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What Can We Learn from the Saga of Chitosan Gums in Hyperphosphatemia Therapy?

Man S. Oh and Jaime Uribarri†*

Abstract

Control of high serum phosphorus, a marker of poor outcome, is still a poorly achieved goal in dialysis therapy. Therefore, the 2009 study (Savica *et al.*, *J Am Soc Nephrol* 20: 639–644, 2009) showing a significant drop of serum phosphate (2.35 mg/dl) after only 2 weeks of chewing a chitosan-containing gum two times per day was received with great hopes by the renal community. Chitosan is a polymer of glucosamine, similar to sevelamer, which allegedly would bind phosphate present in high concentrations in the saliva of renal patients. Recent randomized studies, however, have been unable to duplicate these results. A systematic and detailed quantitative analysis of the available data was performed. It concluded that the amount of chitosan contained in the chewing gum (20 mg) is too little to account for the originally observed reduction in serum phosphate and be of any use as a phosphate binding agent in the management of hyperphosphatemia. It was postulated that the original marked drop in serum phosphate may have been caused by the Hawthorne effect, which is frequently observed in nonrandomized clinical trials. Two important lessons derived from this analysis are emphasized. The first lesson is the demonstration of the importance of randomized, placebo-controlled studies in clinical research. If randomization had been performed in the original study, the Hawthorne effect would have been detected. The second lesson is showing the importance of quantitative analysis, which in this case, would have avoided the time and effort expended in several randomized clinical trials that eventually concluded the ineffectiveness of the chitosan-containing chewing gums as a phosphate binder.

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*Nephrology Division, Department of Medicine, State University of New York Downstate Medical Center, Brooklyn, New York; and †Nephrology Division, Department of Medicine, Mount Sinai School of Medicine, New York, New York

Correspondence: Dr. Jaime Uribarri, Department of Medicine, Mount Sinai School of Medicine, One Gustave Levy Place, New York, NY 10029. Email: jaime.uribarri@mssm.edu

Introduction

There is significant epidemiologic evidence linking hyperphosphatemia and mortality among dialysis patients (1). Therefore, control of hyperphosphatemia is an essential part of the routine clinical care of these patients. In clinical practice, however, average serum phosphorus levels in dialysis patients remain higher than those levels recommended by practice guidelines (2), except in patients on long nocturnal hemodialysis (3), despite the widespread use of high-efficiency dialyzers, new phosphorus binders, and the usual nutritional education. Thus, it is not surprising that the results of a study by Savica *et al.* (4) published in 2009 stirred up hope and excitement in the area of phosphate management of dialysis patients. The study allegedly showed that chewing a gum containing chitosan for 1 hour two times per day was highly effective in controlling hyperphosphatemia in patients on maintenance hemodialysis (4). The study reported that chitosan, a glycosamine polymer, is an effective binder of phosphate in saliva and that phosphate concentration in saliva is very high in hyperphosphatemic uremic patients (4). The reduction in mean serum phosphate concentration with the treatment was rather impressive; over a 2-week period, the serum phosphate concentration decreased from 7.60 to 5.25 mg/dl after chewing a gum containing 20 mg chitosan for 1 hour two times per day. During the same period, the concentration of phosphate in saliva decreased from 73.21 to 33.19 mg/dl (4). The effect of a chitosan-containing chewing gum in reducing serum phosphate was thought to be so dramatic and promising that, in 2011, Nestle purchased the small pharmaceutical company that produced the medication (5), and over the past few years, six clinical trials attempting to confirm the effect on serum phosphate have been carried out (www.clinicaltrials.com). Results of four of these randomized clinical trials have been published (6), but the results were quite different from the results of the original report (4), showing a reduction in serum phosphate of only 0.16–0.20 mg/dl and no reduction in salivary phosphate concentration. The magnitude of the observed reduction in serum phosphate in these more recent studies is of negligible clinical importance and may have been caused by some other effects of the study rather than the drug effect, which will become clear in our subsequent quantitative analysis. How can we explain this striking difference in results with the same medication? We will systematically address this question. What seems clear from the outset, however, is that a careful quantitative analysis of the data should have cast serious doubts on the plausibility of the initial findings (4), because it would be nearly impossible to quantitatively attribute the reduction in serum phosphate to the phosphate binding effect of the chitosan contained in the chewing gum. A quantitative analysis would also have demanded a much higher dose of chitosan in each gum than 20 mg, which was selected

for the study, although a higher dose would not have been any more effective for other reasons.

To put it simply, the amount of chitosan contained in each chewing gum (20 mg) is much too little to account for the observed reduction in serum phosphate (4). Binding of phosphate by chitosan, results from the ionic interaction between positively charged amine moieties of the glucosamine polymer and negative charges of phosphate. Hence, the absolute theoretical limit on the amount of phosphate binding will depend on the quantity of amines in chitosan. Chitosan is a glucosamine polymer, and each glucosamine unit contains one amine unit. The molecular mass of glucosamine is 179 Da, but because of the loss of one water molecule for each glucosamine unit in the polymer formation, the actual molecular mass of each glucosamine unit in the polymer is 161 Da (179−18=161). Hence, 20 mg chitosan would contain 0.124 mmol amines that are potentially capable of binding phosphate.

Chitosan is deacetylated glucosamine made from chitin and acetyl glucosamine. The chitosan used in the study is described as ≥75% deacetylated (4). If we assume that it was 80% deacetylated, the chitosan content of each chewing gum would be 0.1 mmol (0.124×0.8=0.10). Even if we assume 90% deacetylation, the numbers will not change much (0.124×0.9=0.11). Furthermore, acetylated chitosamine (*i.e.*, chitin) weighs more than chitosan, because it contains the additional weight of the acetyl group. Hence, the actual content of amines in 80% deacetylated chitosan would be slightly less than the above calculation.

Phosphate exists in biologic fluid as $HPO_4^{=}$ and $H_2PO_4^{-}$, and the ratio of these two components depends on the pH of the solution. At pH 7.4, the ratio of $HPO_4^{=}$ to $H_2PO_4^{-}$ is 4:1, and hence, the average charge of phosphate is 1.8 mEq/mmol at pH 7.4. For this reason, 1.8 mmol glucosamine units are needed to bind 1 mmol phosphate at pH 7.4. Hence, the maximum phosphate binding capacity of the 20 mg chitosan contained in each gum would be 0.056 mmol (0.1/1.8=0.056) or 1.74 mg phosphate.

When chitosan binds phosphate, there is competition with other anions, such as chloride and bicarbonate, present in saliva in addition to phosphate. According to the *in vitro* study by Savica *et al.* (4), the actual phosphate binding capacity of chitosan was about 50% of the maximum binding capacity. Thus, the actual amount of phosphate that could be bound by 20 mg chitosan in each gum would have been 0.028 mmol or 0.87 mg phosphate if we assume that the condition *in vivo* is as ideal as *in vitro*. Therefore, chewing a chitosan-containing gum two times per day could have removed, at best, only 1.74 mg phosphate/d. One can appreciate how little this amount of phosphate removal is when it is compared with a single hemodialysis treatment, which removes about 900–1000 mg phosphate (7). Another example may illustrate this point more dramatically; because the concentration of salivary phosphate at the start of the chitosan gum treatment was 73 mg/dl, simply spitting out 3 ml saliva would have removed more phosphate (2.19 mg) than the maximum likely amount of phosphate that could have been removed by the daily treatment with chitosan chewing gum (1.74 mg)

The mathematical impossibility of the chitosan-containing gum having any clinically meaningful effect in removing phosphate from the body can be further illustrated by a comparative analysis with another phosphate binding polymer, sevelamer. Sevelamer chloride and sevelamer carbonate are widely used clinically as oral phosphate binders. Like chitosan, the phosphate binding ability of sevelamer depends on the amine moieties of the polymer. However, the relative content of amines in sevelamer for a given weight of the polymer is much greater than the relative content of amines in chitosan. The mean molecular mass of the amine-containing unit of sevelamer is 75 Da (8). Hence, 1 g sevelamer contains 13.3 mmol amine units, and 1 g sevelamer should be able to bind 7.4 mmol phosphate (1.8 mmol amine units are needed to bind 1 mmol phosphate). Because the usual dose of sevelamer in the management of hyperphosphatemia is 4.8 g/d, the total maximum phosphate binding capacity of 4.8 g sevelamer would be 35.52 mmol or 1100 mg phosphate. In reality, the actual phosphate binding by sevelamer *in vivo* is only about 200 mg/d (less than one fifth of the maximum potential binding capacity) (9,10). The likely reason for the discrepancy is competition with other anions. In that sense, the calculations that we have made above for the maximum phosphate binding capacity of chitosan-containing chewing gum are overly generous. It must be noted that, for the same amount of medication, the total phosphate binding capacity of sevelamer is greater than the total phosphate binding capacity of chitosan because of the higher amine content, but more importantly, the usual dose of 4.8 g/d sevelamer is some 120 times the chitosan dose of 40 mg/d. Thus, when the calculation is made on the basis of equivalent phosphate binding capacity on weight basis, 10.3 g chitosan would have been equivalent to 4.8 g sevelamer, and the selected dose of chitosan of 40 mg/d was underestimated 500-fold.

Another calculation based on the phosphate content in saliva further reinforces our conviction on the impossibility of achieving reduction in serum phosphate by 2.35 mg/dl by chewing chitosan-containing gum for 2 hours each day. If we assume that the concentration of phosphate in saliva during the treatment period is 33 mg/dl (4) and the salivary secretion rate is 1000 ml/d, the volume of saliva secreted in 2 hours would be 83 ml, with a total phosphate content of 27 mg. Thus, the removal of every molecule of phosphate in saliva produced during the 2-hour gum-chewing period would have been far insufficient to maintain a drop of serum phosphate by 2.35 mg/dl, because changes of serum phosphate of this magnitude are observed in patients treated with oral phosphate binders that remove about 200 mg phosphate/d.

There is yet another reason that would make chitosan-containing gum very ineffective. As stated earlier, phosphate binding capacity of chitosan depends on the positive charge of the amine group of the glucosamine unit, and the dissociation constant of the glucosamine unit is quite unfavorable for the reaction at the usual pH of saliva. The glucosamine monomer has a p*K* of 7.8, and therefore, at neutral pH, most of the amine would be in a charged state. However, as a glucosamine polymer (*i.e.*, chitosan), its p*K* is reduced to about 6.6 (11). As a result, chitosan will be mostly unionized and quite insoluble at the neutral or alkaline pH of saliva (11,12). In contrast, sevelamer has a much more favorable p*K*, and therefore, at any given time, 40% of all amines are positively charged, balanced by chloride, or bicarbonate (13).

The quantitative analysis performed above makes it clear that the reduction in serum phosphate during treatment with chitosan-containing gum observed in the original study (4) could not have been caused by phosphate binding to the chitosan in the gum. What was the reason for the observed dramatic reduction in serum phosphate? There are several possible explanations. One possibility is a laboratory error. This possibility is not very likely. Why would laboratory errors occur only during the treatment period and then mysteriously disappear at the end of the study? Another possibility is that the subjects' dietary patterns changed during the treatment period. That is, the subjects were consuming a low phosphate diet only during the treatment period, which is possible but not very plausible. We all know that changing the dietary intake of phosphate is very difficult, especially without intensive dietary counseling. The third possible explanation (the most likely one) is the Hawthorne effect of the study on compliance with the preexisting medication (14).

It must be pointed out that the original study (4) was not a randomized controlled trial. The subjects were already being treated with sevelamer hydrochloride at doses ranging from 3.2 to 4.8 g/d, and chitosan-containing gum was given as an add-on treatment. It is noteworthy that, before the treatment with chitosan, although the patients were treated with 3.2–4.8 g/d sevelamer, serum phosphate concentration was unusually and remarkably high (7.6 mg/dl), suggestive of poor compliance with sevelamer. During the treatment period of the study, the subjects were followed weekly with pill counts and perhaps, counseling, and this following may have improved the compliance with sevelamer. We postulate that the most likely reason for the sharp reduction in serum phosphate during chitosan gum treatment is the Hawthorne effect on the compliance with sevelamer, which would have been easily detected if the study had been randomized.

The Hawthorne effect is one of the most famous effects in sociological studies. It means that subjects alter an aspect of their behavior when they realize that they are being studied, and the change is not in response to any particular intervention. The term was coined in 1950 by Henry A. Landsberger (14) in the analysis of experiments from 1924 to 1932 at the Hawthorne Works (a Western Electric factory outside Chicago, IL). Hawthorne Works had commissioned a study to see if its workers would become more productive with higher levels of lighting. Initially, it was thought that workers' productivity improved with better lighting, but later, it was shown that the observed improvement in productivity was unrelated to levels of light. It was suggested that the productivity gain occurred because of the motivational effect on the workers as a result of the interest being shown in them rather than changes in the level of lighting.

In summary, it is our contention based on the quantitative analyses described above that the chitosan-containing gum could not have led to the level of reduction in serum phosphate that was shown in the work by Savica *et al.* (4) and that the amount of chitosan used in the chewing gums is too small to be of any use as a phosphate binding agent in clinical medicine. These findings are clearly shown in double blind randomized studies using a chitosan-containing chewing gum (6). At least two important lessons can be derived from this analysis. First, our analysis showed the importance of randomized, placebo-controlled studies in clinical research. Randomization in the original study (4) would have eliminated the Hawthorne effect. Unfortunately, appropriately designed randomized, blinded studies are very expensive undertakings that require at least some baseline clinical evidence of success. The study by Savica *et al.* (4) would have represented baseline evidence of success provided that some quantitative analyses of binding capacity of the medication had been performed. The second and more compelling point of our review is the importance of quantitative analysis in clinical science.

If quantitative analysis had been applied to the original study findings (4), it would have avoided the time and effort expended in several randomized clinical trials that eventually concluded the ineffectiveness of the chitosan-containing chewing gums as a phosphate binder (6). Interestingly, the original United States patent application for chitosan as a phosphate binder describes the use of much larger amounts of chitosan (15). Obviously, those parties involved in the patent application performed quantitative analysis. How this information got lost when the chewing gum was manufactured for clinical use escapes our knowledge. Moreover, it is unclear whether using even a larger amount of chitosan would be effective given the poor solubility of the compound at the usual salivary pH and the limited availability of phosphate in saliva secreted during the period of gum chewing for 2 hours.

Disclosures

None.

References

1. Block GA, Klassen PS, Lazarus JM, Ofsthun N, Lowrie EG, Chertow GM: Mineral metabolism, mortality, and morbidity in maintenance hemodialysis. *J Am Soc Nephrol* 15: 2208–2218, 2004
2. Tentori F, Blayney MJ, Albert JM, Gillespie BW, Kerr PG, Bommer J, Young EW, Akizawa T, Akiba T, Pisoni RL, Robinson BM, Port FK: Mortality risk for dialysis patients with different levels of serum calcium, phosphorus, and PTH: The Dialysis Outcomes and Practice Patterns Study (DOPPS). *Am J Kidney Dis* 52: 519–530, 2008
3. Walsh M, Manns BJ, Klarenbach S, Tonelli M, Hemmelgarn B, Culleton B: The effects of nocturnal compared with conventional hemodialysis on mineral metabolism: A randomized-controlled trial. *Hemodial Int* 14: 174–181, 2010
4. Savica V, Calò LA, Monardo P, Davis PA, Granata A, Santoro D, Savica R, Musolino R, Comelli MC, Bellinghieri G: Salivary phosphate-binding chewing gum reduces hyperphosphatemia in dialysis patients. *J Am Soc Nephrol* 20: 639–644, 2009
5. Sonne P: Nestlé Buys 'Medical Food' Start-Up. Available at: http://online.wsj.com/article/SB100014240527487-04124504576118273251942458.html. Accessed June 1, 2013
6. Block GA, Persky MS, Shamblin BM, Baltazar MF, Singh B, Sharma A, Pergola P, Smits G, Comelli MC: Effect of salivary phosphate-binding chewing gum on serum phosphate in chronic kidney disease. *Nephron Clin Pract* 123: 93–101, 2013
7. Kjellstrand CM, Ing TS, Kjellstrand PT, Odar-Cederlof I, Lagg CR: Phosphorus dynamics during hemodialysis. *Hemodial Int* 15: 226–233, 2011
8. Mazzeo JR, Peters RM, Hanus MR, Chen X, Norton KA: A phosphate binding assay for sevelamer hydrochloride by ion chromatography. *J Pharm Biomed Anal* 19: 911–915, 1999
9. Block GA, Wheeler DC, Persky MS, Kestenbaum B, Ketteler M, Spiegel DM, Allison MA, Asplin J, Smits G, Hoofnagle AN,

Kooienga L, Thadhani R, Mannstadt M, Wolf M, Chertow GM: Effects of phosphate binders in moderate CKD. *J Am Soc Nephrol* 23: 1407–1415, 2012
10. Oliveira RB, Cancela AL, Graciolli FG, Dos Reis LM, Draibe SA, Cuppari L, Carvalho AB, Jorgetti V, Canziani ME, Moysés RM: Early control of PTH and FGF23 in normophosphatemic CKD patients: A new target in CKD-MBD therapy? *Clin J Am Soc Nephrol* 5: 286–291, 2010
11. Filion D, Lavertu M, Buschmann MD: Ionization and solubility of chitosan solutions related to thermosensitive chitosan/glycerol-phosphate systems. *Biomacromolecules* 8: 3224–3234, 2007
12. Pillai CKS, Paul W, Sharma CP: Chitin and chitosan polymers: Chemistry, solubility and fiber formation. *Prog Polym Sci* 34: 641–678, 2009
13. Braunlin W, Zhorov E, Guo A, Apruzzese W, Xu Q, Hook P, Smisek DL, Mandeville WH, Holmes-Farley SR: Bile acid binding to sevelamer HCl. *Kidney Int* 62: 611–619, 2002
14. McCarney R, Warner J, Iliffe S, van Haselen R, Griffin M, Fisher P: The Hawthorne Effect: A randomised, controlled trial. *BMC Med Res Methodol* 7: 30, 2007
15. Phosphate-Binding Chitosan and Uses Thereof. US Patent 7943597. Available at: http://patft.uspto.gov/netahtml/PTO/search-bool.html. Accessed June 1, 2013

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