Disparity between studies of the stability of BNP in blood: comparison of endogenous and exogenous peptide

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Measurement of plasma concentrations of the natriuretic peptides has recently been recognised as a potentially useful means of identifying patients with left ventricular systolic dysfunction (LVSD). Most studies, including our own, suggest that brain natriuretic peptide (BNP) may be superior to N-terminal atrial natriuretic peptide for diagnostic purposes. The widespread applicability of BNP would, nevertheless, be greatly diminished if the blood sample required special storage or handling. We have previously shown in a mixed population—including patients with LVSD and healthy volunteers—that endogenous BNP is stable in whole blood at room temperature for three days. However, other groups have published conflicting results, which at first sight cast doubt on our data; therefore, our results have not gained universal acceptance. We, therefore, repeated our study and confirmed our original findings. Interestingly, our study is in agreement with the only other one to examine the stability of the endogenous peptide. This led us to believe that the disparity may have arisen because other groups have looked at the stability of exogenous rather than endogenous BNP. We report the results of a study directly comparing the stability of endogenous and exogenous BNP.

**Methods**

Blood was withdrawn from a forearm vein of 10 healthy volunteers (eight men, mean age 38 years) and divided directly into three chilled polypropylene tubes containing EDTA (1 mg/ml blood) and aprotinin (50 kIU/ml blood). The first sample was stored without the addition of any exogenous BNP; 350 pg/ml of human BNP was added to the second sample; and the third sample was separated in a refrigerated centrifuge before the addition of the same concentration of exogenous BNP. Each sample was subsequently divided again into two aliquots; the first being separated, if necessary, and frozen immediately to −20°C (control), and the second stored at room temperature (22°C) for 72 hours. All samples were analysed in a single batch, in duplicate, without prior extraction of plasma using a direct, specific, monoclonal antibody kit as previously described. The aliquots frozen immediately were used to compare the data for samples stored at room temperature.

**Results**

Endogenous plasma BNP concentrations in samples separated and frozen immediately ranged from 1.0 to 35.8 pg/ml (mean 14.8), and from 1.0 to 30.0 pg/ml (mean 11.7) following 72 hours’ storage at room temperature. The corresponding concentrations in spiked whole blood were 227.0 to 356.0 pg/ml (mean 298.5) immediately and 114.4 to 232.8 pg/ml (mean 188.6) after 72 hours’ storage; and for spiked plasma 303.0 to 396.0 pg/ml (mean 349.9) immediately, and 132.1 to 244.0 pg/ml (mean 195.4) following 72 hours’ storage.

These data confirm our previous observations that only a minor decline in endogenous BNP concentration occurs over 72 hours at room temperature in whole blood: mean change in concentration −8.0% (95% confidence intervals (CI) −28.9 to 13.0; p = 0.02; paired two tailed t test). A more pronounced decline in BNP concentration was noted in spiked samples over the same time period: whole blood −38.3% (95% CI −27.7 to −48.8; p < 0.0001); plasma −44.0% (95% CI −37.0 to −51.0; p < 0.0001). The decline in exogenous BNP was significantly greater than that for endogenous BNP whether stored as whole blood (p = 0.008, two sample t test % change) or plasma (p = 0.004).

**Discussion**

The decline in endogenous BNP reported here was slightly greater than we have previously found, but fewer subjects were studied, and the low endogenous BNP concentration from healthy volunteers were near the sensitivity
limits of the assay. The much more dramatic decline in exogenous BNP over 72 hours in spiked blood and plasma samples, however, remains unexplained. Possibilities include adherence of the peptide to platelets, inherent instability of the manufactured peptide, or enzymatic degradation not inhibited by aprotinin. Interestingly, the 40% decline in BNP concentrations that we observed in spiked samples over 72 hours was still substantially less than the 90% fall over 24 hours previously reported.

Our studies continue to support the feasibility of the assay of BNP for diagnosis of LVSD in routine clinical practice.