lytic bands were confirmed as MMP activity because they were completely inhibited by 5 mmol/L EDTA and 2 mmol/L 1,10-phenanthroline (1).

In accordance with previous quantitative studies, we detected for the first time on zymography that fibroblast-derived MMP-2 did not differ in serum and heparin plasma but was lower in EDTA plasma, whereas the activities of all neutrophil-derived MMP-9 isoforms were 2- to 10-fold higher in serum than in heparin and EDTA plasma (Fig. 1A). To evaluate the effect of anticoagulants on gelatinolytic activity, we added EDTA and lithium heparin to the enzyme incubation buffer at concentrations equivalent to those in the above-mentioned Monovette systems (2 g of EDTA or 30 kIU of heparin/L of sample). The addition of EDTA produced almost complete inhibition of blood gelatinolytic activities (Fig. 1B, lanes 1 and 2), whereas heparin did not affect MMP enzymatic activities (recovery, 95–105%; Fig. 1B, lanes 3 and 4). At present, there is no explanation for this effect, and the inhibitory effect of EDTA could be one reason for the decreased MMP activities in EDTA-plasma samples, although a preanalytical effect of EDTA during blood collection is more likely.

Our results confirm higher MMP concentrations in serum than in plasma, as previously quantified (5, 6), but they also demonstrate that the concentrations of all neutrophil-derived MMP isoforms (92, 130, and 225 kDa) are higher in serum than in plasma, without apparent influence on the proportion of active to latent isoforms. Discrepancies in serum samples prepared in tubes with and without clot activator were also detected: MMP-9 isoforms appeared to be twofold higher with silica-gel coagulation accelerator (Fig. 1A, lanes 3 and 4). Platelet activation or neutrophil mobilization during clotting could produce these differences (9).

The discrepancies between MMP forms in sera and plasma should also be considered to avoid preanalytical misinterpretation. We recommended that only heparin-plasma samples be used.

References

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Effect of Sulfamethoxazole on Clinical Capillary Zone Electrophoresis of Serum Proteins

To the Editor:

Capillary zone electrophoresis (CZE) using fused-silica capillaries has become a well-accepted method for the separation of serum proteins and for

Fig. 1. Effect of sulfamethoxazole on serum CZE.

Panels A and B show the CZE electropherograms of samples from two patients receiving intravenous sulfamethoxazole-trimethoprim (400 mg of sulfamethoxazole/80 mg of trimethoprim, 12 ampoules/day for 6 days). Panel C shows the CZE electropherogram of a normal sample to which sulfamethoxazole (Roche) dissolved in methanol (final concentration, 240 mg/L) was added. The arrows indicate the abnormal peak.
the detection of monoclonal components in human serum (1–4). In earlier methods, such as those that use agarose gels, quantification of the protein fractions was based on dye binding, whereas CZE uses ultraviolet detection at 214 nm for direct protein quantification via the peptide bonds. Any substance or drug that is present in serum and that absorbs at 214 nm potentially can interfere with CZE analysis. Few interfering substances have been reported. Radiocontrast media that absorb at 214 nm interfere with CZE and can simulate a monoclonal component (5–7), and the antibiotic piperacillin-tazobactam (Tazocin®; Wyeth Lederle) produces a small peak in the β-globulin fraction (8). In the present report, we describe that the sulfamide sulfamethoxazole produces a small peak at the anodal site of the albumin fraction.

Shown in panels A and B of Fig. 1 are CZE electropherograms (Beckman Coulter Paragon CZE 2000, software Ver. 1.6) of two samples obtained from patients who received intravenous sulfamethoxazole-trimethoprim (400 mg of sulfamethoxazole/80 mg of trimethoprim, 12 ampoules/day for 6 days). In the sample shown in Fig. 1B, there was a small monoclonal protein in the gamma region. In each case, a distinct peak was observed at the anodal site of the albumin fraction. No such peak is present in a typical CZE electropherogram, and none was seen in the CZE electropherograms of specimens from the same patients as in Fig. 1, A and B, collected 2 or 3 days after sulfamethoxazole-trimethoprim administration. After this time period, the antibiotic has been cleared from the blood stream. In patients with normal kidney function, the elimination half-life of sulfamethoxazole is 9 h and that of trimethoprim is 10 h. Protein binding for sulfamethoxazole is 66%, whereas for trimethoprim, it is 42–46%.

When we added sulfamethoxazole but not trimethoprim to a normal serum sample, an abnormal peak appeared in the CZE electropherogram (Fig. 1C) in the same region as the extra peak observed in the electropherograms from patients receiving the antibiotic. The addition of various concentrations of sulfamethoxazole (final concentrations of 480, 240, 120, 60, 30, 15, 7.5, and 3.75 mg/L) to a normal serum sample led to the appearance of a dose-dependent peak. The peak was largest at a sulfamethoxazole concentration of 480 mg/L, with a gradual reduction of the size of the peak with decreasing sulfamethoxazole concentrations. No interference was observed with sulfamethoxazole concentrations ≤7.5 mg/L.

We observed a peak similar to the abnormal peaks in panels A and B of Fig. 1 in 10 other patients. All of these patients had received sulfamethoxazole. The position of the peak was the same for all patients. The size of the peak, however, varied slightly. Oral administration of sulfamethoxazole-trimethoprim produced the appearance of the abnormal peak on CZE. Removal of nonprotein components by use of D-Salt™ Dextran Plastic Desalting Columns (Pierce) (7) removed the sulfamethoxazole peak.

In summary, sulfamethoxazole produces a small peak at the anodal site of the albumin fraction with CZE. Medical technologists, clinical pathologists, and clinicians should be aware of this interference, which is not seen with classic agarose gel electrophoresis of serum proteins.

References


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