Silicate Increases the Release of MMP-9 Forms in Peripheral Blood: Why Gelatin Zymography Differ Significantly in Citrate Plasma and Serum Obtained with or without Clot Activators

To the Editor:

Whether serum or plasma is the best specimen for determination of matrix metalloproteinases (MMPs) is a matter of debate, as are the influences of sample collection and processing on MMP concentrations (1–3). MMP-2 concentrations do not differ significantly in plasma and serum, whereas MMP-9 concentrations are significantly higher in serum than in plasma and serum with and without silicate were analyzed by use of Western blot (75-77F and GE-213 monoclonal antibodies against MMP-2 and -9, respectively; Calbiochem) and gelatin zymography (7.5% polyacrylamide gels containing 2 g/L gelatin 90 Bloom type A from porcine skin; Sigma) (2). U-937 cells were cultured in serum-free conditions (to avoid endogenous bovine serum gelatinases) and treated for 3 h with silicate (54 mg/L). Calibrators were prepared from capillary WB (2). The accuracy/precision of gelatinolytic activities were evaluated by zymogram densitometry with Image Pro-Plus software (Cybernetics) (4). Differences were compared using the Mann–Whitney U-test; P values <0.05 were considered statistically significant. All study participants gave informed consent, and the work was carried out in accordance with the ethics standards of the Helsinki Declaration of 1975, as revised in 1983.

Western blots identified pro-MMP-2 (Gelatinase A, EC 3.4.24.24, 72 kDa) and pro- and complexed forms of MMP-9 (Gelatinases B, EC 3.4.24.35, of 92, 130, and 225 kDa) in whole PB (Fig. 1, lanes 2 and 3). Citrate plasma results showed that pro-MMP-2 expression did not differ significantly between serum and plasma, nor did it change with silicate treatment in any of the paired sample types. MMP-9 forms were found in significantly higher amounts in serum (mean 5-fold higher; P <0.001) than in citrate samples (Fig. 1, lanes 6 vs 4 serum). However, addition of silicate to previously separated citrate plasma and serum did not noticeably change the zymographic profile of MMP-9 with respect to untreated samples (Fig. 1, lane 4 vs 5, and lanes 6 vs 7).

Addition of silicate to citrate plasma tubes before PB collection increased MMP-9 (Fig. 1, lanes 8 and 9) a mean of 4-fold (P <0.001). Addition of silicate into empty plastic tubes for serum collection before PB addition also significantly increased MMP-9 concentrations (P <0.001) (data not shown). Addition of silicate to citrate and serum tubes before PB addition resulted in similar trends of MMP increase vs silicate concentration: MMP-9 activity (μg/L) = 28.0 × silicate (mg/L) − 9.4, r² = 0.94, and MMP-9 activity (μg/L) = 30.6 × silicate (mg/L) + 91.6, r² = 0.88, respectively.

When samples were collected into empty serum tubes to which buffered silicate (silicate dissolved in PBS containing 137 mmol/L NaCl, 10 mmol/L phosphate, 2.7 mmol/L KCl, pH 7.4) was added before PB collection, all MMP-9 forms were increased, and the zymographic profile was similar to that of Sca (Fig. 1, lanes 10 and 11). Samples collected in the presence of nonbuffered silica (nonsoluble silica particles sprayed into plastic tubes or with silica-gel) (http://catalog.bdm.com/ecat/msds/d01/vs60313.pdf) showed MMP-9 release 1.5-fold higher than for buffered silicate (data not shown). Serum collected in plastic tubes with clot accelerators showed the highest MMP-9 activities (Fig. 1, lane 11). The addition of silicate to BCs isolated from citrate PB significantly enhanced MMP-9 release in buffered solution (Fig. 1, lanes 12 and 13). Similarly, silicate addition to U-937 cells cultured in serum-free media significantly increased MMP secretion (Fig. 1, lanes 14 and 15). Thus silicates increase in vitro release of MMP-9 forms from leukocytes. Our observations are consistent with the findings that during silicosis both macrophages and lymphocytes secrete enhanced amounts of MMP-9 forms (5).

To optimize the diagnostic accuracy of PB MMPs as biomarkers, we strongly recommend avoiding the use of serum samples, particularly in serum with clot activators containing silica/silicate. We believe the increased MMP-9 observed in these specimens reflects both the interfering effects of the coagulation/fibrinolysis processes (4) and the induction by silicates of MMP-9 release from leukocytes.
Grant/funding support: None declared.
Financial disclosures: None declared.

References


Fig. 1. Gelatinzymograms of MMPs in human WB, citrate plasma (Cit), serum (S), BC, and U-937 conditioned medium treated with silicate.
Lane 1, calibrators; molecular masses (kDa) are indicated. Western blots of pro-MMP-2 and pro- and complexed forms of MMP-9 (lanes 3 and 2, respectively). PB was collected into plastic tubes with no additives (S), with a silica gel-coated surface (Sca), and with buffered citrate (Cit). Sodium silicate (54 mg/L) was added into Cit (lane 9) and S (lane 10) devices before PB collection and after separation of Cit plasma (lane 5) and S serum (lane 7). BCs (lane 13) and myelomonocytic U-937 cells (lane 15) were treated with silicate (54 mg/L).

© 2007, American Chemical Society
DOI: 10.1373/clinchem.2007.090548