CROATICA CHEMICA ACTA CCACAA 53 (2) 389—396 (1980)

CCA-1216

YU ISSN 0011-1643
UDC 548.547.461.2:546.41
Conference Abstract

In-Vitro-Experiments on the Dissolution of Ca-Oxalate and Ca-Phosphate Renal Calculi by the Tricarboxylic Acids of the Krebs-Cycle. Results and in Vivo Perspectives*

P. Leskovar, R. Hartung, R. Allgayer, and A. Siebert

Urologische Klinik und Poliklinik rechts der Isar der Technischen Universität München, München, BR Deutschland

Received September 6, 1979

INTRODUCTION

The nephrolithiasis is the most frequent urological disease.[1,2] The stone incidence in general is 1—4% and can be compared with that of diabetes mellitus.

Hedenberg called attention to the fact that the published data represent pure hospital statistics and don't include the 70—80% of stone patients which were not sent to a hospital. Ljunghall and Hedstrand[3] estimated the real percentage of stone-formers in the general population to be much higher (13.7%).

An additional problem is the high stone-recurrence rate. Following Alken and Herrmann[4] respectively Kollwitz et al.,[5] this recurrence rate is 50%. Ljunghall and Hedstrand[3] calculated even a 72% probability of stone recurrence in absence of any metaphylactic precautions.

In the last years, the stone-incidence is increasing. Schneider[2] speaks of a weavy course of the stone-incidence: the stone-frequency increased after 1927, dropped to a fifth during and after the world war II and rised steeply since 1948. The increased stone-frequency seems to be related to the raised living standard, social state, increased protein consumption as well as to the raised serum uric acid level.

The high actuality of the prevention and dissolution of urinary stones is obvious. Nowadays only the uric acid calculi and to some extent the cystine calculi, representing 15 to 25% of all calculi, can be dissolved perorally. The residual 75 to 85% of renal stones, being composed of Ca-oxalate and phosphate, are not accessible to the peroral chemolitholysis.

Using a new quantitative measuring technique, based on the Coulter principle, we were able to test in vitro about 80 substances with respect to their inhibitory and crystal dissolving properties.

---

* Based on an invited lecture presented at the 5th »Ruđer Bošković« Institute's International Summer Conference *Chemistry of Solid/Liquid Interfaces*, Cavtat/Dubrovnik, Croatia, Yugoslavia, June 1979.

Here, only the tricarboxylic acids of the Krebs-cycle, the citric, the aconitic and the isocitric acid as well as the structurally similar tricarballylic acid will be presented.

METHODS

Meta- and instable Ca-oxalate solutions as well as suspensions of oxalate and phosphate crystals were treated for 48 hours with test substances of a definite concentration and the size distribution of crystals both in the test and in the reference suspension was evaluated quantitatively by Coulter Counter and Channelyzer. Here, the results can be presented only in a very simplified manner as numbers respectively volumes of crystals in the particular size classes. The efficacy of the tested substances can be deduced directly from the reduction of crystal number and crystal volume after the incubation with the test substance.

In schedules to be presented, the crystal number in the reference suspension is designated by n_{blank}, the corresponding volume by V_{blank}. The crystal number in the test solution after the 48-hour-incubation was denoted (signified) by n_{sample} and the corresponding crystal volume by V_{sample}.

S_{big} represents the sum of big crystals and S_{tot} the sum of all crystals measured.

RESULTS

In Figure 1, the crystal growth-inhibition from an oversaturated Ca-oxalate solution in presence of aconitate is demonstrated. The lower values for n_{sample} compared with those for n_{blank} indicate the inhibited respectively the prevented precipitation and growth of oxalate crystals by aconitate. The aconitate proved to be efficaceous not only in the inhibition of precipitation but also in the dissolution of preformed Ca-oxalate crystals (Figure 2).

INHIBITED GROWTH OF CA-OXALATE CRYSTALS IN THE PRESENCE OF CIS-ACONITATE

d(um)	n_{blank}	n_{sample}
8,4 - 10,6	4854 ± 380	4336 ± 340
10,6 - 12,1	4028 ± 316	3614 ± 308
12,1 - 13,3	3822 ± 300	2788 ± 218
13,3 - 14,3	3924 ± 308	-
14,3 - 15,2	3924 ± 308	-
15,2 - 16,0	3822 ± 300	-
16,0 - 16,8	3718 ± 292	-
16,8 - 17,4	3512 ± 276	-
17,4 - 18,1	3098 ± 242	-
S_{big}	25.820	2.788
S_{tot}	34.702	10.738

Figure 1.

DISSOLUTION OF STONE-FORMING CRYSTALS BY CIS - ACONITATE

- Ca-oxalate crystals

	n_{blank}	n_{sample}	V_{blank}	V_{sample}
S_{big}	16.732	11.671	25.504	16.457
S_{tot}	41.416	31.398	39.262	27.416

- Hydroxylapatite crystals

	n_{blank}	n_{sample}	V_{blank}	V_{sample}
S_{big}	10.018	4.338	13.214	5.058
S_{tot}	28.712	14.770	23.854	11.118

Figure 2.

DISSOLUTION OF STONE-FORMING CRYSTALS BY D,L-ISOCITRATE

- Ca-oxalate crystals

	n_{blank}	n_{sample}	V_{blank}	V_{sample}
S_{big}	16.732	4.441	25.504	5.328
S_{tot}	41.416	15.802	39.262	11.690

- Whewellite/weddellite crystals

	n_{blank}	n_{sample}	V_{blank}	V_{sample}
S_{big}	2.168	-	2.337	-
S_{tot}	12.636	2892	8.223	1.463

Figure 3.

Again, a direct comparison of n_{blank} and n_{sample} clearly shows the high efficacy of aconitate.

Aconitate proved to dissolve not only the oxalate but also the phosphate crystals and disintegrated urinary stones (Figure 2). The difference between the number and size of crystals before and after incubation with aconitate is obvious.

In Figure 3, the dissolution of oxalate crystals and of disintegrated oxalate stones (whewellite and weddellite) by isocitrate is demonstrated. Crystal number and volume are markedly reduced after treatment by the sodium salt of isocitric acid.

Isocitrate is also highly efficaceous in the dissolution of various kinds of phosphate crystals, as can be deduced from the lower values both of S_{big} and S_{tot} in the test suspension, compared with the reference suspension (Figure 4).

The high efficiency of sodium citrate in dissolution of artificial oxalate crystals and disintegrated oxalate stones is evident. Like other active substances, citrate is much more effective in inhibiting the precipitation and growth of crystals than in dissolution of existing crystals (Figure 5).

PARTIAL DISSOLUTION OF PHOSPHATE CRYSTALS BY D,L-ISOCITRATE

- Brushite crystals

	n_{blank}	n_{sample}	V_{blank}	V_{sample}
S_{big}	7.022	-	9.063	-
S_{tot}	15.904	4.234	14.375	2.305

- Hydroxylapatite crystals

	n_{blank}	n_{sample}	V_{blank}	V_{sample}
S_{big}	10.018	-	13.214	-
S_{tot}	28.712	2.276	23.854	1.113

- Struvite crystals

	n_{blank}	n_{sample}	V_{blank}	V_{sample}
S_{big}	7.748	-	10.069	-
S_{tot}	18.904	-	16.591	-

Figure 4.

PARTIAL DISSOLUTION OF STONE-FORMING CRYSTALS BY CITRATE

- Ca-oxalate crystals

	n_{blank}	n_{sample}	V_{blank}	V_{sample}
S_{big}	16.732	7.538	25.504	9.683
S_{tot}	41.416	19.520	39.262	16.587

- Whewellite/weddellite crystals

	n_{blank}	n_{sample}	V_{blank}	V_{sample}
S_{big}	2.168	-	2.337	-
S_{tot}	12.636	1.240	8.222	573

Figure 5.

PARTIAL DISSOLUTION OF PHOSPHATE CRYSTALS BY CITRATE

- Brushite crystals

	n_{blank}	n_{sample}	V_{blank}	V_{sample}
S_{big}	7.022	-	9.063	-
S_{tot}	15.904	-	14.375	-

- Hydroxylapatite crystals

	n_{blank}	n_{sample}	V_{blank}	V_{sample}
S_{big}	10.018	-	13.214	-
S_{tot}	28.712	3.614	23.854	1.765

- Struvite crystals

	n_{blank}	n_{sample}	V_{blank}	V_{sample}
S_{big}	7.748	-	10.069	-
S_{tot}	18.904	826	16.591	382

Figure 6.

INFLUENCE OF TRICARBALLYLATE ON NUCLEATION AND GROWTH OF CA-OXALATE CRYSTALS (IN PRESENCE OF CRYSTAL SEEDS)

d(μm)	n_{blank}	n_{seeds}	n_{sample}
8,4 - 10,6	14.046 ± 1102	1.756 ± 157	3.408 ± 368
10,6 - 12,1	12.394 ± 972	1.032 ± 92	2.478 ± 194
12,1 - 13,3	9.192 ± 722	749 ± 67	516 ± 40
13,3 - 14,3	6.300 ± 494	516 ± 46	-
14,3 - 15,2	5.060 ± 396	206 ± 18	-
15,2 - 16,0	4.142 ± 324	-	-
16,0 - 16,8	3.408 ± 268	-	-
16,8 - 17,4	2.996 ± 234	-	-
17,4 - 18,1	1.550 ± 120	-	-
S_{big}	32.638	1.471	516
S_{tot}	59.078	4.259	6402

Figure 7.

PARTIAL DISSOLUTION OF STONE-FORMING CRYSTALS BY TRICARBALLYLATE

Ca-oxalate crystals

	n_{blank}	n_{sample}	V_{blank}	V_{sample}
S_{big}	16.732	9.915	25.504	13.517
S_{tot}	41.416	26.647	39.262	22.901

Brushite crystals

	n_{blank}	n_{sample}	V_{blank}	V_{sample}
S_{big}	7.022	3.512	9.063	4.294
S_{tot}	15.904	10.742	14.375	8.525

Figure 8.

DISSOLUTION OF PHOSPHATE CRYSTALS BY TRICARBALLYLATE

Hydroxylapatite crystals

	n_{blank}	n_{sample}	V_{blank}	V_{sample}
S_{big}	10.018	2.374	13.214	2.718
S_{tot}	28.712	11.360	23.854	7.951

Struvite crystals

	n_{blank}	n_{sample}	V_{blank}	V_{sample}
S_{big}	7.748	2.375	10.068	2.560
S_{tot}	18.904	11.154	16.591	7.729

Figure 9.

In Figure 6, the dissolution of phosphate crystals and crystalline stone--material by citrate is demonstrated.

The sodium tricarballylate presented in Figure 7, was of interest because of its structural similarity with the tricarboxylic acids described and because of its possible renal elimination in an unchanged, litholytically active form. Here, the influence of tricarballylate on growing oxalate crystals is demonstrated. Again, the n_{sample}-values are distinctly lower than the n_{blank}-values.

In Figure 8, the dissolution of Ca-oxalate crystals in presence of sodium tricarballylate is shown. The reduced n_{sample}-values indicate the partial dissolution of stone-forming crystals.

Sodium tricarballylate proved to be efficaceous also in dissolving phosphate crystals, as can be concluded from the distinctly decreased crystal number in the test suspension in comparison to the reference solution (Figure 9).

CONCLUSION

From our experimental data, it can be concluded that all the tricarboxylic acids tested showed a clear inhibitory effect on growing oxalate crystals. There was also a distinct dissolution of oxalate and phosphate crystals as well as of the disintegrated renal calculi.

This seems to be of special importance in connection with our observation of a significantly reduced citric acid excretion in reccurrent stone-formers. In animal experiments, we were able to increase the urinary citric acid level 7.2 to 7.8 fold by malonate and 8 fold by equimolar mixture of oxalacetate and acetate.

REFERENCES

1. W. Lutzeyer and H. Melchior, *Therapiewoche* **26** (1976) 5851.
2. H. J. Schneider and H. Hesse, *Therapiewoche* **26** (1976) 5881.
3. S. Ljunghall and H. Hedstrand, *Acta Med. Scand.* **197** (1975) 439.
4. C. E. Alken and G. Hermann, *Urol. Int.* **4** (1957) 335.
5. A. A. Kollwitz, H. Kracht, R. Brauer, and E. Löhe, *Urol. Int.* **24** (1969) 318.

SAŽETAK

Laboratorijski eksperimenti otapanja bubrežnih kamenaca kalcijeva oksalata i kalcijeva fosfata u trikarboksilnim kiselinama Krebsova ciklusa. Rezultati istraživanja i perspektive in vivo

P. Leskovar, R. Hartung, R. Allgayer i A. Siebert

Opisani su pokusi koji dokazuju da trikarboksilne kiseline Krebsova ciklusa inhibiraju rast oksalatnih kristala. Isto tako opažen je znatan utjecaj tih kiselina na otapanje i oksalatnih i fosfatnih kristala, kao i samih bubrežnih kamenaca.

UROLOGISCHE KLINIK UND POLIKLINIK
RECHTS DER ISAR
TECHNISCHE UNIVERSITÄT MÜNCHEN
MÜNCHEN, B. R. DEUTSCHLAND

Prispjelo 6. rujna 1979.