Heparin Lowers the Blood Pressure in Hypertensive Rats

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SUMMARY This study describes the effect of heparin on blood pressure, cardiac output, and total peripheral resistance in spontaneously hypertensive and one-kidney, one-clip Goldblatt hypertensive rats. Administration of heparin (200 units/day/rat) for 8 weeks to young (6-week-old) spontaneously hypertensive rats (SHR) resulted in an attenuated rise in blood pressure; mean blood pressure in heparin-treated SHR (180 ± mm Hg) was significantly lower (p < 0.05) than that in control SHR (205 ± 7 mm Hg). Similar heparin treatment started immediately after the induction of one-kidney, one-clip (Goldblatt) hypertension reduced the rise in blood pressure. After 4 weeks of treatment, heparin-treated Goldblatt hypertensive rats had much lower blood pressure (150 ± 4 mm Hg) than did control rats (178 ± 8 mm Hg). The difference was highly significant (p < 0.01). Similarly, heparin treatment also lowered the blood pressure in rats with developed Goldblatt hypertension. After the cessation of heparin treatment, the blood pressure returned to pretreatment level in these rats. When compared to vehicle-treated rats, heparin-treated animals with either spontaneous or Goldblatt hypertension concomitantly exhibited a significant increase in cardiac output, and significant decreases in total peripheral resistance and packed cell volume. Further, the left ventricular weight to body weight ratio was significantly lower (p < 0.05) in heparin-treated than control animals. Since a relationship seems to exist between an increase in packed cell volume and blood viscosity and the rise in arterial pressure, this blood-pressure-lowering effect of heparin may be attributed to a decrease in packed cell volume.

KEY WORDS  • spontaneous hypertension • one-kidney, one-clip hypertension • cardiac output • peripheral resistance • packed cell volume (hematocrit)

ALTHOUGH heparin has been known for a long time, reports on its cardiovascular effects are scarce. Mandal et al.¹ have reported that prolonged heparin treatment normalizes the blood pressure in young and old spontaneously hypertensive rats (SHR), but no information with regard to cardiovascular hemodynamics was available from this work. The study of Wilson et al.,² despite some differences, has essentially confirmed these results, since they found that chronic administration of heparin attenuates the rate of rise in systolic blood pressure and prevents severe fibrinoid vascular lesions in stroke-prone SHR. Heparin also has been found to inhibit rat arterial smooth muscle cell proliferation in vivo and in vitro.³,⁴

Materials and Methods

Two different models of hypertension in rats were studied: SHR and one-kidney, one-clip (Goldblatt) hypertensive rats. All rats were given tap water ad libitum and standard pellet rat food (Veterinarski Zavod, Zemun) throughout the study.

Spontaneous Hypertension

Eighteen 6-week-old male rats with spontaneous hypertension were used. Animals were bred (brother to sister mating) at our institution; rats used in this study are the second generation descending from the breeders originally obtained from Taconic Farms, Inc., Germantown, New York. Baseline body weights were recorded, and rats were randomly divided into two

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groups. For the following 8 weeks, rats from the first group (10 rats) were treated with heparin (Galenika) (200 units of heparin dissolved in 0.2 ml of saline per rat, once a day, s.c.), while control animals from the second group (8 rats) received vehicle (0.2 ml of 0.9% NaCl solution).

After 8 weeks of the respective treatments, hemodynamic variables were studied in eight rats from each group. Cannulation was unsuccessful in two heparin-treated SHR. Animals were anesthetized with pentobarbital (35 mg/kg), and a tracheal cannula was inserted.

Blood pressure was measured directly through a femoral artery cannula (PE-50, Clay Adams), using a pressure transducer (P23Db, Statham) and a direct writing recorder (Physiograph Four, Narco Bio Systems, Inc.). Mean pressure was obtained by electronic integration. Cardiac output was determined using a previously described modification of Coleman's application of the dye dilution technique. Indocyanine green (Hynson, Westcott and Dunning) was used as an indicator, and a recording densitometer (Beckman) was used for detection of dye in the blood and registration of dilution curves. Total peripheral resistance was calculated from the mean arterial pressure (assuming that mean right atrial pressure is 0) and cardiac output.

Packed cell volume (hematocrit) was determined from samples of arterial blood. At the end of the experiment, rats were exsanguinated and their hearts removed. The atria were excised, the right ventricular septum, and their weights were immediately determined on an automatic analytic balance (Mettler).

One-kidney, One Clip Hypertension

Male Wistar rats, 3 to 3.5 months old and weighing about 300 g, were used. Two separate experiments were performed. In the first experiment, rats were randomly divided into two groups with 21 animals in each group. An initial measurement of systolic blood pressure (tail-cuff) was made in all rats. Rats were anesthetized with pentobarbital (35 mg/kg), right nephrectomy was performed, and a silver clip (0.2 mm in diameter) was placed on the left renal artery as described by Brooks et al. For the 4 weeks following surgery, rats in the first group were treated with heparin while animals from the second (control) group received vehicle similar to that given SHR. Systolic blood pressures (tail-cuff) were recorded once a week. Seven rats from the control group and five rats from the heparin-treated group died during the experiment. After 4 weeks of treatment, cardiac hemodynamic studies were done in 11 animals from each group. Cannulation was unsuccessful in the remaining animals. A relatively high percentage of unsuccessful cannulations resulted because the cannulas needed for cardiac output determination were too large for the carotid artery and jugular vein.

In the second experiment, one-kidney, one clip hypertension was induced in 30 rats, as already described. Five weeks after the surgery 15 rats with systolic blood pressure (tail-cuff) ranging between 160 and 180 mm Hg were selected for the study, while the remaining rats were excluded. These 15 rats were randomly divided into two groups; rats in the first group (eight animals) received heparin (200 units/day/rat), while the animals from the control group (seven rats) received vehicle for a period of 4 weeks. Systolic blood pressures were measured by the tail-cuff technique once a week during the 4 weeks of treatment and for 3 weeks after termination of treatment.

Statistical analyses (t test, unpaired, correlation and regression coefficients) were made according to Steel and Torrie. Results are expressed as means ± 1 SEM.

Results

Spontaneous Hypertension

No significant difference in average body weight was observed between the groups either at the beginning (148 ± 8 g vs 140 ± 8 g, heparin-treated and control group, respectively) or at the end of the study (323 ± 8 g vs 304 ± 7 g, heparin and control group, respectively).

As shown in figure 1, a significant decrease in mean arterial blood pressure, an increase in cardiac output, a decrease in total peripheral resistance, and a decrease in packed cell volume were found in heparin-treated rats compared to those in control group after 8 weeks of heparin treatment. No difference in heart rate was observed (399 ± 7 beats/min vs 407 ± 8). Left ventricular hypertrophy was less pronounced in the heparin group, as indicated by a significantly (p < 0.05) reduced left ventricular weight/body weight ratio (0.280 ± 0.004 vs 0.293 ± 0.004 in heparin-treated and control group, respectively). No correlation was found between packed cell volume and mean blood pressure when either separate data for heparin-treated (r = 0.152, t = 0.378) and control group (r = 0.247, t = 0.625) were used or when combined data for both groups were plotted (r = 0.463, t = 1.956).

![Figure 1. Mean femoral artery pressure (MAP), cardiac output (CO), total peripheral resistance (TPR), and packed cell volume (PCV) from control (n = 8) and heparin-treated (n = 8) spontaneously hypertensive rats are shown. Results are expressed as means ± 1 SEM. *p < 0.05; **p < 0.001.](image-url)
One-kidney, One Clip Hypertension

No significant difference in average body weight was observed between the groups either at the beginning (330 ± 6 g vs 319 ± 10 g, heparin-treated and control group, respectively) or at the end of the study (358 ± 6 vs 359 ± 9 g, heparin-treated and control group, respectively).

The effects of heparin on the development of one-kidney, one clip hypertension are shown in figure 2. During the third and fourth week after the induction of hypertension, systolic blood pressure was found to be significantly lower in heparin-treated rats compared to control rats. As shown in figure 3, a significant decrease in mean arterial pressure, a significant increase in cardiac output, a significant decrease in total peripheral resistance, and significantly lowered packed cell volume also were found in heparin-treated rats compared to those in the control group at 4 weeks after the induction of hypertension. No difference in heart rate was observed (400 ± 8 beats/min vs 407 ± 10). Left ventricular hypertrophy was less pronounced in the heparin group, as indicated by a significantly (p < 0.05) reduced ratio of left ventricular weight/body weight (0.222 ± 0.005 vs 0.253 ± 0.009 in heparin-treated and control rats, respectively). When combined data for both groups were used, a significant positive correlation (r = 0.536, r = 2.84, p < 0.05) and linear regression (fig. 4) between packed cell volume and mean arterial blood pressure was observed. However, when separate data for each group were analyzed, no relationship could be found (fig. 4) (r = 0.105 for heparin-treated group and r = 0.257 for the control group).

Administration of heparin to rats with chronic one-kidney, one clip hypertension induced a significant (p < 0.01) decrease in blood pressure (fig. 5). While at the end of heparin treatment, packed cell volume was found to be significantly (p < 0.01) lower in heparin-treated rats (44.5% ± 0.8%) than in control rats (49.7% ± 1.1%), no difference in packed cell volume between groups (46.9% ± 1.2% vs 48.1% ± 0.7%; heparin-treated and control group, respectively), was observed 2 weeks after the cessation of heparin treatment. After termination of heparin therapy, the blood pressure gradually reached the pretreatment level (fig. 5).

Discussion

The results of the present study demonstrate that prolonged heparin treatment alleviates both spontaneous and one-kidney, one clip hypertension. The present data confirm previous observations by Mandal et al.1 and Wilson et al.2 in SHR and further demonstrate that heparin has a similar hypotensive effect in another hypertensive model such as Goldblatt hypertension. An additional finding in this study is a significant decrease in packed cell volume in heparin-treated rats as compared to control rats.

Hemodynamically, the heparin-induced decrease in blood pressure in both hypertensive models appears to...
be related to a fall in peripheral resistance, cardiac output being increased in heparin-treated rats. However, our results do not point to the intimate mechanism by which heparin may induce the observed decrease in total peripheral resistance and blood pressure. The observed decrease in packed cell volume may account for the decrease in peripheral resistance and blood pressure in heparin-treated rats. The in vitro and dog hind limb studies of Whittaker and Winton have shown that blood viscosity increases significantly with increases in hematocrit. Since, from Poiseuille's equation, blood pressure is directly proportional to viscosity, blood pressure should also rise, if all other factors in the equation remain constant. Therefore, a possibility remains that a pharmacologic agent such as heparin, which is found to lower packed cell volume in our study, may also lower the viscosity and in this way induce a decrease in total peripheral resistance and blood pressure.

Several reports have confirmed the idea that changes in blood viscosity may alter circulatory hemodynamics, although the results do not always point to the same direction. Recently, a direct relationship between blood pressure and blood viscosity has been shown in normal and hypertensive subjects, but a causal relationship between these two hemodynamic variables has not been documented. A positive correlation between packed cell volume and blood pressure observed in one-kidney, one clip hypertensive rats in our study is in agreement with this report. On the other hand, studies in normal dogs revealed that experimentally induced changes in blood viscosity (when either the hematocrit value or the plasma viscosity was altered) were associated with a quantitatively similar change in total peripheral resistance and a reciprocal change in venous return and cardiac output, blood pressure being unchanged. Applied to our study, these results would indicate that a decrease in packed cell volume in heparin-treated rats accounts for the increase in cardiac output and, in part, for a decrease in peripheral resistance, but the decrease in blood pressure would be unaccounted for. Furthermore, the magnitude of the blood pressure decrease observed in heparin-treated rats in our study (about 25 mm Hg) is of the same order as in the study of Wilson et al., although they noted a much greater decrease in packed cell volume (from 46.9 to 28.7 in their study compared to 48.6 to 44.5 in the SHR in our study).

Therefore, if there is a simple and direct relationship between packed cell volume and blood pressure, one should expect heparin to have a more pronounced effect on blood pressure in the study by Wilson et al., or a less pronounced effect in our study. The fact that a correlation between packed cell volume and blood pressure could not be firmly established in our study (found only in one-kidney, one clip hypertensive rats when combined data for both groups were used), together with the foregoing discussion, does not support the idea that the heparin-induced decrease in packed cell volume is the sole factor responsible for the heparin-induced decrease in blood pressure.
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Other possibilities also appear plausible in explaining the hypotensive effect of heparin. As already mentioned, heparin inhibits arterial smooth muscle cell proliferation and, in this way, may decrease peripheral resistance and hence blood pressure. Furthermore, heparin is known to inhibit the renin angiotensinogen reaction, and the consequent reduction in the amount of generated angiotensin II may explain the observed decrease in peripheral resistance and blood pressure. Since heparin is considered a lipolytic agent, it may also be suggested that, due to heparin-induced lipolysis, more arachidonic acid is available for prostaglandin synthesis. As prostaglandins are known for their antihypertensive activity, this may also explain the observed decrease in peripheral resistance and blood pressure in heparin-treated hypertensive rats in the present study.

Although gross hemorrhage has not been observed in heparin-treated rats, the possibility that capillary bleeding is responsible for lower packed cell volume in heparin-treated rats cannot be excluded. Since splenic contraction and emptying has been shown to increase packed cell volume in experimental animals, a possibility of inhibited splenic function may also be suggested in explaining low packed cell volume in heparin-treated rats. This idea is supported further by the prompt rise in packed cell volume following withdrawal of heparin.

Acknowledgments

The authors are grateful to Dr. Lerner B. Hinshaw for his most helpful suggestions. The expert technical assistance of Gordana Funduk and Zaga Jovanovic and secretarial assistance of Jovana Susic are greatly appreciated.

References

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D Susic, A K Mandal and D Kentera

_Hypertension._ 1982;4:681-685
doi: 10.1161/01.HYP.4.5.681

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1982 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

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