Use of Novel Preparations of Heparin to Eliminate Interference in Ionized Calcium Measurements: Have All the Problems Been Solved?

A long-standing concern in rapid measurement of ionized calcium is the interference from heparin used as an anticoagulant. Although serum may be obtained and analyzed without use of anticoagulant, clotting and centrifugation add to the turnaround time and can present variable artifacts (1, 2). Very little heparin (−1 IU/mL) is required to inhibit coagulation. One molecule of heparin catalyzes the binding between many molecules of antithrombin III and thrombin, thereby preventing the conversion of fibrinogen to fibrin.

"Liquid heparin" (i.e., heparin in solution) readily mixes with blood, but use of liquid heparin in blood-collection devices is declining for a variety of reasons. Now that instruments have been developed for analyzing blood gases, electrolytes, glucose, lactate, magnesium, etc., in whole blood, the addition of liquid heparin could cause dilutional errors in these other tests. Thus, the ideal anticoagulant would be dry, free of interference in laboratory tests, inexpensive, and completely reliable as an anticoagulant. Although lyophilized heparin does not produce a dilution of blood, time for exposure to the anticoagulant is required for it to dissolve and mix completely with blood to inhibit coagulation. Small entrapped air bubbles may prevent the contact of heparin with blood and its subsequent dissolution; consequently, greater amounts of lyophilized (than of "liquid") heparin are generally used in syringes to ensure that sufficient heparin becomes dissolved.

Salts of heparin such as sodium or lithium have been used in both liquid and lyophilized form as anticoagulants for many years; however, heparin binding of calcium ions can artifactually lower the ionized calcium concentration by an amount proportional to the heparin concentration. For example, 15 IU of sodium heparin per milliliter of blood lowers the measurement of ionized calcium by −0.03 mmol/L; 25 IU/mL lowers ionized calcium by −0.05 mmol/L.

To minimize or eliminate the interference of heparin on ionized calcium results, many preparations of modified heparin have been developed. Liquid calcium-titrated heparins were produced by Radiometer in the late 1970s. This product virtually eliminated any effect on ionized calcium results at concentrations of 1.0–1.5 mmol/L but, outside that range, slightly increased lower ionized calcium results and slightly decreased higher ionized calcium results, both by −0.03 mmol/L. These changes are due to the relatively large amount of calcium heparin present, which is designed to have a normal value of ionized calcium. Although these changes were of little clinical consequence in most situations, the liquid calcium-titrated heparin had to be manually added to the blood-collection device, making the product impractical for routine use.

More recently, calcium (electrolyte)-balanced heparin has become available in syringes. An evaluation of such a product (1), Smooth E (Radiometer), showed that ionized calcium results were within ±0.02 mmol/L of results from uncoagulated whole blood with ionized calcium concentrations ranging from 0.90 to 1.60 mmol/L. However, as with the liquid product, ionized calcium values below this range were slightly increased and values above this range were slightly decreased by the presence of the heparin. Furthermore, total calcium results were now increased by an average of 0.06 mmol/L (1).

At about the same time, Marquest produced a syringe (Gas-Lyte) that contained only 2–3 IU of heparin per milliliter. The novel aspect of this product was that the heparin was prepared in a "puff" of inert filler material having two important properties: (a) the puff dissolved rapidly and presumably was dispersed throughout the sample with proper mixing, and (b) the puff could be dispensed during production to deliver an accurate and precise amount of heparin to each syringe. "Pure" crystals of lithium heparin cannot be so precisely dispensed.

Another recent heparin product that has been developed for use with ionized calcium measurements is zinc heparin. The rationale for developing this product is that (a) zinc ions, by binding to divalent cation-binding sites on heparin, prevent binding by calcium ions, (b) low and high ionized calcium concentrations are not affected, and (c) total calcium results are not affected. Although these conditions appear to be met, the presence of excess zinc ions causes positive interference in both ionized calcium determinations (3) and total magnesium measurements by widely used methods (4).

Because lithium heparin lowers ionized calcium and zinc heparin increases ionized calcium, a so-called calcium-neutralized lithium zinc (CNLZ) heparin has been developed (Sherwood Medical Co.). As shown by Landt et al. (3) in this issue, this product virtually eliminates effects on ionized calcium, total calcium, potassium, sodium, pH, PCO2, and PO2. In my laboratory, we too have evaluated a similar product from Martell Medical Co. that does not influence ionized calcium (unpublished).

Although these lithium–zinc heparin products provide a relatively high amount of heparin without the previously encountered problems, Wilhite et al. (4) report (see next month's issue) that pure zinc heparin interferes with plasma total magnesium measured by widely used instrumentation. Whether blood collected
in syringes containing both lithium and zinc heparin will be subject to significant interference with magnesium measurements remains to be determined.

Swanson et al. (5) compared six collection methods for ionized calcium (and for Na, K, and pH). The Gas-Lyte syringe containing 2.8 IU/mL heparin dispersed in an inert puff of filler showed no bias in ionized calcium compared with samples collected without heparin. The very low heparin content of these syringes should cause little or no interference in most laboratory tests, given that lithium heparin at ~15 IU/mL has been used for years in samples tested for routine chemistry analytes. One concern with this product is that, in routine use, the low amount of heparin may be insufficient to prevent coagulation in all samples. However, both our evaluation (unpublished), and that by Swanson et al. (5), as well as routine use at a large medical center for over two years (personal communication, Donald Forman, North Carolina Memorial Hospital, Chapel Hill, NC) have not indicated that clotting of samples is a problem. Nevertheless, one should keep in mind that this product contains a proprietary inert filler material that could potentially interfere with other routine laboratory tests.

At least three types of syringe products containing novel preparations of heparin are now available that practically eliminate the interference of heparin in ionized calcium measurements. Other anticoagulants under development, e.g., recombinant hirudin (6), appear to have no effect on ionized calcium measurements. Given that some of the heparin preparations appear to affect other tests—e.g., total calcium (1) and magnesium (4)—it will be important to test these new anticoagulants for their effect on routine chemistry tests that might be requested on samples collected primarily for ionized calcium and (or) blood gas measurements. Because this combination of requests is at present relatively uncommon, an interference in some analyses does not necessarily preclude the use of the anticoagulant. However, continuing efforts to minimize the amount of blood collected, especially from pediatric and elderly patients, may increase the frequency of requests for additional tests on samples originally collected for ionized calcium/blood gas measurements.

References

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