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Response

To the Editor: We have read the comment from Professors Laudañski and Reduta with great interest, and we would like to respond hoping that our experience will be beneficial and of help for them and others in their future studies.

Their major concern is about the levels of active MMP-9. In the beginning of our study, we measured both total (pro- and active) MMP-9 and active MMP-9 levels using Biotrak MMP-9 activity assay system (Amersham Pharmacia Biotech, Uppsala, Sweden). When samples are not activated, only the 68kDa fully active MMP-9 is measured. However, when samples are activated with p–aminophenylmercuric acetate (APMA), the 92kDa latent (pro-) MMP-9 is converted to the 68kDa active form, which is subsequently measured. Resulting activity corresponds with the levels of total (both pro- and active) MMP-9. The kit measures human, mouse, and rat MMP-9.

However, we had problems using the above-noted assay. The first problem was undesirable activation of MMP-9 in the urine by the freezing-thawing process and variable composition of urine (pH, osmolality), giving us non-reproducible results in terms of active MMP-9. In our hands, after one freezing-thawing cycle, MMP-9 was variably activated. Some values were then as high as twice values before the freezing. To get reliable results of active MMP-9, it seems to be essential to analyze only fresh samples. Our conclusion was further affirmed by discussions with the technical support of the company producing the kit.

Another drawback of the kit seemed to be the measurement of total MMP-9 levels. In certain samples, the levels of total MMP-9 were surprisingly lower as compared with those of active MMP-9. The explanation for these findings by Amersham Pharmacia Biotech, was that the already active MMP-9 (68kDa) can be "over-activated" by APMA and hence processed into too small molecular weight form that is either not immobilized by the antibody, or is no longer truly active. If the majority of the MMP-9 content of this sample is naturally endogenous active MMP-9, this will result in high endogenous active MMP-9 readings but low total MMP-9, once the active MMP-9 has been over-activated.

After this experience we decided to focus only on total MMP-9 and TIMP-1 for the protein levels since our samples from the clinical study were already frozen and for cell experiments also on mRNA levels (1).

For the measurement of total MMP-9 levels in human urine, a commercial ELISA kit (R&D systems, Abingdon, UK), which recognizes total human MMP-9, was used. We employed the kit from Amersham only for the measurement of total mouse MMP-9 levels since there was no such kit available from another company at the time of analyzing the results.

We hope that our experience will also help the commenting authors in the evaluation of their results since they were using the same kit and frozen samples in their previous study (2) giving them different results in terms of active MMP-9 as compared with total MMP-9.

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