Author's response to reviews

Title: Study of the factors that affect the regrowth of calcium oxalate dihydrate fragmented calculi

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Author's response to reviews: see over
Dear Editor,

I send you the paper entitled “Study of the factors that affect the regrowth of calcium oxalate dihydrate fragmented calculi” revised according to all the comments and suggestions raised by the reviewers. I also include a cover letter describing the modifications made to the manuscript.

I look forward to hearing from you.

Being at your disposal, yours sincerely

Prof. Dr. F. Grases
RESPONSE TO THE REVIEWERS’ COMMENTS

Reviewer 1. Nagaraja Rao

- The effect of citrate on calcium salts crystallization reduction is based on two different effects. First of all, citrate forms soluble complexes with calcium, thus reducing the effective supersaturation of the calcium salt and reducing the rate of precipitation. On the other hand, citrate is a crystallization inhibitor that can avoid crystallization by selfadsorption on the surface of the nucleus in formation or crystal in growth. The objective of this paper was to study the inhibitory effects of different substances (not the chelant effects), and for this reason supersaturation was corrected by adding calcium to obtain the same activity as without citrate. The differences found when comparing the results observed here with the ones obtained by other authors must be attributed to the different experimental conditions used in each case.

- The study presented here demonstrated a serie of important differences when compared with the previous ones in which we studied the regrowth of calcium oxalate monohydrate post-ESWL residual fragments. We think that it is important to develop studies using the two types of substrates (COD stone fragments and COM stone fragments) that constitute the important group of calcium oxalate stones (around 60-70% of all stones, 34% COD and 29% COM), in order to be able to compare the results and to dispose of information on the emerging problem that suposes stone fragment regrowth. On the other hand, these studies also supply important information about the general mechanism of renal stone formation and development, and of course we consider that this is “per se” of interest.
Reviewer 2. Alberto Trinchieri

- When mM and µM is used in the manuscript it refers to mmol/L and µmol/L respectively.

- We consider that hypercalciuria as metabolic risk corresponds to a calcium excretion superior to 250 mg / 24h for female and superior to 300 mg / 24h for male, whereas we consider that hypercalciuria as lithogenic urinary risk corresponds to a urinary calcium concentration superior to 170 mg/l (for both, female and male). For this reason, we chose a concentration of 150 mg/l as no lithogen risk associated to calcium and 250 mg/l as lithogenic.

Reviewer 3. David Goldfarb

- In the abstract (page 2, line 5) the word “real” has been changed by “renal”.

- The entire surface of the fragments was exposed to the artificial urine, and now it is specified (page 4, lines 22-23; page 5, line 1).

- The reason of working with 48h and 192h is now explained (page 5, lines 9-11).

- Fragments were weighed until constant weight (after drying the samples). It is specified in page 5, lines 3-5.

- The mass increase uniformization is detailed in page 5, lines 5-9.

- At the studied pH values, urea is a neutral molecule that does not affect to the ionic strenght and for this reason does not affect to the activities of calcium, oxalate and phosphate ions and consequently does not affect to the supersaturation of the corresponding insoluble salts. For this reason, urea was not included as a component of synthetic urine.

- Page 7, line 8 has been corrected following the reviewer’s suggestion.

- The crystal phases that appear in figures 4 and 5 are now labelled.

- The y axis scale is now the same for figures 6a and 6b.

- Statistical analysis has been performed in table 2.
The effect of citrate on calcium salts crystallization reduction is based on two different effects. First of all, citrate forms soluble complexes with calcium, thus reducing the effective supersaturation of the calcium salt and reducing the rate of precipitation. On the other hand, citrate is a crystallization inhibitor that can avoid crystallization by self-adsorption on the surface of the nuclei in formation or crystal in growth. The objective of this paper was to study the inhibitory effects of different substances (not the chelant effects), and for this reason supersaturation was corrected by adding calcium to obtain the same activity as without citrate. The differences found when comparing the results observed here with the ones obtained by other authors must be attributed to the different experimental conditions used in each case.