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Heparin and derivatized heparin inhibit zymosan and cobra venom factor activation of complement in serum

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Abstract

Heparin has been shown to inhibit activity of the alternative, classical and terminal pathways of complement by regulating C1, C1 inhibitor, C4 binding protein, C3b, factor H and S-protein. In vivo, heparin inhibits cobra venom factor activation of complement in a dose-related manner in guinea pigs. However, the ability of heparin and of modified heparin to inhibit complement activation in serum has not been examined systematically. The present study compared commercial heparin with a modified heparin that has reduced anticoagulant activity (*N*-desulfated, *N*-acetylated heparin) for ability to inhibit cobra venom factor and zymosan-induced complement activation in guinea pig and human serum. Both heparins inhibited cobra venom factor and zymosan-induced consumption of C3 activity in both human and guinea pig serum. In both serum types, commercial heparin was about twice as active as modified heparin on a weight basis for ability to inhibit cobra venom factor-induced complement activation. Both heparins also inhibited zymosan-induced complement activation in human serum. About four times more heparin was required to inhibit cobra venom factor-induced complement activation in guinea pig serum than in human serum while heparin was more than ten times more active in human serum than in guinea pig serum when zymosan was used as the activator of complement. This study suggests that heparin is considerably more effective in regulating complement activity in humans than in guinea pigs, an animal model in which heparin clearly has in vivo capacity to regulate complement activity. These observations represent an important step in the development of new clinically relevant oligosaccharide-derived pharmacologic agents to regulate complement activity.

Key words: Heparin; Heparin derivative; Complement; Inhibition; Serum

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Abbreviations: *N*-desulfated, *N*-acetylated heparin, Hep-NAc; cobra venom factor, CoVF; guinea pig serum, GPS; pooled human serum, PHS; half-isotonic veronal-buffered saline, pH 7.5, containing 0.1% gelatin, 0.15 mM calcium, 0.5 mM magnesium and 2.5% dextrose, $DGVB^{++}$; ethylene glycol-bis-(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid, EGTA; 10 mM $MgCl_2$, 10 mM EGTA in $DGVB^{++}$, MgEGTA-$DGVB^{++}$.

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1. Introduction

Ecker and Gross (1929) first reported that heparin inhibits complement activity in 1929. Since then, studies in many laboratories have shown that heparin inhibits complement at multiple sites in the alternative (Weiler et al., 1978; Maillet et al., 1983; Weiler, 1983; Sharath et al., 1985; Linhardt et al., 1988; Weiler and Linhardt, 1989), classical (Loos et al., 1976b; Loos et al., 1976a; Raepple et al., 1976; Rent et al., 1976; Weiler and Linhardt, 1989) and terminal pathways of complement (Baker et al., 1975; Edens et al., 1993). Heparin has been shown to inhibit C4 binding protein (Scharfstein et al., 1978), augment the activity of C1 inhibitor (Caughman et al., 1982); inhibit C3b (Weiler et al., 1978; Linhardt et al., 1988), inhibit S-protein (Tschopp et al., 1988), and augment (Boackle et al., 1983) and inhibit (Weiler et al., 1978) factor H.

We recently demonstrated that heparin has the ability to inhibit cobra venom factor (CoVF)-induced complement activation in guinea pigs (Weiler et al., 1992). Animals that were pretreated with heparin had less consumption of C3 hemolytic activity after CoVF administration than did animals pretreated with saline. Furthermore, about 100 times less heparin was required to inhibit in vivo hemolysis than to inhibit C3 consumption in these animals. These studies showed that heparin is able to inhibit complement activation in vivo and that it is possible to modify heparin to decrease greatly anticoagulant activity and to retain complement inhibitory activity (Weiler et al., 1992).

In the present study, we compared the ability of heparin and a chemically modified heparin to inhibit CoVF and zymosan induced complement activation in guinea pig serum (GPS) and pooled human serum (PHS); we sought to determine whether the phenomenon we saw in guinea pigs might also occur in humans. We found that heparin was considerably more active in regulating complement activation in PHS than in GPS and should be studied clinically to determine if it has a role in the regulation of complement activation in clinical conditions in which complement plays a pathogenic role.

2. Materials and methods

2.1. Preparation of heparin and derivatized heparin

Porcine mucosal sodium heparin was obtained from Hepar Industries Inc., Franklin, OH. Derivatized heparin was prepared by a modification of the protocol described first by Kazatchkine (Kazatchkine et al., 1981; Weiler et al., 1992). Briefly, 2.5 g sodium heparin (Hepar Industries) was dissolved in 50 ml distilled water and passed through a column containing 30 g Dowex AG-50W-X8 cation exchange resin (the H^+ form) producing H^+-heparin. The H^+-heparin was then neutralized using pyridine to yield the pyridinium salt of heparin. After dialysis and lyophilization, the heparin salt was dissolved in 120 ml dimethyl sulfoxide/5% methanol and heated at 50 °C for 1.5 h to *N*-desulfate the heparin. The mixture was diluted with 125 ml distilled water, the pH was adjusted to 9.1 using sodium hydroxide and the mixture was dialyzed and lyophilized. This *N*-desulfated heparin was dissolved in 100 ml 10% methanol/0.05 M sodium carbonate and *N*-acetylated by adding 0.8 ml acetic anhydride over 2 h (115 μl every 20 min) at 25 °C. This solution was diluted with two volumes of distilled water, exhaustively dialyzed (3500 M_w cut off dialysis tubing) against distilled water and lyophilized, resulting in 1.8 g (70% yield) of *N*-desulfated, *N*-acetylated heparin (Hep-NAc) (Weiler et al., 1992).

2.2. Complement buffers and components

Half-isotonic veronal-buffered saline, pH 7.5, containing 0.1% gelatin, 0.15 mM calcium, 0.5 mM magnesium and 2.5% dextrose ($DGVB^{++}$) was used as buffer in the hemolytic assays. Ethylene glycol-bis-(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid (EGTA) and $MgCl_2$ were made 10 mM in $DGVB^{++}$ (MgEGTA-$DGVB^{++}$). Guinea pig C2 (C2gp) was isolated from GPS (Rockland, Inc., Gilbertsville, PA) as described by Nelson et al. (1966); rat complement (used in factor B assays) was also obtained from Rockland. Human C5, C6, C7, C8 and C9 were purchased from Diamedix (Miami, FL). Complement factors B, D and P were isolated from PHS as previously described (Weiler and Linhardt, 1991). EAC1,4b cellular intermedi-

ates were prepared as described previously (Harrison and Lachmann, 1986). EAC4b,3b were prepared as previously described (Weiler and Linhardt, 1991).

2.3. Overview of experimental approach

The experiments were conducted in two phases. Briefly, in the first phase, we treated serum (human and guinea pig) with either cobra venom factor or with zymosan to activate the alternative pathway of complement in the presence or absence of heparin. Then, in the second phase we assayed these treated sera for hemolytically active C3 or factor B as described below. In this second phase, we adjusted the dilutions of all of the aliquots so that the samples withdrawn at zero time would have about 1 Z of lysis, to assess lysis in the linear portion of the hemolytic assay system. Then, we set the amount of hemolytic activity at the zero time point (about 1 Z of lysis) equal to 100% and calculated activity remaining based upon this value. Finally, data were summed at each time point and the mean and standard error of the mean were calculated. Data at each time point were analyzed using the fully factorial (M)ANOVA (analysis of variance) procedure (where activity remaining was the dependent variable and heparin concentration was the factor) of SYSTAT 5.01 FOR WINDOWS (Evanston, IL) on a Hewlett Packard Vectra 486/66U.

2.4. CoVF activation of serum

Naja naja siamensis cobra venom was purchased from Miami Serpentarium, (Puntaguoro, FL). CoVF was purified from cobra venom as described by Ballow and Cochrane (1969) and had an activity of 250 units/mg. Human serum was drawn from healthy donors and pooled (PHS). Pooled GPS was purchased from Rockland.

To examine complement activation in PHS, we added 100 μl of PHS at 0 °C to 200 μl of $DGVB^{++}$ containing varying concentrations of heparin or Hep-NAc (0, 25, 50, 100, or 200 μg/200 μl at 0 °C). Tubes were placed in a 37 °C shaking water bath and 2.21 units CoVF in 60 μl $DGVB^{++}$ were added at 0 min. Then, 20 μl aliquots were removed every 10 min from 0 to 60 min and added to 980 μl of $DGVB^{++}$ that had been kept on ice. Tubes were kept on ice until assay or frozen at −70 °C for later assay. Activation of GPS was examined in the same manner except that only 0.02 units CoVF in 5 μl $DGVB^{++}$ was required to achieve a comparable level of C3 consumption over time.

2.5. Zymosan activation of serum

Zymosan from ICN (Cleveland, OH) (20 mg/ml saline) was boiled for 1 h, washed until supernatant was clear and resuspended to 20 mg/ml saline. Again, PHS was drawn from healthy donors and GPS was obtained from Rockland. To examine zymosan-induced activation of complement in PHS, we added 200 μl of PHS at 0 °C to 200 μl of $DGVB^{++}$ containing varying amounts of heparin or Hep-NAc (0, 25, 50, 100 or 200 μg/200 μl at 0 °C). Tubes were placed in a 37 °C shaking water bath and 10 μl of the zymosan stock suspension was added at 0 min. Then, 20 μl aliquots were removed at intervals from 0 to 90 min and added to 780 μl $DGVB^{++}$ that had been kept on ice. The tubes were centrifuged for 5 min in an Eppendorf microfuge (model 5413) at 4 °C. 50 μl of the supernatant was removed and added to 950 μl of $DGVB^{++}$ for subsequent C3 hemolytic activity determination; and 50 μl of the supernatant was removed and added to 950 μl MgEGTA-$DGVB^{++}$ to assay factor B activity. In both cases, samples were kept on ice until assay or frozen at −70 °C for later assay. In each assay system the amount of lysis was set to be about 1 Z, an average of 1 hemolytic event per cell, for the zero time point upon which the % activity remaining was calculated.

To assess zymosan-induced activation of complement in GPS we added 200 μl GPS at 0 °C to 200 μl $DGVB^{++}$ containing varying amounts of heparin or Hep-NAc (0–2.4 mg/200 μl at 0 °C). Tubes were placed in a 37 °C shaking water bath and 10 μl of the zymosan stock suspension was added at 0 min. Then, 30 μl aliquots were removed at intervals and added to 470 μl $DGVB^{++}$ that had been kept on ice. The tubes were centrifuged for 5 min in an Eppendorf microfuge at 4 °C. 50 μl of the supernatant was removed and added to 950 μl $DGVB^{++}$ for subsequent determination of C3 hemolytic activity. Samples were kept on ice until assay or frozen at −70 °C for later assay.

2.6. Determination of C3 hemolytic level

Activated serum samples were assayed for C3 hemolytic activity. Briefly, the serum was further diluted in $DGVB^{++}$, added to 1×10^7 EAC1,4b, 660 ng C2gp, and 10 units each of C5, C6 and C7 to a final volume of 400 μl in $DGVB^{++}$ and incubated with shaking for 30 min at 30 °C. Then, 10 units each of C8 and C9 in 200 μL $DGVB^{++}$ were added and incubated at 37 °C for 60 min. Finally, 1.5 ml saline were added, the tubes were shaken and centrifuged for 5 min and lysis was determined by measuring A_{414} of the supernatant. Again, in each assay system, the serum was diluted sufficiently in order that the amount of lysis would be about 1 Z, an average of 1 hemolytic event per cell, for the zero time point upon which the % activity remaining was calculated.

2.7. Determination of factor B levels remaining in activated serum

Serum was diluted in MgEGTA-$DGVB^{++}$ and then assayed for factor B hemolytic activity as described previously (Weiler and Linhardt, 1991).

3. Results

3.1. Regulation of CoVF activation of serum

We compared heparin (Fig. 1a) and Hep-NAc (Fig. 1b) for ability to regulate CoVF-induced complement activation in GPS and found that both heparins inhibited CoVF-induced consumption of C3 hemolytic activity in a dose-related manner. As seen in Fig. 1, 77% of C3 was consumed in the

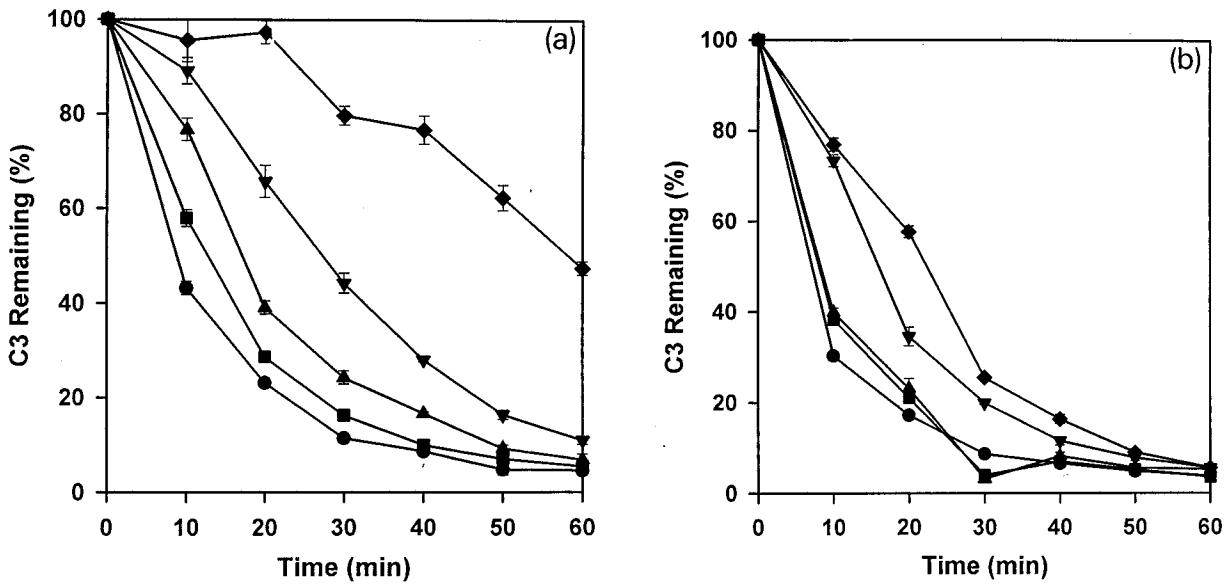

Fig. 1. C3 hemolytic activity remaining over time when guinea pig complement was activated with CoVF in the presence of heparin (a) or Hep-NAc (b). (a) CoVF was used to activate guinea pig complement in the presence of 0 (circle), 25 (square), 50 (triangle up), 100 (triangle down) or 200 (diamond) μg of heparin. Aliquots were removed over time and assayed for C3 hemolytic activity. Heparin inhibited CoVF-induced activation of guinea pig complement in a dose-related manner. (b) CoVF was used to activate guinea pig complement in the presence of 0 (circle), 25 (square), 50 (triangle up), 100 (triangle down) or 200 (diamond) μg of Hep-NAc. Aliquots were removed over time and assayed for C3 hemolytic activity. Hep-NAc also inhibited CoVF-induced activation of guinea pig complement in a dose-related manner. Each experiment presented here was performed three times. Using one way ANOVA, $p < 0.001$ at all time points in (a); $p < 0.001$ at 10, 20, 30 and 40 min, $p = 0.002$ at 50 min and $p = 0.033$ at 60 min in (b).

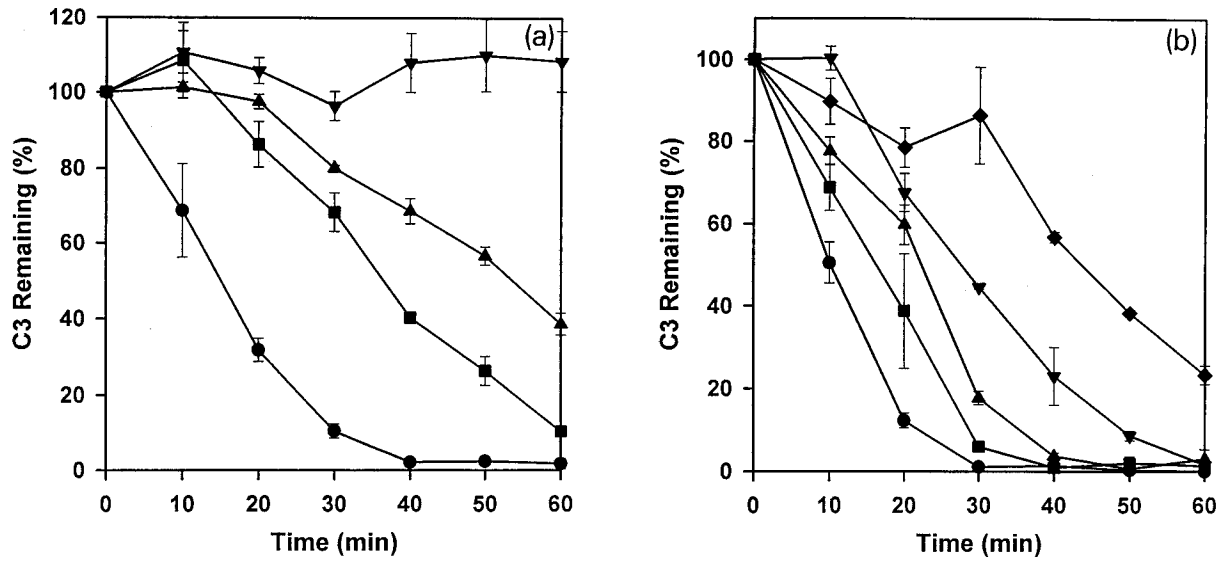

Fig. 2. C3 hemolytic activity remaining over time when human complement was activated with CoVF in the presence of heparin (a) or Hep-NAc (b). (a) CoVF was used to activate human complement in the presence of 0 (circle), 25 (square), 50 (triangle up) or 100 (triangle down) μg of heparin. Aliquots were removed over time and assayed for C3 hemolytic activity. Heparin inhibited CoVF-induced activation of human complement in a dose-dependent manner. (b) CoVF was used to activate human complement in the presence of 0 (circle), 25 (square), 50 (triangle up), 100 (triangle down) or 200 (diamond) μg of Hep-NAc. Aliquots were removed over time and assayed for C3 hemolytic activity. Hep-NAc also inhibited CoVF-induced activation of human complement in a dose-related manner. Each experiment presented here was performed three times. Using one way ANOVA, $p = 0.014$ at 10 min and $p < 0.001$ at all other time points in (a); $p < 0.001$ at all time points in (b).

absence of heparin and 62% in the presence of 50 μg heparin at 20 min. About 100 μg Hep-NAc were required to achieve the same level of inhibition of C3 consumption. Thus, Hep-NAc inhibited C3 consumption in GPS about half as well as commercial heparin on a weight basis.

We also compared heparin (Fig. 2a) and Hep-NAc (Fig. 2b) for ability to regulate CoVF-induced activation of complement in PHS. Again we found that both heparins inhibited CoVF-induced consumption of C3 hemolytic activity in a dose-related manner. As seen in Fig. 2a, C3 consumption was almost totally inhibited in the presence of 100 μg of heparin for 60 min. In the presence of a comparable amount of Hep-NAc, 32% of C3 was consumed, whereas in the absence of heparin or Hep-NAc 88% of the C3 was consumed at 20 min. Thus, Hep-NAc also inhibited C3 consumption in PHS about half as well as commercial heparin on a weight basis. As Figs. 1a and 2a show, approximately the same degree of complement activation was achieved in the two serum types (because the percentage drop in C3 was about the same in both). However, about four times more heparin was required to inhibit comparable C3 consumption in GPS than in PHS.

3.2. Regulation of zymosan activation of serum

We compared heparin (Fig. 3a) and Hep-NAc (Fig. 3b) for ability to regulate zymosan-induced complement activation in PHS and found that both heparins inhibited zymosan-induced consumption of C3 hemolytic activity in a dose-related manner. As shown in Fig. 3 about 100 μg heparin caused the same amount of inhibition of C3 consumption over 90 min, as 200 μg Hep-NAc. Thus, Hep-NAc inhibited C3 consumption in PHS about half as well as commercial heparin on a weight basis. Similarly, heparin (Fig. 4a) and Hep-NAc (Fig. 4b) both inhibited zymosan-induced consumption of factor B

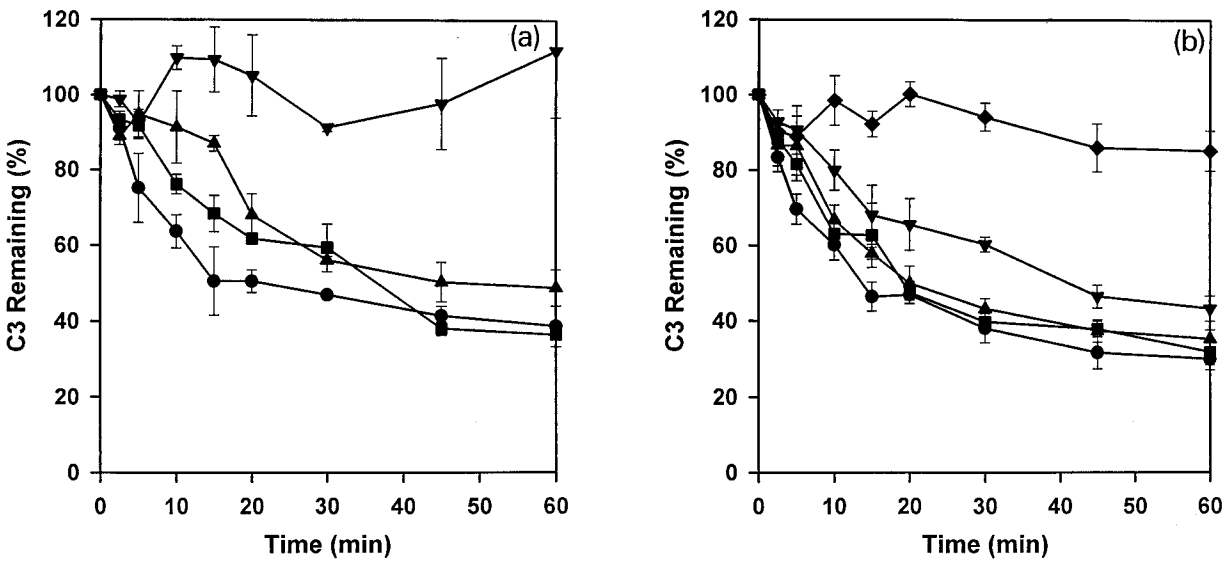

Fig. 3. C3 hemolytic activity remaining over time when human complement was activated with zymosan in the presence of heparin (a) or Hep-NAc (b). (a) Zymosan was used to activate human complement in the presence of 0 (circle), 25 (square), 50 (triangle up) or 100 (triangle down) μg of heparin. Aliquots were removed over time and assayed for C3 hemolytic activity. Heparin inhibited zymosan-induced activation of human complement in a dose-related manner. (b) Zymosan was used to activate human complement in the presence of 0 (circle), 25 (square), 50 (triangle up), 100 (triangle down) or 200 (diamond) μg of Hep-NAc. Aliquots were removed over time and assayed for C3 hemolytic activity. Hep-NAc also inhibited zymosan-induced activation of human complement in a dose-related manner. Each experiment presented in (a) was performed two times and in (b) six times. Using one way ANOVA, $p = 0.221$ at 2.5 min, $p = 0.302$ at 5 min, $p = 0.093$ at 10 min, $p = 0.202$ at 15 min, $p = 0.141$ at 20 min, $p = 0.001$ at 30 min, $p = 0.011$ at 45 min, $p = 0.018$ at 60 min, $p = 0.039$ at 75 min and $p = 0.003$ at 90 min in (a); $p = 0.481$ at 2.5 min, $p = 0.074$ at 5 min and $p < 0.001$ at all other time points in (b).

activity in a dose-related manner. Again, commercial heparin was about twice as active as Hep-NAc on a weight basis (Fig. 4).

We also examined both heparins for ability to regulate zymosan-induced activation of complement in GPS. Again, we found that heparin was able to inhibit complement activation in a dose-related manner (Fig. 5). In the presence of 600 μg of heparin, 47% of C3 was consumed at 10 min, whereas in its absence, 82% of C3 was consumed, but this inhibitory activity of heparin was completely lost by 60 min (Fig. 5). Hep-NAc was unable to inhibit zymosan-induced complement activation in doses as high as 2.4 mg. We compared the effects of heparin on zymosan-induced complement activation in PHS with that in GPS (Figs. 3a and 5) and found that heparin had approximately ten times less activity in inhibiting zymosan-induced consumption of C3 hemolytic activity in GPS than in PHS.

4. Discussion

We compared commercial heparin with a derivatized heparin (Hep-NAc) for ability to regulate complement activation in whole guinea pig and human sera. The M_r of Hep-NAc was similar to that of commercial heparin yet Hep-NAc was only minimally digested by heparin lyase I (Weiler et al., 1992) indicating that the conversion of the *N*-sulfated glucosamine residues in heparin to *N*-acetylated glucosamine had been complete or almost complete. We found that both heparin and Hep-NAc were able to inhibit CoVF-induced activation of complement in a dose related manner (Figs. 1, 2) and that heparin had about twice as much complement inhibitory activity as Hep-NAc on a weight basis. As shown, CoVF in either serum type consumed C3 hemolytic activity over time at a comparable rate. However, only about 25% as much heparin was required to

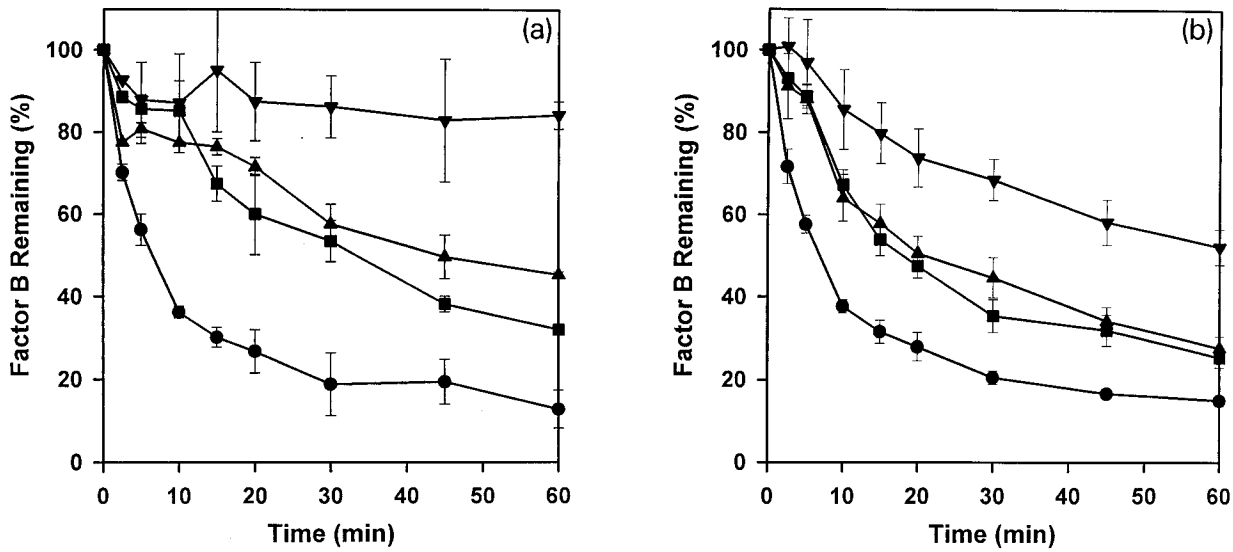

Fig. 4. Factor B hemolytic activity remaining over time when human complement was activated with zymosan in the presence of heparin (a) or Hep-NAc (b). (a) Zymosan was used to activate human complement in the presence of 0 (circle), 25 (square), 50 (triangle up) or 100 (triangle down) μg of heparin. Aliquots were removed over time and assayed for factor B hemolytic activity. Heparin inhibited zymosan-induced activation of human complement in a dose-related manner. (b) Zymosan was used to activate human complement in the presence of O (circle), 25 (square), 50 (triangle up) or 100 (triangle down) μg of Hep-NAc. Aliquots were removed over time and assayed for factor B hemolytic activity. Hep-NAc also inhibited zymosan-induced activation of human complement in a dose-related manner. Each experiment presented in (a) was performed two times and in (b) five times. Using one way ANOVA $p = 0.004$ at 2.5 min, $p = 0.044$ at 5 min, $p = 0.010$ at 10 min, $p = 0.010$ at 15 min, $p = 0.038$ at 20 min, $p = 0.005$ at 30 min, $p = 0.015$ at 45 min, $p < 0.001$ at 60 min, $p = 0.001$ at 75 min and $p = 0.003$ at 90 min in (a); $p = 0.026$ at 2.5 min and $p < 0.001$ at all other times points in (b).

achieve similar inhibition of C3 consumption in PHS as compared to GPS (Figs. 1a, 2a). Thus, these experiments demonstrate that about four times more heparin is required to inhibit CoVF-induced complement activation in GPS than in PHS.

Likewise, we found that both heparin and Hep-NAc were able to inhibit zymosan-induced complement activation in PHS in a dose-related manner (Figs. 3, 4). Higher concentrations of heparin caused inhibition of complement activation in GPS (Fig. 5). Comparison of the effects of heparin on complement in GPS with that in PHS revealed that heparin was greater than 10 times more active in inhibiting zymosan-induced complement activation in PHS than in GPS (Figs. 3a, 5). This result is particularly interesting because heparin has clearly been shown to regulate complement activation in vivo in guinea pigs. Thus, we hypothesize that heparin would be capable of inhibiting complement activation in humans at much lower doses than were required to inhibit complement activation in the guinea pig animal model. Again Hep-NAc possessed about half as much complement inhibitory activity on zymosan activation as commercial heparin on a weight basis (Figs. 3, 4).

The ability of heparin to regulate complement activity in vitro is well established (Ecker and Gross, 1929; Baker et al., 1975; Loos et al., 1976a; Loos et al., 1976b; Raepple et al., 1976; Rent et al., 1976; Scharfstein et al., 1978; Weiler et al., 1978; Caughman et al., 1982; Boackle et al., 1983; Maillet et al., 1983; Weiler, 1983; Sharath et al., 1985; Linhardt et al., 1988; Tschopp et al., 1988; Weiler and Linhardt, 1989; Edens et al., 1993). Heparin binding regions have been described on C4 binding protein (Hessing et al., 1990) and factor H (Pangburn et al., 1991). Clearly, the effect of heparin on complement is multifaceted (Edens et al., 1993). Heparin regulates the classical pathway (Loos et al., 1976a, 1976b; Raepple et al., 1976; Rent et al., 1976; Weiler and Linhardt, 1989) by augmenting C1 inhibitor (Caughman et al., 1982), inhibiting C4 binding pro-

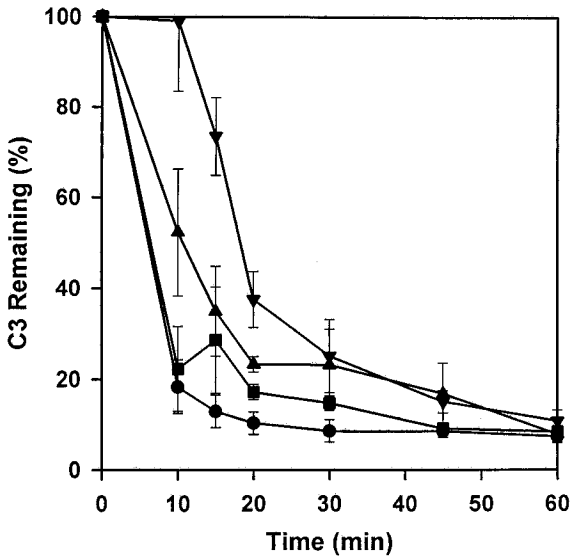

Fig. 5. C3 hemolytic activity remaining over time when guinea pig complement was activated with zymosan in the presence of heparin. Heparin was used to activate guinea pig complement in the presence of 0 (circle), 300 (square), 600 (triangle up) or 1200 (triangle down) μg of heparin. Aliquots were removed over time and assayed for C3 hemolytic activity. Heparin inhibited zymosan-induced activation of guinea pig complement in a dose-dependent manner. Each experiment presented here was performed three times. Using one way ANOVA, $p = 0.137$ at 5 min, $p = 0.008$ at 10 min, $p = 0.003$ at 15 min, $p = 0.003$ at 20 min, $p = 0.237$ at 30 min, $p = 0.364$ at 45 min and $p = 0.621$ at 60 min.

tein (Scharfstein et al., 1978) and inhibiting C1$\bar{s}$ interaction with C4 and C2 (Loos et al., 1976a, 1976b). Heparin acts on the alternative pathway (Weiler et al., 1978; Maillet et al., 1983; Weiler, 1983; Sharath et al., 1985; Linhardt et al., 1988; Weiler and Linhardt, 1989) by inhibiting (Weiler et al., 1978) and augmenting (Boackle et al., 1983) factor H and by inhibiting C3b interaction with factor B (Weiler et al., 1978). Finally, heparin acts on terminal lysis (Baker et al., 1975; Edens et al., 1993) by inhibiting S-protein (Tschopp et al., 1988) and by interfering with C5,6,7 (Baker et al., 1975). These previous studies suggest that the net effect of heparin is to inhibit complement activity and this is indeed what we observed in the present study and in the in vivo study in guinea pigs. Thus, the inhibitory activity of commercial heparin and of heparin derivatives in whole serum should be useful in predicting in vivo activity. Similarly, other polyanions that have been shown to have in vivo activity to inhibit complement activation (Weiler and Linhardt, 1989) could be tested in whole serum to predict the in vivo activity of these substances.

Comparison of the current in vitro study in serum with the results from our previous in vivo study in guinea pigs reveals that: (1) Heparin and Hep-NAc inhibit CoVF-induced activation of complement; (2) Heparin is about twice as potent in inhibiting complement activity as Hep-NAc on a weight and a molar basis; and (3) Heparin and Hep-NAc are about three times less active in vivo than in vitro (Weiler et al., 1992). Interestingly, about four times less heparin was required to inhibit CoVF-induced activation in PHS than in GPS suggesting that heparin may inhibit complement activity in humans at much lower doses than required in the guinea pig animal model. Furthermore, heparin was even more active in PHS than in GPS when the activator was zymosan, raising the possibility that heparin may be even more active in vivo depending on the activating surface.

These data demonstrate an in vitro model to predict the in vivo complement inhibitory activity of heparin. This model suggests that much less heparin would be required to regulate the complement system in humans than is required in guinea pigs, an animal in which heparin has clearly been shown to play a role in vivo. This strongly suggests a role for heparin as a pharmacological agent to inhibit complement activation in patients in conditions in which complement activation is detrimental. For example, heparin might find clinical usefulness in the treatment of C1 inhibitor deficiency (Weiler et al., 1991; Levine and Stechschulte, 1992). Heparin might also be used to inhibit complement activation in an inflamed joint. The in vitro screening of heparin and heparin derivatives for the capacity to regulate complement is an important step prior to use of heparin in animal and human studies.

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