ratios between calcium and citrate as well as between calcium and magnesium are much lower than those found in human urine.

The AP(CaOx) $index_{RAT}$ is acceptably accurate provided the different urine variables vary within reasonable limits relative to the basic urine composition. As for the human index, excessive increments or reductions in any of the included variables will result in deviations from the expected AP_{CaOx}. This is particularly evident in case of very low or high citrate levels. In addition, the index will reflect a fairly accurate estimate of AP_{CaOx} up to the heterogeneous formation product at a level of $2\text{–}3 \cdot 10^{-8}$ $(mol/l)^2$. At higher levels, the AP(CaOx) $index_{RAT}$ will slightly overestimate or underestimate the ion-activity product, but at those levels an exact estimate of AP_{CaOx} is usually not necessary. It is also less likely that these high levels of supersaturation are encountered in rats, even in experimental conditions. The distribution of basic values of AP(CaOx)$index_{RAT}$ recorded in 24-h urine samples (Fig. 3) show that 70% of the animals had AP(CaOx)$index_{RAT}$ values below 0.8, which is a level close to the solubility product of CaOx trihydrate $(0.82 \cdot 10^{-8}(mol/l^2))$ [9].

In summary, we thus have derived a simplified estimate of the ion-activity product of calcium oxalate for rat urine. The index provides an approximate expression of AP_{CaOx} from analysis of calcium, oxalate, citrate and magnesium. Further experimental studies will disclose the usefulness of the index.

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