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Viewpoint

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Using Medicinal Chemistry To Solve an Old Medical Mystery

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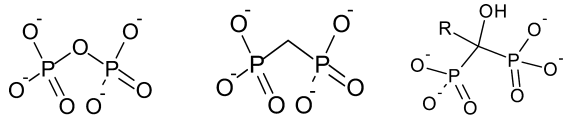
ABSTRACT: The logic behind the traditional medicinal chemistry technique of designing a synthetic enzyme substrate to mimic a natural one is used to uncover the identity of the unknown cause of two legacy industrial diseases by comparing the reported symptoms to two side effects of a modern synthetic.

Medicinal chemists frequently employ synthetics resembling natural substrates to influence enzymatically catalyzed biologic processes. Two such agents, with analogous molecular structures, known to affect the same biologic system, may be expected to possess similar additional effects. By comparing the side effects prevalent in two 19th and 21st century cohorts, we have identified the previously unknown cause in the legacy cohort by searching for a chemically and biologically rational candidate, which resembles the known agent associated with the more recent group. In the 19th century, two white phosphorus match-industry diseases[1] provoked an early confrontation between government regulators and industrialists, quite reminiscent of some in our present era. The cause of these diseases has been the subject of much speculation. No rational molecular explanation has been forthcoming. We believe that a satisfactory explanation can be found in the extant chemical literature.

In the late 1950s, Herbert Fleisch[2] began reporting on the inorganic chemical inhibition, by pyrophosphoric acid (PPi) in buffered solutions, of the dissolution of hydroxyapatite, essentially the mineral portion of bone matrix. Because the loss of bone mineral appeared to be a cause of acquired bone fragility, he reasoned that this effect might be beneficial in human medicine. His inorganic studies were expanded to show PPi stabilization of bone mineral in ex vivo systems. On the basis of the lack of this effect with orally administered PPi salts and data interpreted as suggesting a short plasma half-life, he concluded that hydrolysis limited the beneficial potential in vivo and sought a nonhydrolyzable molecular analogue. He selected the bisphosphonic acids (BPs) as candidates that might mimic the action of PPi and might be, in addition, orally active. These objectives were indeed achieved, although the mechanism of this achievement proved quite different from the original expectation.

The synthetic analogues and natural PPi have similar positioning of the two phosphate groups. The structural similarity is illustrated by comparing PPi (a) to the simplest BP, methylenediphosphonate (b), which shares the bone affinity of the FDA-approved carbon-substituted amino-bisphosphonate medications (c), including among others, the compounds widely used for the treatment of postmenopausal osteoporosis: alendronate, risedronate, ibandronate, and zoledronic acid. The illustrations show representative anionic forms possibly present at physiologic pH.



Fleisch soon discerned that the inhibition of hydroxyapatite dissolution was not the cause of the bone-sparing effects of the BPs in living systems. While it is not germane to our deduction, the effect is due to the inhibition of resorption by the bone-absorbing osteoclast (see below) during restorative bone remodeling. It was elsewhere demonstrated[3] that parenterally administered PPi had this same biological effect.

The assumption that preserved or increased bone mineral density would be associated with decreased fragility fractures was borne out in a series of 36 month pivotal trials starting in the 1990s and leading to the introduction of the four mentioned BPs between 1995 and 2007. By 2003, reports began to appear of two unusual side effects of these medications, with most reports identifying association with alendronate, which was approved first. These effects are rare but have a defining character, and it is their occurrence in the same cohort of exposed patients that provides the clue to the earlier match-industry diseases. BP-associated osteonecrosis of the jaw (ONJ) and atypical transverse subtrochanteric/midshaft low-impact femur fractures (AFF) are now widely reported in the medical literature. Conservative definitions of these two problems have been provided by Task Forces convened by the American Society of Bone and Mineral Research in 2007 (ONJ) and 2010 (AFF). A recently available large study[4] of long-term outcomes of AFF patients found that the average interval of BP exposure before the AFF was approximately 9 years, well outside the reach of the large 36 month trials.

The 19th century industrial match-industry diseases, which caused so much political and public concern,[1] were *also* a necrosis of the bones of the jaw and a tendency of the midfemoral shaft of long-time workers to snap with negligible trauma.[5] The modern ONJ was recognized very quickly to be analogous to the industrial disease, which had become known as "phossy jaw". These problems were uncommon in the match industry but were concentrated in those workers most exposed to the smoke and fumes emanating from the pastes used to

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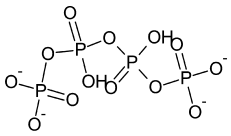
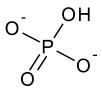
form the match heads. The inflammable component of this paste was white phosphorus, which ignites spontaneously on exposure to moist air. Frequent small and occasional large ignitions are well-documented to have been common in the factories. White phosphorus is very toxic, but ingestion is associated with major organ failure, not these exotic lesser ailments. It was recognized by the turn of the century that elemental phosphorus was not likely the source of pathogenesis. Suspicion fell[5] on the oxides that were assumed, based on the chemical knowledge of the day, to constitute the white smoke that the workers were breathing. Because P_4O_{10}, the P(V)oxide predominantly formed in the presence of adequate oxygen, apparently reacts rapidly with moisture to form phosphoric acid, it has since been argued that there is no logical connection between the industrial exposure and the BP therapy.

In 1985, the U.S. Army analyzed the smoke from white phosphorus burning in air. The smoke consists of the hydrolysis products of P_4O_{10}, the major initial component being PPi.[6] The terminal hydrolysis moiety, phosphoric acid, is not the initial product. Longer chain polyphosphoric acid polymers (d) were shown to slowly hydrolyze to PPi even as it was being hydrolyzed to the relatively innocuous phosphoric acid (e). PPi (b) was a measurable component of the smoke for 24 h.



d



e

It hitherto has not been appreciated that PPi, which becomes pyrophosphate at physiologic pH, was in the flume inhaled by the match workers. The remarkably parallel nature of the sets of complications provides mutually supportive evidence for the proximal cause of each set of cases: proven PPi bioanalogues in the modern cases and repetitively inhaled PPi itself in the legacy cases. Complexed PPi is known from radionuclide studies to be absorbed through the lungs, about 10% of the complex being in the circulation after 30 min. A similar behavior for BP tin-radionuclide complexes had been demonstrated by Fleisch. Understanding the connection lies in recognizing the exposure of the match workers to PPi. It is the only substance generated in the chain of oxidation and hydrolysis from white phosphorus to the stable end-product phosphoric acid that has been proved to have an antiresorptive impact on bone metabolism. PPi is the sole link between the otherwise markedly dissimilar groups of younger laborers in the early match industry and contemporary elderly persons at risk for fragility fractures taking BPs.

The mechanism of action of the amino-bisphosphonates listed above has been defined. They concentrate in exposed remodeling bone mineral and become resident there, are partially absorbed by osteoclasts during reparative resorption, inhibit an osteoclastic intracellular mevalonate pathway enzyme, and consequently inhibit the action of the bone-resorbing cell. The inhibition of the same enzyme, farnesyl pyrophosphate synthase (FPPS), by PPi has been demonstrated.[7] Pyrophosphate is in fact a product of the condensation of dimethylallylpyrophosphate and isopentenylpyrophosphate by FPPS in the process of forming farnesylpyrophosphate, a critical substrate in the osteoclast metabolism. Nonetheless, PPi inhibits the enzyme, albeit to a lesser degree than the BP drugs, probably due to the irreversible distortions induced in the enzyme by the nonphysiologic carbon analogues.[8] It seems probable that the abundance of PPi inhaled from the smoke and flume would at least equal the effect of the very small amounts of therapeutic BPs that reach the systemic circulation. Pyrophosphate is stable at the pH of the blood into which it would pass directly upon absorption by the pulmonary route, and it is physiologically active in vivo in a parallel manner to the BPs.[2]

No direct a priori deductive evidence has been produced that the amino-bisphosphonates are the true cause of the modern pair of side effects, despite hundreds of case reports of association. However, the remarkable similarity of the two rare and extremely uncommon sets of disease associated with the BP medications and the exposure of the match workers strongly suggest a common cause. When the analytical chemical analysis of the smoke that pervaded the match factories is examined, the most likely candidate, PPi, also proves to be the most abundant initial component. Because no evidence exists suggesting the possibility of ad hoc phosphonic acid synthesis in mammalian systems,[8] PPi remains the sole candidate, a candidate already proved to be the biological equivalent of the BPs.[3] The additional information that this moiety and the BPs both inhibit FPPS, which is thought to be the mechanism by which the amino-bisphosphonates exert their effect on bone osteoclasts, leads us to propose that this parallel double analogy offers persuasive inductive evidence both that the BPs are the true ultimate causative agents of the modern complications of ONJ and atypical femoral fractures and that the mysterious 19th century syndromes of phossy jaw and fractured femurs are attributable to inhalation of PPi.

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Author Contributions

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Notes

Views expressed in this editorial are those of the authors and not necessarily the views of the ACS.

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ABBREVIATIONS

PPi, inorganic pyrophosphoric acid/pyrophosphate; BP, bisphosphonate; ONJ, osteonecrosis of the jaw; AFF, atypical femur fracture; FPPS, farnesyl pyrophosphate synthase

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■ NOTE ADDED AFTER ASAP PUBLICATION

This paper was published on the Web on December 7, 2012, with an incorrect version of structure d. The corrected version was reposted on December 18, 2012.