Time Course of B-Type Natriuretic Peptide (BNP) and N-Terminal ProBNP Changes in Patients with Decompensated Heart Failure

To the Editor:
The short-term infusion of levosimendan (Simdax™), a calcium sensitizer, improves the hemodynamic function in patients with decompensated heart failure (1–3). Blood concentrations of B-type natriuretic peptide (BNP) and the amino-terminal fragment of its precursor hormone (NT-proBNP) have been reported to reflect the severity of heart failure (4), and BNP concentrations have been shown to decrease with improving hemodynamic function during tailored intravenous treatment of decompensated heart failure (5). Because of the shorter biological half-life of BNP compared with NT-proBNP (6), we hypothesized that BNP would show a faster response to hemodynamic improvement during intravenous levosimendan therapy. This could be of relevance considering the possible role of natriuretic peptides for guiding therapy in acutely decompensated heart failure.

The present observational study, carried out prospectively at the Division of Internal Medicine, St. John of God Hospital (Linz, Austria), was approved by the local ethics committee in accordance to the Helsinki Declaration. Eligible patients were those admitted with acute decompensation of chronic heart failure who were judged to require hemodynamic monitoring and intravenous treatment. Inclusion criteria were defined as follows, based on a previous report (1): documented left ventricular ejection fraction ≤30% by echocardiogram and a pulmonary artery catheter placed for clinical purposes that demonstrated a pulmonary capillary wedge pressure (PCWP) ≥15 mmHg along with a cardiac index (CI) ≥2.5 L·min⁻¹·m⁻². Exclusion criteria were angina-limited exercise; unstable angina or acute myocardial infarction with urgent need for invasive procedure; obstructive myocardial; uncorrected primary stenotic valve; history of ventricular flutter, fibrillation, or symptomatonic ventricular tachycardia; symptomatonic primary pulmonary disease; supine systolic blood pressure <85 or >200 mmHg; resting heart rate >120 beats/min; serum creatinine >25 mg/L; liver transaminases >2 times the upper limit of the reference interval; and uncorrected hypo- or hyperkalemia (serum potassium <3.5 or >5.5 mmol/L). Between January and July 2003, we recruited 11 consecutive patients after obtaining informed written consent.

In all study participants, levosimendan (Simdax) was initiated with an intravenous bolus of 24 µg/kg of body weight (delivered over 10 min) followed by a continuous infusion of 0.1 µg·(kg body weight)⁻¹·min⁻¹ for the next 50 min. Thereafter, the continuous infusion rate was increased in all patients to 0.2 µg·(kg body weight)⁻¹·min⁻¹ for the next 23 h. None of the following dose-limiting events occurred: symptomatic hypotension with a systolic blood pressure <75 mmHg; tachycardia with a heart rate >140 beats/min for at least 10 min or increased by >25 beats/min; need for rescue therapy with intravenous positive inotropic or vasodilator drugs; or any adverse event that, in the opinion of the investigators, required dose modification. During infusion, the dose of concomitant oral medications (i.e., angiotensin-converting enzyme inhibitors, beta-blockers, digitals glycosides, amiodarone) was held constant, but diuretics were stopped.

Hemodynamic measurements were made by means of a pulmonary artery catheter and included PCWP, pulmonary artery pressure, right atrial pressure, and cardiac output (thermodilution). Heart rate was determined from the electrocardiogram, and blood pressure was determined by intraarterial monitoring. CI, stroke volume, systemic vascular resistance, and pulmonary vascular resistance were calculated by standard equations. Hemodynamic measurements were recorded at baseline (before start of levosimendan administration), at 10 min (at the end of initial bolus), at 60 min (at the end of first infusion period), at 120 min (2 h after initiation of infusion), at 6 h (6 h after initiation of infusion), and at 24 h (the end of levosimendan infusion). Blood samples were drawn at those same time points for determination of BNP and NT-proBNP. Plasma BNP was assayed on an ADVIA Centaur analyzer (Bayer Diagnostics), and serum NT-proBNP was assayed on an Elecsys 2010 analyzer (Roche Diagnostics), both according to the manufacturers’ recommendations.

Statistical calculations were performed with the MedCalc (Ver. 7.2.0.2) software. The Kolmogorov–Smirnov test was used to assess gaussian distribution of all relevant data. Time-dependent changes in hemodynamics and the BNP and NT-proBNP values were evaluated by paired t-tests. Values are presented as the mean (SD). All probabilities were two-tailed, and P values <0.05 (not corrected for multiple comparisons) were regarded as statistically significant.

The mean age of the study population (10 males and 1 female) was 62 (16) years. All patients had New York Heart Association class III (n = 3) to IV (n = 8) heart failure of either ischemic (n = 7) or nonischemic origin (n = 4) with a CI ≤2.2 L·min⁻¹·m⁻² and a PCWP >15 mmHg. Mean left ventricular ejection fraction as determined by echocardiography was 20 (4)%. Relevant hemodynamic measurements (i.e., CI and PCWP) and BNP and NT-proBNP values at baseline as well as absolute/relative changes to baseline during levosimendan administration are shown in Table 1. Main finding was a different response of BNP and NT-proBNP to a standardized levosimendan therapy in decompensated heart failure. When comparing relative changes with baseline measurements, BNP concentrations significantly decreased within 60 min, whereas NT-proBNP showed a significant decrease not earlier than 24 h after initiation of levosimendan infusion. In contrast, hemodynamic indices (PCWP and CI) changed immediately after initiation of levosimendan administration.

The faster response of BNP to therapy compared with NT-proBNP may be attributable to their different biological half-lives. Although there...
Currently there are data only on the half-life of NT-proBNP in sheep (7), the half-life of human NT-proBNP is considered to be 60–120 min (probably depending on renal function) (6), suggesting that meaningful changes in hemodynamics could be reflected by this test theoretically every 12 h, which is in good accordance with our observations. Thus, NT-proBNP concentrations lag behind the clinical picture, given its longer time for clearance. The half-life time of human NT-proBNP is considered to be 60 min (proba-

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### Table 1. Hemodynamic measurements and BNP and NT-proBNP values at baseline and absolute/relative changes relative to baseline during levosimendan administration.

<table>
<thead>
<tr>
<th>Hemodynamic indices</th>
<th>Baseline</th>
<th>10 min</th>
<th>60 min</th>
<th>120 min</th>
<th>6 h</th>
<th>24 h</th>
</tr>
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<tbody>
<tr>
<td>mmHg</td>
<td>31(9)</td>
<td>27(7)</td>
<td>24(6)</td>
<td>23(7)</td>
<td>24(7)</td>
<td>19(6)</td>
</tr>
<tr>
<td>%</td>
<td>100(0)</td>
<td>90(19)</td>
<td>78(15)</td>
<td>75(19)</td>
<td>79(21)</td>
<td>62(17)</td>
</tr>
<tr>
<td>L · min⁻¹ · m⁻²</td>
<td>1.8(0.3)</td>
<td>2.5(0.5)</td>
<td>2.7(0.5)</td>
<td>2.6(0.7)</td>
<td>2.8(0.5)</td>
<td>3.0(0.7)</td>
</tr>
<tr>
<td>%</td>
<td>100(0)</td>
<td>139(26)</td>
<td>151(28)</td>
<td>145(31)</td>
<td>158(31)</td>
<td>169(29)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biochemical markers</th>
<th>ng/L</th>
<th>%</th>
<th>ng/L</th>
<th>%</th>
</tr>
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<tbody>
<tr>
<td>BNP</td>
<td>1099(696)</td>
<td>100(0)</td>
<td>1003(622)</td>
<td>93(10)</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td>4486(4553)</td>
<td>100(0)</td>
<td>4572(4689)</td>
<td>102(11)</td>
</tr>
</tbody>
</table>

**a-c** Difference from baseline values: *a* P < 0.01; *b* P < 0.001; *c* P < 0.05.
Incidental Clostebol Contamination in Athletes after Sexual Intercourse

To the Editor:

Clostebol is a synthetic androgenic steroid with anabolic effects that is frequently used in sports to increase physical performance. Because of medical and ethical reasons, the use of clostebol is prohibited by the International Olympic Committee (IOC) (1), and its misuse would fall under the strict liability rule of the IOC and the World Antidoping Agency. It is therefore the responsibility of athletes to submit evidence contrary to any ruling issued against them by the appropriate sports body. Despite the prohibition against the use of clostebol, abuse of this steroid is increasing, mainly in Brazilian athletes. In Brazil, clostebol acetate is present in medicines for dermatologic and gynecologic treatments, whereas in the US, the Food and Drug Administration does not approve of the use of medicines that contain anabolic agents.

Our laboratory, LABDOP, is accredited by the IOC and in the past 3 years has encountered four urine samples that contained clostebol metabolites. One male athlete whose urine tested positive for traces of clostebol metabolites claimed that he was contaminated as a result of sexual intercourse with a woman taking a medication containing clostebol. The IOC did not exonerate him from the results reported by LABDOP. The remaining athletes maintained that the presence of clostebol metabolites in their urine was the result of using clostebol-containing medications. Despite this controversy, the directive from the IOC has been followed, and positive results are always enforced. A previous publication by Debruyckere et al. (2) showed the presence of clostebol metabolites in human urine after oral intake of contaminated meat, but did not mention sexual intercourse.

LABDOP undertook the present study to determine whether the urine of men exposed to intravaginal clostebol acetate during sexual intercourse contains clostebol metabolites. A gas chromatographic–mass spectrometric method (3, 4) was used to test for the presence of two metabolites of clostebol, clostebol-M1 (4-chloroandrost-4-en-3α-ol-17-one) and clostebol-M2 (4-chloroandrost-3α-ol-17-one), and other steroids in urine samples. The procedure involves preextraction with XAD-2 resin, elution with tert-butyl methyl ether, hydrolysis with β-glucuronidase from Escherichia coli, extraction with n-pentane, and derivatization at 60 °C for 60 min with a solution containing 1 mL of N-methyl-N-(trimethylsilyl)trifluoroacetamide, 2 μg of NH₄I, and 6 μL of 2-mercaptoethanol (3, 4). The analytes were monitored in selected-ion monitoring mode.

In Brazil, clostebol acetate is available for intravaginal administration. One such preparation (Trofodermin™, Searle) contains 200 mg of clostebol acetate and 200 mg of neomycin sulfate per 40-g blister. The package insert states that Trofodermin is indicated for cervicitis, postoperative vaginitis, and ulcerative vaginitis, and the recommended dose is 5 g once or twice a day. Two healthy couples (group I) and two healthy men (group II) were involved in the study. A baseline urine was obtained from all volunteers before exposure to clostebol acetate. Participants were healthy and without a history of drug use or gynecologic disease. The study was approved by the University ethics committee (protocol 168/02). Immediately after intravaginal application of 5 g of clostebol acetate, group I had sexual intercourse lasting ~20 min (experiment I). In experiment II, the men in group II applied 200 mg of clostebol acetate topically to their penis for 20 min. Urine samples were collected from all participants volunteers for the following 2 days.

The urine of the men in experiment I contained trace amounts of clostebol-M1 (0.9–3.5 μg/L) with a t_{max} of 16 h. The concentration of clostebol-M1 in the urine of the females reached a maximum of 35 μg/L after 23 h. Small amounts of clostebol-M2 were also detected. The urine of the men in experiment II contained higher amounts of clostebol-M1, with a peak concentration of 22 μg/L after 3.5 h, and was detectable for 15 h. The baseline urines contained no clostebol, clostebol-M1, or clostebol-M2. The possibility of incidental contamination from sexual intercourse was confirmed, despite the fact that the amount of clostebol-M1 (long-term metabolite) was near the limit of detection (μg/L). Because the IOC does not make a distinction among circumstances or means of administration of anabolic compounds, athletes should be warned not to use clostebol-containing medications and to be aware of their partner’s medical treatments.

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