Effects of Bemiparin and Heparin on blood pressure, renal and liver function tests and platelet indices of salt-loaded uninephrectomized rats

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Abstract

Low-molecular-weight Heparins (LMWHs) are being preferred to unfractionated Heparin (UFH) because of their superior convenience and comparable or slightly better toxicity profile. This study was designed to investigate and compare the effects of LMWH (Bemiparin) and Heparin on hemodynamic parameters, liver and renal function tests and platelet indices of salt-loaded uninephrectomized hypertensive rats. The experimental rats divided into two groups. The first group included 18 hypertensive rats. Hypertension induced by unilateral nephrectomy and high NaCl loading with 4% NaCl in diet for 4 weeks. The rat models were subdivided into three groups, each subgroup consists of six rats. The first subgroup served as a positive control. The second subgroup received a daily intraperitoneal (I.P) injection (250 unit/kg) of Bemiparin for thirty days. The third subgroup received daily I.P injection (250 unit/kg) of Heparin for thirty days. The second group included six rats underwent sham operated surgery and served as a control group. Blood pressure was recorded in conscious rats by the tail-cuff plethmography method. At the end of the experiments, blood samples were collected from the rats for determination of serum alanine aminotransferase (ALT), alkaline phosphatase (ALP) concentrations and platelet indices. Compared to sham control underwent sham operated surgery and served as a control group. Blood pressure was recorded in conscious rats by the tail-cuff plethmography method. At the end of the experiments, blood samples were collected from the rats for determination of serum alanine aminotransferase (ALT), alkaline phosphatase (ALP) concentrations and platelet indices. The results suggest that Bemiparin has more beneficial effects than Heparin in improving blood pressure and renal functions by affecting serum levels of sodium, creatinine and urea. Unlike Heparin, Bemiparin did not lead to hyperkalemia in hypertensive rats.

Keywords: Uninephrectomized hypertensive rats, Bemiparin, Heparin, Blood pressure, Liver and Renal function tests.

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Introduction

Heparin, a commonly used anticoagulant agent, is frequently used for the prophylaxes and treatment of deep venous thromboembolism. As with most medications, Heparin has a significant side effect profile. Two of its most important side effects, major bleeding and hyperkalemia, may be devastating without immediate diagnosis and treatment (1,2).

Treatment with Heparin has beneficial effects in diabetic nephropathy (3). Heparin also has inhibitory effects on smooth muscle cell proliferation and may be useful for long term treatment of patients with atherosclerosis with the aim of regression of atherosclerotic lesions (4). Reantragoon et al (5) demonstrated that Heparin suppresses thrombin stimulated endothelin-1 production in endothelial cells which is consistent with its reported effects of lowering blood pressure.

LMWHs are being preferred to unfractionated UFH because of their superior convenience and comparable or slightly better toxicity profile. Bemiparin sodium is a new second-generation LMWH. Bemiparin has the lowest mean molecular weight (3600 Da), the longest half-life (5.3 h) and the largest antifactor Xa: antifactor IIa ratio (8:1) of all LMWHs (6). The distinctive characteristics of LMWH have resulted in decreased rates of bleeding and equivalent rates of thrombocytopenia compared with UFH (7). LMWH has evident advantages over conventional Heparin with chronic renal failure and concomitant arterial hypertension (8). LMWH showed favorable outcome in come in bleeding time, whole blood clotting time prothrombin time, platelet count, fibrinogen, blood urea and serum creatinin (9). Furthermore Deepa and Varalakshmi found that decreased concentration of serum albumin and increased serum urea, uric acid and creatinin concentration were normalized by LMWH treatment (10). Animal studies have shown that both Heparin and LMWH decrease bone necrosis (11). Moreover, Olayinka, (12) concluded that LMWH can lead to hyperkalemia. However, Korea-Michowitz et al (13) explained that the effect of LMWH on serum potassium may be aldosteron independent.

Abdel Salam et al. (14) demonstrated that total serum bilirubin was increased in rats treated with conventional Heparin; while alkaline phosphates was higher after LMWH treatment. Their results suggested a beneficial effect for nadroparin and enoxaparin in the treatment of patients with obstructive jaundice or cholestatic liver disorders (14). Few studies have yet been conducted to test the effects of UFH and LMWH on blood pressure, electrolytes, and liver and renal function tests in nephrotoxicized salt loaded rats. The aim of this study was to investigate the beneficial and adverse effects of LMWH (Bemiparin) and Heparin in experimentally induced hypertensive rats.

Materials and methods

Animals

Male albino rats (Rattus norvegicus) weighing 300-375 grams were used in the present study. During the experimental period, six animals were kept in each cage. The room temperature was maintained at 25 °C. A 12 hr light/dark cycle was set (15). Rodent food rich in nutrient and tap water were supplied.
Experimental design
The experimental rats were divided into two groups. The first group included 18 rats which were fed a high-salt diet (4% NaCl), for 4 weeks and underwent uninephrectomy (16). The animals were allowed to survive after each operation and were monitored in the postoperative period with daily weights and close observations of their behavior. The rat models were subdivided into three groups, each subgroup having six rats. The first subgroup served as a positive control. The second subgroup received a daily I.P injection (250 mg/kg) of Bemiparin for thirty days. The third subgroup received daily I.P injection (200 mg/kg) of UFH for thirty days. The second group included six rats underwent sham operated surgery and served as a control group.

Blood pressure and heart rate measurements
Systolic blood pressure (SBP) and heart rate were measured by the tail-cuff plethymography method in unanaesthetized rats prewarmed for 20 minutes at 37 °C in a thermostatically control heating cabinet. The tail pressure pulsations were detected with a pneumatic pulse transducer (ADInstruments, power lab 2/25).

Animals were adapted to the blood pressure measurement procedure three times before the first pressure recording was made. Pressure and heart rate values were obtained by averaging 3-4 individual readings.

Chemical analysis
At the end of the experiment, rats were sacrificed, blood samples were collected and sera were separated from whole blood samples immediately by centrifugation. Serum AST, ALT, ALP, creatinine, urea, total bilirubin and calcium were estimated spectrophotometrically by their standard biochemical kits (BIOLABO, France). Serum sodium and potassium were determined by flame photometry (JENWAY, Model 8515 PFP 7).

Statistical analysis
All data are expressed as mean± standard error (M±SE) and statistical analysis was carried out using statistically available software (SPSS Version 11.5). Data analysis was made using one-way analysis of variance (ANOVA). The comparison among groups done using Duncan test. P<0.05 was considered as statistical significance.

Results
Statistical analysis revealed that, SBP was significantly increased (P<0.01) in salt-loaded uninephrectomized rats (132.16 ± 2.414 mm Hg) compared to the control group (110.86 ± 2.963 mm Hg). Administration of Heparin (250 units/kg/day) and Bemiparin (250 units/kg/day) for thirty days to hypertensive rats resulted in significantly decreasing blood pressure in both Bemiparin-treated hypertensive rats (110.63 ± 2.777 mm Hg) and Heparin treated hypertensive rats (116.61 ± 3.44 mm Hg) (Table 1). Compared to the control group (297.6 ± 7.05 BPM), the heart rate was also significantly higher in salt-loaded nephrectomized rats (349.08 ± 15.55 BPM). Both Bemiparin and Heparin administration were unable to significantly change heart rate of salt-loaded nephrectomized rats (Table 1).

Table 1: Effects of Bemiparin and Heparin on systolic blood pressure and heart rate in salt loaded nephrectomized rats (n=24).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Systolic BP (mm Hg)</th>
<th>Heart rate (beat/minute.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>110.86±2.9630a</td>
<td>297.6±7.0506a</td>
</tr>
<tr>
<td>Salt loaded uninephrectomized rats</td>
<td>132.16±2.4141b</td>
<td>349.08±15.5551b</td>
</tr>
<tr>
<td>Salt loaded nephrectomized rats + Bemiparin</td>
<td>110.63±2.7774a</td>
<td>316.2±16.9557b</td>
</tr>
<tr>
<td>Salt loaded nephrectomized rats + Heparin</td>
<td>116.61±3.4433a</td>
<td>314.8±9.44966b</td>
</tr>
</tbody>
</table>

Different letters indicate significant difference at P<0.05.

Serum calcium tended to increase in salt-loaded nephrectomized rats, Bemiparin and Heparin failed to attenuate the rise in serum calcium (Table 2). Serum sodium levels of salt-loaded nephrectomized rats were markedly increased. Administration of a daily I.P injection of (250 mg/kg) of Bemiparin significantly (P<0.05) lowered serum sodium. Heparin significantly increased the serum potassium (5.580 ± 0.1356 meq/l) level of salt loaded nephrectomized rats. Compared to the positive control group (4.520 ± 0.2332 meq/l) Bemiparin did not significantly change serum potassium (4.780 ± 0.1530 meq/l) concentration.

Neither salt-loaded nephrectomized rats nor Bemiparin and Heparin significantly changed serum AST and ALT, while serum alkaline phosphatase tended to increase in salt-loaded nephrectomized rats from 35.175± 3.7929 in the control group to 66.00± 4.3302 in the salt-loaded nephrectomized rats. Bemiparin treatment of salt-loaded nephrectomized rats did not alter serum alkaline phosphatase, while daily I.P injection of Heparin (200 mg/kg) for thirty days caused a small insignificantly
reduction in its serum level, whereas, no statistical differences were detected in serum total bilirubin in all the treated groups (Table 3).

Our results demonstrate that both blood urea and serum creatinine were reduced significantly (P<0.05) by Bemiparin and Heparin administration (Table 3).

Unilateral nephrectomized and NaCl treated rats did not show significant change in platelet count, mean platelet volume (MPV) and plateletcrit (PCT) relative to control group. Moreover, Bemiparin and Heparin treated salt-loaded nephrectomized rats did not change platelet indices as seen in (Table 4).

Table 2: Effects of Bemiparin and Heparin on serum calcium sodium and potassium in salt loaded nephrectomized rats (n=24).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serum calcium (mg/dl)</th>
<th>Serum Na⁺ (meq/l)</th>
<th>Serum K⁺ (meq/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.1500±0.1284⁺</td>
<td>149.4±2.4113⁺</td>
<td>5.120 ± 0.243⁹</td>
</tr>
<tr>
<td>Salt loaded uninephrectomized rats</td>
<td>8.7000±0.1140⁹</td>
<td>165.0±0.9486⁹</td>
<td>4.520 ± 0.233²</td>
</tr>
<tr>
<td>Salt loaded nephrectomized rats + Bemiparin</td>
<td>8.6250±0.0487⁹</td>
<td>155.8±2.9223⁹</td>
<td>4.780 ± 0.150⁰</td>
</tr>
<tr>
<td>Salt loaded nephrectomized rats + Heparin</td>
<td>8.5400±0.1600⁹</td>
<td>159.6±3.3407⁹</td>
<td>5.580 ± 0.135⁶</td>
</tr>
</tbody>
</table>

Different letters indicate significant difference at P<0.05.

Table 3: Effects of Bemiparin and Heparin on liver and kidney function tests in salt loaded – nephrectomized rats (n=24).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ALT (U./ml)</th>
<th>AST (U./ml)</th>
<th>ALP (U./L)</th>
<th>Blood urea mg/dl</th>
<th>Serum creatinine mg/dl</th>
<th>Serum total bilirubin mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16.1501±</td>
<td>20.125±</td>
<td>35.175±</td>
<td>25.805±</td>
<td>0.3000±</td>
<td>0.1250±</td>
</tr>
<tr>
<td></td>
<td>0.2224ᵃ</td>
<td>0.2416ᵃ</td>
<td>3.792ᵃ</td>
<td>0.3489ᵇ</td>
<td>0.0836ᵃ</td>
<td>0.0193ᵇ</td>
</tr>
<tr>
<td>Salt loaded uninephrectomized rats</td>
<td>16.6001±</td>
<td>20.300±</td>
<td>66.00±</td>
<td>25.300±</td>
<td>2.0400±</td>
<td>0.1200±</td>
</tr>
<tr>
<td></td>
<td>0.8074ᵃ</td>
<td>1.1410ᵃ</td>
<td>4.330ᶜ</td>
<td>1.1887ᵇ</td>
<td>0.7187ᵇ</td>
<td>0.0200ᵃ</td>
</tr>
<tr>
<td>Salt loaded nephrectomized rats + Bemiparin</td>
<td>16.750±</td>
<td>20.325±</td>
<td>58.200ᶜ</td>
<td>15.400±</td>
<td>0.5000ᶜ</td>
<td>0.1250ᵃ</td>
</tr>
<tr>
<td></td>
<td>0.3041ᵃ</td>
<td>0.1391ᵃ</td>
<td>3.8131ᵇ</td>
<td>1.3293ᵃ</td>
<td>0.0316ᵃ</td>
<td>0.0194ᵃ</td>
</tr>
<tr>
<td>Salt loaded nephrectomized rats + Heparin</td>
<td>16.100±</td>
<td>20.160±</td>
<td>54.040ᵇ</td>
<td>14.300±</td>
<td>0.7800ᵇ</td>
<td>0.1200ᵃ</td>
</tr>
<tr>
<td></td>
<td>0.6753ᵃ</td>
<td>1.1655ᵃ</td>
<td>1.1289ᵇ</td>
<td>0.4147ᵃ</td>
<td>0.2709ᵃ</td>
<td>0.0200ᵃ</td>
</tr>
</tbody>
</table>

Different letters indicate significant difference at P<0.05, MPV = Mean platelet volume, PCT = Plateletcrit.

Discussion

The results of the present study showed that unilateral nephrectomy along with salt diet increased SBP. This elevation in blood pressure may be result from peripheral vasoconstriction and a decrease in renal blood flow and GFR (17). Several studies found that the mechanisms by which salt increases BP may result from an alteration in renin-angiotensin and nitric oxide (NO) levels, increased oxidative stress and damage to kidneys (18,19). Whereas, Vasdev et al. (20), suggested that insulin resistance may be a major mechanism by which high salt intake increases blood pressure. High salt intake increases insulin resistance, a condition strongly associated with hypertension due to excess production of endogenous aldehyde.

Bohlerder et al. (21) and Kobori et al. (22) concluded that renovascular and salt loaded hypertension may be due to increased Ag II rather than rennin. A previous study demonstrated that high salt intake along with unilateral nephrectomy (UNx) produced accelerated hypertension within 3-4 weeks in dogs (23). Experimental studies confirmed that renal damage greatly enhanced by unilaterial nephrectomy and high salt diet (24). Hypertension that was detected in salt-loaded nephrectomized rats was reduced and returned close to the

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control values by both Bemiparin and Heparin administration. Reantragoon et al. (5) demonstrated that Heparin suppresses thrombin-stimulated endothelin-1 production in endothelial cells which is consistent with its reported effect of lowering blood pressure. On the other hand, the available evidence showed that LMWH has advantages over conventional Heparin in the conservative treatment of patient with glomerulonephrits, with chronic renal failure and concomitant arterial hypertension (6).

The mechanism by which Bemiparin reduces blood pressure is not well understood. One possible hypothesis is that Heparin could reduce blood pressure through rennin-angiotensin-aldosteron system rather than the involvement of sympathetic nervous system which can increase heart rate elevation. Both Bemiparin and Heparin administration did not significantly change heart rate.

Results of the current study revealed that serum sodium and potassium were increased in uninephrectomized rats that received 4% NaCl. Johnson et al. (25) demonstrated interstitial accumulation of Angiotensin II positive cells as the reason for primary sodium retention in patients with nephrotic syndrome.

It is well known that renal effects of Angiotensin II include a decreased glomerular filtration rate which will reduce the filtered sodium load, an increase in tubular sodium reabsorption and impairment of pressure natriuresis (26). In addition, decreased NO level in uninephrectomized-salt loaded rats resulted in decrease in urinary excretion of sodium, potassium and water which in turn increase serum levels of sodium and potassium (27). Moreover, Schiffrin and St-Louis, (28) found that atrial natriuretic peptide receptor are down regulated after sodium loading in uninephrectomized rats.

Administration of Bemiparin significantly lowered serum sodium and returned close to the control values. However, unfactionated Heparin could not reduce serum sodium significantly. Hyperkalemia that was induced by Heparin was not observed in Bemiparin group. This result may be due to that Heparin induces hypoaldosteronism leading to hyperkalemia (1). Interestingly, for the first time; this result showed that Bemiparin unlike other LMWH did not cause hyperkalemia. Olaynka, concluded that LMWH like UFH can lead to hyperkalemia (12). Moreover, short-term treatment with LMWH (nadoparin) induces a significant increase in serum potassium level but the related incidence of relevant hyperkalemia is low (29).

Serum calcium tended to increase in salt-loaded nephrectomized rats, this is in agreement with the finding of Beevers et al. who reported that contraction of smooth muscle cells is related to a rise in intracellular Ca²⁺ concentration (30). Tierney et al. elucidated that an increase in intracellular Na⁺ may lead to increased intracellular Ca²⁺ concentrations as a result of facilitated exchange and might explain the increase in vascular smooth muscle cells (VSMCs) tone that is characteristic of established hypertension (31). Eiam-Ong et al. observed that hypercalcemia could influence BP by direct action on the VSMCs, or by inducing increments in blood levels of various vasopressive substances (32).

Neither salt-loaded nephrectomized rats nor Bemiparin and Heparin significantly changed serum AST and ALT, while serum alkaline phosphatase tended to increase in salt-loaded nephrectomized rats. The reason behind this elevation may be due to the necrotic and oxidative action of liver tissues which cause leakage of these enzymes from hepatocytes as a result of hepatocytes membrane damage (33). The results presented that both blood urea and serum creatinine were reduced significantly by daily intraperitoneal injection of both Bemiparin or Heparin. Reports related to the effects of LMWH on blood urea and serum creatinine are very limited. However, there is evidence that decreased concentration of serum albumin, urea, uric acid and creatinine were normalized by LMWH treatment, Bemiparin and Heparin treated salt-loaded nephrectomized rats did not change platelet indices relative to control group. This finding is consistent with the observation of Paul et al. (9) who found a favorable outcome in bleeding time, whole blood clotting time, prothrombin time and platelet count.

**Conclusions**

The results suggest that Bemiparin has more beneficial effects than Heparin in improving blood pressure and renal functions by affecting serum levels of sodium, creatinine and urea. Unlike Heparin, Bemiparin did not lead to hyperkalemia in nephrectomized salt loaded hypertensive rats.

**References**


12. Olayinka, A.O: Low molecular weight Heparins can lead to hyperkalemia, the interner Journal of Geriatrics and Grontology, 2005, volum 2, number 2


16. Rothermund, L; Susanne, L; Peter, K; Martin, and Reinhold, K: Renal endothelia ETALETB receptor imbalance differentiates spontaneous hypertension. Hypertension, 200137: 275-290


