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Efficacy of Combined CHAp and Lanthanum Carbonate in Therapy for Hyperphosphatemia

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***Abstract*—**Although, lanthanum carbonate has not been approved by the FDA for treatment of hyperphosphatemia, we prospectively evaluated the efficacy of the combination of Calcium hydroxyapatite (CHAp) and Lanthanum Carbonate (LaC) for the treatment of hyperphosphatemia on mice. CHAp was prepared by co-precipitation method using $Ca(OH)_2$, H_3PO_4, NH_4OH with calcination at 1200ºC. Lanthanum carbonate was prepared by chemical method using $NaHCO_3$ and $LaCl_3$ at low pH environment, below 4.0. The structures were characterized by FTIR spectra and SEM -EDX analysis. The study group included 16 subjects-mice divided into four groups according to the administered substance: lanthanum carbonate (group A), CHAp (group B), lanthanum carbonate + CHAp (group C) and salt water (group D). The results indicate a phosphate decrease when subjects (mice) were treated with CHAp and lanthanum carbonate (0.5% CMC), in a single dose of 1500 mg/kg. Serum phosphate concentration decreased [(from 4.5 ± 0.8 mg/dL) to 4.05 ± 0.2 mg/dL), $P < 0.01$] in group A and in group C (to 3.6 ± 0.2 mg/dL) at 12 hours from the administration. The combination of CHAp and lanthanum carbonate is a suitable regimen for hyperphosphatemia treatment because it avoids both the hypercalcemia of $CaCO_3$ and the adverse effects of CHAp.

***Keywords*—**Calcium hydroxyapatite, hyperphosphatemia, lanthanum carbonate, phosphatebinder, structures.

I. Introduction

LANTHANUM carbonate exhibits a considerable ability to bind phosphate and the substitution of Ca^{2+} ions by divalent or trivalent lanthanide metal ions attracted researcher's attention during the past few years [1]-[3]. Lanthanum carbonate (LaC) is a calcium-free oral phosphate binder that can control hyperphosphatemia without adding to the patient's calcium load [4]-[7]. The phosphate control remains an important therapeutic target in management of chronic kidney disease (CKD) patients, not only to halt progression to secondary hyperparathyroidism but also to reduce the risk of vascular calcification and cardiovascular mortality; although no prospective interventional studies currently exist to demonstrate that this is achievable. LaC and calcium carbonate (CaC) have an additive effect; the phosphate binder potency is almost two times higher when is used LaC compared with CaC [8]-[9]. The LaC without Ca as a Pi binder may inhibit vascular calcification in CKD patients [10].

Lanthanum Carbonate (LaC) is an ideally phosphate liant and it prevents the adsorption of food phosphate forming insoluble lanthanum phosphate. LaC usually binds phosphate ionically optimally at pH 3-5, while retaining its phosphate-binding capacity across the full pH range from 1 to 7 [11]. Studies *in vitro* suggest that the phosphate bind efficacy of the lanthanum phosphate is comparable with the aluminum salts. FDA approved that LaC can be use as a phosphate binder for patients with renal insufficiency. Lanthanum carbonate is promising to be a new non-aluminium, calcium-free phosphate binder [4], [6].

The capacity to bind phosphate was validated through spectroscopic methods. There are some results published on LaC obtained by different methods, but some unresolved problems still remain. So, reaction between Na_2CO_3 and $LaCl_3$ could produce a secondary compound like $La(OH)CO_3$ if pH is higher. It was even reported that $La(OH)CO_3$ can bind phosphate and that it has clinical safety. Lanthanum carbonate induced as well lower ionized calcium levels within 2 days of treatment [12]. The noncalcium-containing phosphate-binding agents showed a differential effect on gastrointestinal calcium absorption. In healthy human volunteers was confirmed lower net calcium absorption after a single dose of lanthanum carbonate compared with sevelamer carbonate.

Calcium hydroxyapatite commonly referred as CHAp is a bioceramic material and is one of the most important implantable materials due to its biocompatibility and osteoconductivity [13]. CHAp biomaterial represents a mainly mineral component of hard tissue at vertebrae, mostly biocompatible with excellent bioactivity to be used in clinical applications. It can be also used for soft tissue. This material could be biologic integrated when is directly implanted into bone defect and it hasn't any harmful effect to immune system, no toxic and an osteconductive behaviour. There are different methods to synthesize CHAp: sol-gel precipitation, microundes, fish scale (FHAp), but chemical co precipitations is more economical, versatile.

A recent study indicates that La^{3+} ion can be incorporated into the crystal lattice of hydroxyapatite as result of the production of La-containing apatites. La^{3+} content plays important roles in both the physicochemical properties and biocompatibilities of the La-containing apatites [14]-[15]. The application of CHAp with LaC offers the advantages of a favorable bioactivity combination as a greater alkaline phosphatase activity, preferable osteoblast morphology and comparable cytotoxicity. Furthermore they possess attractive

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mechanical properties as higher thermal stability, higher flexural strength or lower dissolution rate [16].

In this work we report the synthesis and characterization of the lanthanum carbonate (LaC) and calcium hydroxyapatite (CHAp), both substances are bioceramic materials with medical applications. As part of their pre-clinical safety evaluation, the study was conducted in mice with normal renal function to compare the phosphate-binding efficacy of the lanthanum carbonate directly and with the CHAp contribution after a single dose administration.

The proposed research is a novel research direction, and according to the applicants' knowledge, there is no previously published research on what is proposed to be a new research of the efficacy of the LaC - CHAp system as a potential pharmacology application to examine the phosphate-binding efficacy.

II. Experimental

A. Synthesis of Lanthanum Carbonate

The lanthanum carbonate has been synthesized from $LaCl_3$ 1M and $NaHCO_3$ 1M. Initial 8.14 g La_2O_3 (99.92% purity) was treated with HCl 35% until a pH of 0 value was obtained. Solution of $NaHCO_3$ 1M was added in acidic solution of $LaCl_3$ 1M trough permanently agitation.

Two reactions took place, a precipitation (1) and neutralization (2) respectively:

$$2La^{3+}+3HCO_3^-=La_2(CO_3)_3\downarrow+3H^+ \quad (1)$$

$$H^++HCO_3^-=H_2O+CO_2\uparrow \quad (2)$$

The initial nuclei of LaC were generated when $NaHCO_3$ was added slowly. Initial smaller quantities of $NaHCO_3$ solution were added faster, pH was around 3 and the initiation of LaC nuclei was favorable. The pH in system increased constantly by adding $NaHCO_3$ solution and the neutralization reaction was provided simultaneously. To prevent to form a lanthanum carbonate hydroxide the pH was monitorized permanently to keep it constantly lower than pH 4.0.

The small quantity of supernatant was treated with NaOH 1M and no precipitate was observed, so La^{3+} was completely consummated. The lanthanum carbonate formed after 40 min. and was kept on pH between of 4 – 6. Finally the white precipitate was filtrated and dried at 110ºC during 90 min. The LaC powder structure was characterized by FTIR technique (equipment Bruker) and Scanning Electron Microscopy (SEM-EDX, equipment Qanta 2000).

B. Synthesis of Calcium Hydroxyapatite

Calcium hydroxyapatite (CaHAp, $Ca_{10}(PO_4)_6(OH)_2$) is the principal inorganic component of bone and teeth, one of the most important bioceramics. CHAp powders of high purity was synthesized by chemical coprecipitation method using aqua solution started from $Ca(OH)_2$, H_3PO_4 and NH_4OH according to reaction (3).

$$10Ca(OH)_2 + 6H_3PO_4 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 18H_20 \quad (3)$$

48 g $Ca(OH)_2$ was dissolved in 28.5 mL distillate water. Separately, 2.25 mL of H_3PO_4 were dissolved in 19.5 mL distillate water. NH_4OH was used to adjust the pH to be higher than 10.5, in order to obtain a stoichiometric CHAp (Ca/P = 1.67). In order to obtain a CHAp suspension, solution was added during 10 minutes and it was magnetically stirred intensively (800 rpm) on a water bath with constant temperature of 75°C. This suspension was mentioned to maturate 48 h. Finally it was filtrated vide, washed with distillate water, dried at 110ºC for 24 h, triturated to reduce particle size and calcinated at 1200ºC during 3 h. Small nanosize particles of CHAp were obtained with high specifically surfaces and high purity. The structure of CHAp was characterized by scanning electron microscopy (SEM) Fourier transform infrared spectroscopy (FTIR). The chemical composition was made by Energy Dispersive Spectroscopy (EDX).

III. Results and Discussion

A. Structural Characterization of LaC and CHAp

LaC contains lanthanum, a typical lanthanide which is seen as clear contrast images in computerized tomography and dual energy X-ray absorptiometry scanning [17]. The molecular formula is $La_2(CO_3)_3$ x 4 H_2O (M 529.9 g) and it is insoluble in water. LaC is a metallic salt that isn't metabolised *in vivo*. The structure of LaC indicates morphology of the particles formed. Spherical carbonate particles combined with small quantity of needle-shaped particles were found (Fig. 1). FT-IR spectra (Fig. 4 (a)) confirm the presence of specifically chemical bonds for LaC relived as in literature. The CHAp morphology is shown in Fig. 2. EDX analysis of both bioceramics confirms the presence of specifically elements of the structures. The composition for LaC is La 42.5 %, O is 33.7 % and C 23.7% (Fig. 3 (a)). For CHAp the composition confirms presence of Ca 40.6%, O 35.6% and P 2.3 % (Fig. 3 (b)).

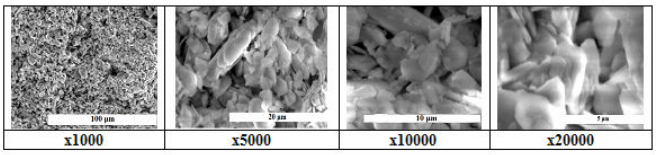

Fig. 1 SEM images of LaC at different magnification

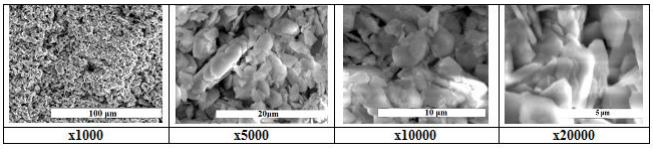

Fig. 2 SEM images of CHAp at different magnification

FT-IR spectra confirm also the presence of specific bonding of LaC and CHAp according to data reported. FT-IR spectrum of LaC is given in Fig. 4 (a). In this case, characteristic peak of LaC has sharp peaks in the fingerprint region at 873 cm^{-1},

822 cm^{-1}, and 668 cm^{-1}. Characteristic peak of H_2O higher of 3200 cm^{-1} can be observed for $La(CO_3)$ $8H_2O$ (Table I).

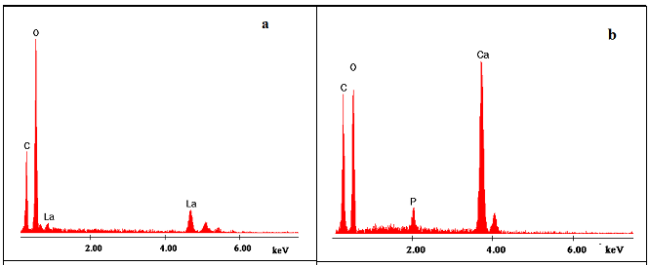

Fig. 3 EDX analysis of LaC (a) and CHAp (b)

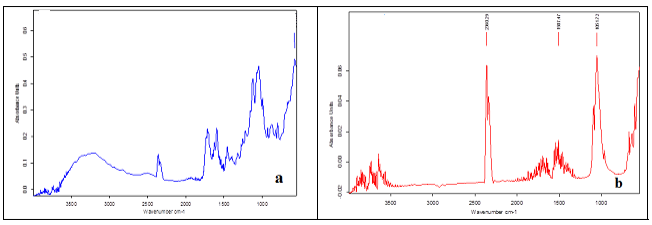

Fig. 4 FT-IR spectra of LaC (a) and CHAp (b)

Presences of all characteristic vibrations of CHAp, for PO_4^{3}, OH, CO_3^{2-} and PO_2 and water associate of this structure were noticed (Fig. 4 (b)). There are small differences compared with literature, from the co-precipitation process with modification of parameters. Carbonate is present at 1420 cm^{-1} (Table I).

TABLE I
CHARACTERISTIC PEAKS FOR LAC AND CHAP

ν (cm^{-1})	LaC	ν (cm^{-1})	CHAp
3219	H_2O associate with LaC	3720	H_2O associate with CHAp
2360; 2341	CO_3^{2-}associate with LaC	3600	H_2O associate with CHAp
1541; 1521	La, CO_3^{2-} unidentately	2360	P
1126; 1046	La;O	1607	H_2O associate with CHAp
873; 822	La; CO_3^{2-}	1061	PO_4^{3-}, P=O, P-OH (OH), PO_2 conjugate (v. symmetric PO_2)
668; 574	La-OH	868	P-OH, valence vibration P-O, $CaCO_3$

B. Evaluation of Phosphate from Biochemical Analysis

The introduction of subsequently lanthanum carbonate could represent a significant development in phosphate management as non aluminium, calcium-free agent [4], [6]. LaC suspended in 0.5% CMC (Carboxymethyl Cellulose) and CHAp were used for the oral administration at mice. Also a mixture of strong biominerals, LaC and CHAp (1:1) was administrated to a distinct mice group to demonstrate that lanthanide have high affinity for inorganic phosphate (Pi).

This animal study directly compares the efficacy of LaC and CHAp at an equivalent phosphate binding potency for controlling the serum phosphate level and suppressing the deterioration of the renal function.

The animal study included 16 subjects-mice divided into four groups according to the administered substance: only lanthanum carbonate (group A), CHAp (groupB), lanthanum carbonate + CHAp (group C) and salt water (group D). Subjects were male *Mus musculus* albino mice, weighing around 30 g. Each lot received a single dose of bioceramics as is presented in Table II. The mice haven't received food with cca. 8-12 hours before tacking the blood samples in order to normalize enzyme concentration and to eliminate fats [18].

TABLE II
SCHEME OF THE DOZE ADMINISTRATION

Lot	LaC (mg/kg)	CHAp (mg/kg)	LaC + CHAp (mg/kg)
A	1500	-	-
B	-	1500	-
C	-	-	1500
D-martor	-	-	-

The blood from subjects was collected after 12 hours from the single dose administration. The binded Pi by lanthanum in the mice blood was analyzed. Blood sampling was done using the technique called cervical dislocation. The blood was drown off in specific biochemical determinations vacutainer with red cap (no additives) and purple cap (EDTA anticoagulant) [12].

The phosphate was analyzed through a spectrophotometric and colorimetric method (MIcroSlide). The phosphate data are shown in Fig. 5. It can be observed that phosphate levels are in normal limits for the martor group D (4.5 ± 0.8 mg/dL). For the group A where mice were treated with LaC it is observed a decrease of cca. 10% of phosphate. The phosphate concentration after the 12 hours from a single dose of administration decreased from 4.5 ± 0.8 mg/dL in martor group D to 4.05± 0.2 mg/dL in group A (only LaC) and to 3.7 ± 0.2 mg/dL in group B, where mice were treated with CHAp.

There are significant differences between the phosphate values obtained for the group C, treated with LaC + CHAp (1:1) and the martor group D, from 4.5 mg/dL to 3.6 ± 0.1 mg/dL. The results indicate a better decrease of phosphate when subjects (mice) were treated with CHAp and LaC, in a single dose of 1500 mg/kg according with scheme from Table II.

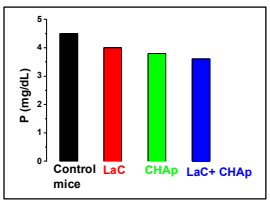

Fig. 5 Variation of phosphate at mice subjects

LaC a powerful Pi binder has a similar effect as aluminum hydroxide in reducing serum Pi levels [8]. In a second large prospective, the efficacy of lanthanum carbonate could be compared with calcium carbonate [12]. Animal studies have shown that lanthanum carbonate has similar phosphate binding efficacy as aluminum, but dramatically lower oral bioavailability [11]. Unlike calcium-based binders and

sevelamer hydrochloride, lanthanum has shown, *in vitro*, to bind phosphate efficiently even at the low pH found in the stomach, as well as the high pH values found in the duodenum and jejunum. This range of pH for binding phosphate is similar to that seen at aluminum salts [7], [8], [11].

We analyzed the efficacy of LaC and CHAp which are both commonly used to reduce the serum phosphate level during a single doze administration. Lanthanum does not cause hypercalcemia, is effective in reducing calcium × phosphate product, and may have a positive effect on bone histology.

Our results of the phosphate levels in this study suggest that the phosphate binding potency of LaC is higher than CHAp potency. Data obtained indicate a decreasing of cca. 20 % of the phosphate level at administration of both ceramics (LaC and CHAp) and this placed them at decreased risk of diseases of CKD.

Conclusion

The goal of this research was to synthesize two chemical biocompounds, LaC and CHAp which have been proven to function as a phosphate binder so that the therapy of hyperphosphatemia would be improved. The structural characterization of the bioceramics synthesized was proved by SEM images and FTIR spectra. The chemical composition was made by EDX.

Lanthanum carbonate represents another step in the way to complete phosphate control. Evidence suggests that it is an effective, well tolerated and safe phosphate binder.

The animal pilot study was made on mice male Mus musculus albino mice. The combination of CHAp and LaC is a suitable regimen for hyperphosphatemia treatment because it avoids both the hypercalcemia of $CaCO_3$ and the adverse effects of CHAp. The results indicated that the phosphate binding potency of LaC is better with CHAp combination. This pilot study suggested that LaC delayed progression of Pi compared with CHAp. A direct comparison of the binding capacities of the currently available phosphate-binders would be useful to guide clinical practice.

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References

[1] S. Damment, "Pharmacology of the Phosphate Binder, Lanthanum Carbonate", *Laboratory Studies*, vol. 33, no. 2, pp. 217-224**,** 2011.

[2] V. P. Persy, G. J. Behets, A. R. Bervoets, M. E. De Broe, P. C. D'Haese, "Lanthanum: a safe phosphate binder", *Semin Dial*, vol. 19, pp. 195-199, 2006.

[3] J. **P**airon, F. Roos, P. Sebastien, B. Chamak, I. Abd-alsamad, J. Bernaudin, J. Bignon, P. Brochard, "Biopersistence of cerium in the human respiratory tract and ultrastructural findings", *Am. J. Ind. Med.* vol. 27, pp. 349-358, 1995.

[4] A. J. Hutchison, M. E. Barnett, R. J. Krause, G. A. Siami, "Lanthanum carbonate treatment, for up to 6 years, is not associated with adverse effects on the liver in patients with chronic kidney disease Stage 5 receiving hemodialysis", *Clin. Nephrol.,* vol. 7, no. 3, pp. 286-295, 2009.

[5] S. M. Sprague, H. Abboud, P. Qiu, M. Dauphin, P. Zhang, W. Finn. "Lanthanum carbonate reduces phosphorus burden in patients with CKD stages 3 and 4: a randomized trial", *Clin J Am Soc Nephrol,* vol. 4, pp. 178-185, 2009.

[6] A. J. Hutchison, M. E. Barnett, R. Krause, J. T. Kwan, G. A. Siami, "Lanthanum Study Group Long-term efficacy and safety profile of lanthanum carbonate: Results for up to 6 years of treatment"*, Nephron Clin Pract.*, vol. 110, no. 1, pp. c15-23, 2008.

[7] G. J. Behets, G. Dams, S. R. Vercauteren, S. J. Damment, R. Bouillon, M. E. De Broe, P. C. D'Haese**,** "Does the phosphate binder lanthanum carbonate affect bone in rats with chronic renal failure?", *J. Am. Soc. Nephrol.,* vol. 15, pp. 2219-2228, 2004.

[8] T. Shigematsu, Y. Nakashima, M. Ohya, Tatsuta K., Koreeda D., Yoshimoto W., Yamanaska S., Sakaguchi T., Hanba Y., Mima T., Negi "The management of hyperphosphatemia by lanthanum carbonate in chronic kidney disease patients", *Inter. J. of Nephrology and Renovascular Disease*, May 2012.

[9] I. Mohammed, A. Hutchison, "Phosphate binding therapy in dialysis patients: focus on lanthanum carbonate", *Ther. Clin. Risk Manag.,* vol 4, no. 5, pp. 887-893, 2008.

[10] W. F. Finn, "Lanthanum carbonate versus standard therapy for the treatment of hyperphosphatemia: safety and efficacy in chronic maintenance hemodialysis patients", *Clin. Nephrol*., vol. 65, pp. 191-202, 2006.

[11] A. J. Hutchison, F. Al-Baaj, "Lanthanum carbonate for the treatment of hyperphosphatemia in renal failure and dialysis patients", *Expert Opin. Pharmacother.*, vol. 6, pp. 319-28, 2005.

[12] G.J. Behets, G. Dams, S.J. Damment, P. Martin, M. E. Broe, De, D'Haese, "Differences in gastrointestinal calcium absorption after the ingestion of calcium-free phosphate binders", *American Journal of Physiology - Renal Physiology*, vol.306, no. 1, pp. F61-F67, 2014.

[13] A. Palasz, P. Czekaj, "Toxicological and cytophysiological aspects of lanthanides action"*,* II Department of Histology and Embryology, Silesian Medical Academy, Katowice, Poland*,* vol. 47, no. 4, pp. 1107-1114, 2000.

[14] Z. Deng, L. Wang, D. Zhang, J. Liu, C. Liu, J. Ma, "Lanthanum-containing hydroxyapatite coating on ultrafine-grained titanium by micro-arc oxidation: A promising strategy to enhance overall performance of titanium", *Med. Sci. Monit.*, vol. 20, pp. 163-166, 2014.

[15] D. G. Guo, A. H. Wang, Y. Han, K. W. Xu, "Characterization, physicochemical properties and biocompatibility of La-incorporated apatites", *Acta Biomater*. vol. 5, no. 9, pp. 3512-3523, November. 2009.

[16] J.C. Zhang, T.L. Zhang, S.J. Xu, K. Wang, S.F. Yu, "Effects of lanthanum on formation and bone-resorbing activity of osteoclast-like cells", *J. Rare Earths*, vol. 22, no. 6, pp. 891-895, 2004.

[17] H. Hayashi, M. Machida, T. Sekine., H. Yamaguchi, Kiriyama, T. Kumita S., "Beam-hardening artifacts on computed tomography images caused by lanthanum carbonate hydrate in a patient on dialysis", *Jpn. J. Radiol*., vol. 28, no. 4, pp. 322-324, May 2010.

[18] G. N. Nadkarni, J. Uribarri, "Phosphorus and the kidney: what is known and what is needed", *Adv. Nutr.,* vol. 5, pp. 98-103, 2014.