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Interaction of heparin with human cardiac troponin complex and its influence on the immunodetection of troponins in human blood samples

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Abstract

Objectives: Heparin is a highly charged polysaccharide used as an anticoagulant to prevent blood coagulation in patients with presumed myocardial infarction and to prepare heparin plasma samples for laboratory tests. There are conflicting data regarding the effects of heparin on the measurement of cardiac isoforms of troponin I (cTnI) and troponin T (cTnT), which are used for the immunodiagnosis of acute myocardial infarction. In this study, we investigated the influence of heparin on the immunodetection of human cardiac troponins.

Methods: Gel filtration (GF) techniques and sandwich fluoroimmunoassay were performed. The regions of cTnI and cTnT that are affected by heparin were investigated with a panel of anti-cTnI and anti-cTnT monoclonal antibodies, specific to different epitopes.

Results: Heparin was shown to bind to the human cardiac full-size ternary troponin complex (ITC-complex) and free cTnT, which increased their apparent molecular weights in GF studies. Heparin did not bind to the low molecular weight ITC-complex and to binary cTnI-troponin C complex. We did not detect any sites on cTnI in the ITC-complex that were specifically affected by heparin. In contrast, cTnT regions limited to approximately 69–99, 119–138 and 145–164 amino acid residues (aar) in the ITC-complex and a region that lies approximately between 236 and 255 aar of free cTnT were prone to heparin influence.

Conclusions: Heparin binds to the ITC-complex via cTnT, interacting with several sites on the N-terminal and/or central parts of the cTnT molecule, which might influence the immunodetection of analytes in human blood.

Keywords: cardiac troponin complex; enoxaparin; heparin; monoclonal antibodies; myocardial infarction; serum and plasma samples

Introduction

Troponin complex consists of troponin I, troponin T, and troponin C (TnC), which are present in a molar ratio of 1:1:1 in a functionally active unit [1, 2] that regulates striated muscle contraction. In heart muscle cells, troponin I and troponin T are present as cardiac isoforms (cTnI and cTnT, respectively) that are specific to the myocardial tissue. The cardiac specificity of cTnI and cTnT makes it possible to use these proteins as biomarkers of various heart diseases accompanied by myocyte necrosis, including acute myocardial infarction (MI) [3].

Heart muscle damage upon MI is accompanied by the release of troponins into the blood flow where they circulate in various forms: full-size ternary complex cTnI-cTnT-TnC (ITC-complex), low molecular weight ternary complex (LMW ITC-complex) in which cTnT comprises only the C-terminal fragment that is bordered by approximately 190–287 amino acid residues (aar; in this study we use the numeration of the aar according to the sequence of cTnI (UniProtKB – P19429) and cTnT (UniProtKB – P45379-6) including the first Met residue) [4], binary cTnI-TnC complex (IC-complex) that comprises full-size or partially proteolyzed cTnI bound to TnC, and various free proteolytic fragments of cTnT and cTnI [5-8]. These proteins are detected in the patients' blood using various anti-cTnI or anti-cTnT immunoassays [9, 10].

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Despite a 30-year history of measuring cardiac troponins in the blood, the influence of some factors on the detection of troponins has not yet been clearly established. One of these factors is heparin, a highly negatively charged polysaccharide used to prepare heparin plasma samples – a common matrix for immunometric measurements of cardiac troponins [11]. Heparin and its synthetic low molecular weight forms (LMW heparin) are widely used as anticoagulants during MI treatment [12]; therefore, they may also be present in the serum and citrate or EDTA plasma samples if taken shortly after heparin administration to patients [13]. Data regarding the influence of heparin on troponin detection are contradictory. Some authors reported that the recovery of cTnT in heparin plasma was commonly slightly lower than that in serum [14], but markedly lower in some patients [15]; in some cases, it depended on the time after the MI onset when the samples were taken for analysis [16]. In a number of studies, no difference was found in the cTnT values between serum and heparin plasma [17, 18], while other studies demonstrated that the detected cTnT levels were higher in heparin plasma than in serum samples [19].

As for cTnI, it was reported that cTnI levels in heparin plasma are 13 % lower than those in serum [20], but most of the modern systems show good agreement in measuring cTnI in serum vs. heparin plasma samples [21–26]. No data are available regarding the influence of LMW heparin on the immunochemical detection of cTnT or cTnI.

In this study, we investigated the effects of unfractionated and LMW heparin on the immunochemical detection of cTnI and cTnT in the plasma samples of patients with MI.

Materials and methods

Unless otherwise stated, all chemicals were purchased from Sigma-Aldrich. All monoclonal antibodies (Table 1), native cardiac ternary troponin complex (ITC-complex), artificial binary IC-complex (IC-complex) constructed from purified native cTnI and TnC, and recombinant cTnT and cTnI were obtained from HyTest Ltd., Finland.

Blood samples

Normal pooled citrate and heparin plasma samples were prepared from the blood of 20 healthy donors using a standard technique with corresponding vacutainers (Greiner Bio-One GmbH). Citrate and heparin plasma samples from three patients with MI were taken simultaneously 3–7 h after MI. Blood samples of MI patients were collected according to the protocol approved by the Independent Ethical Committee of National Medical Research Centre of Cardiology named after academician E.I. Chazov, Moscow, Russia. All participants were informed of the study in accordance with the current revision of the Helsinki Declaration.

Table 1: Specificity of anti-troponin monoclonal antibodies.

Anti-cTnI mAb	Epitope, aar	Anti-cTnT mAb	Epitope, aar	Anti-cTnT mAb	Epitope, aar
14G5	2–16	35	2–20	375	145–164
916	14–23	25	59–68	376	145–164
M18	19–29	7F4	61–68	387	145–164
810	23–32	7G7	61–68	389	145–164
4C2	24–30	175	69–86	1A11	145–164
M155	27–36	1F2	69–86	1F11	145–164
228	27–36	8	80–99	2G3	145–164
10F4	35–38	20	80–99	401	158–164
19C7	42–50	180	80–99	405	158–178
560	84–94	2A7	93–112	1C11	171–190
16A11	87–91	108	119–138	2	184–190
84	118–127	300	119–138	102	184–203
M46	131–146	329	119–138	7E7	223–242
441	150–158	332	119–138	196	236–242
324	162–179	412	132–138	30	236–255
458	170–179	9	132–152	193	236–255
625	170–179	406	132–152	15	250–268
472	184–193	414	132–152	155	263–281
MF4	191–197	408	145–152	199	275–288
p45-10	196–210				
7B9	Specific to TnC, exact epitope is unknown				
Tcom8	Specific to conformational epitope on cardiac IC-complex				

mAb, monoclonal antibody; aar, amino acid residues.

Matrices for troponin study

The ITC-complex, IC-complex, cTnT, and cTnI were spiked into normal citrate and heparin plasma samples or buffer A solution (20 mM Tris-HCl, pH 7.5, containing 150 mM KCl, 5 mM $CaCl_2$, 75 g/L bovine serum albumin (BSA), 0.15 g/L NaN_3).

Unfractionated heparin (>180 IU (international unit)/mL, Sigma) was used to supply Buffer A with 0.01–50 IU/mL heparin. Alternatively, 9 mL of Buffer A was added to a heparin plasma vacutainer (Greiner Bio-One GmbH) to prepare Buffer A with approximately 17 IU/mL heparin (according to the manufacturer's instructions). Clexane® (2000 anti-Xa IU/0.2 mL, Sanofi-Aventis) was used to prepare Buffer A containing 0.01–50 anti-Xa IU/mL of LMW heparin.

Heparinase treatment of plasma samples

1 mL aliquots of normal heparin or citrate plasmas were incubated with *Bacteroides* Heparinase I (New England BioLabs, final concentration of 12 units/mL) for 4 h at 30 °C, and then on ice for 15 min to stop the reaction and later centrifuged for 5 min at 25,000 *g*, +4 °C.

Heparin and citrate plasma samples incubated for 4 h at 30 °C without heparinase were used as controls.

Sandwich fluoroimmunoassay (FIA)

A two-step sandwich FIA was performed as follows: capture antibodies (10 µg/mL in phosphate-buffered saline; 100 µL per well) were incubated in the wells of 96-well polystyrene plate (Corning Costar) for 30 min at

room temperature (RT) under gentle shaking. The plates were washed thrice with the washing solution (50 mM Tris-HCl buffer, 150 mM NaCl, 0.25 g/L Tween 20, pH 7.8). Following washing, 50 µL of the sample and 50 µL of detection antibodies (200 ng/well) labeled with a stable Eu^{3+} chelate in an assay buffer (50 mM Tris-HCl, 150 mM NaCl, 0.01 % Tween 40, 0.5 % w/v BSA, 0.05 % NaN_3, pH 7.5) were added into the wells and incubated for 1 h at RT under shaking. Following six washes with the washing solution, 200 µL of an enhancement solution (50 mM Glycine-NaOH, 5 mM Na_2CO_3, 5 % v/v glycerol, 20 % v/v 1-propanol, 1 M NaCl, 1.75 M NaSCN, pH 10.0) were added into each well and plates were incubated for 3 min under shaking at RT. Fluorescence was measured using a 1420 Multilabel Counter Victor instrument (PerkinElmer).

Gel filtration chromatography

Gel filtration (GF) studies were performed using an AKTA pure chromatography system (GE Healthcare) on a HiLoad Superdex 200 PG 16/60 column (GE Healthcare). One milliliter of each sample was loaded onto the column that was equilibrated with 50 mM Tris-HCl buffer, pH 7.5, 150 mM NaCl, 5 mM $CaCl_2$, 0.1 % BSA and 0.15 g/L NaN_3. Proteins were eluted at a rate of 2 mL/min, 1 mL fractions were collected and analyzed using FIA.

Results

Interaction of ITC-complex with heparin in different matrices

To investigate whether heparin can bind to cardiac ternary ITC-complex, we spiked 1 µg of native ITC-complex into 1 mL of normal citrate or heparin plasmas or into buffer A with or without 5 IU/mL unfractionated heparin and subjected the samples to GF separation. The fractions were analyzed using the Tcom8-7E7 assay, which is specific only to the ternary ITC-complex (mAb Tcom8 interacts with cTnI and TnC bound together, and mAb 7E7 interacts with the C-terminal part of cTnT). The peak location of the ITC-complex in the citrate plasma corresponded to that in the buffer without heparin. In contrast, the peak of the ITC-complex shifted to a higher molecular weight region in both the heparin-containing buffer and heparin plasma samples (Figure 1).

These data suggest that the apparent molecular mass of the ITC-complex increased in the presence of heparin, indicating that heparin interacts with the full-size ITC-complex.

Interaction of IC-complex and cTnT with heparin in different matrices

To determine which component of the ITC-complex heparin interacts with, we repeated the GF experiments separately with the native artificial binary IC-complex and free cTnT. The IC-complex was analyzed by a 19C7-7B9 assay, in which 19C7 recognizes cTnI, and 7B9 reacts with TnC, cTnT was detected by a 9-1A11 assay specific to the central part of the cTnT molecule.

Heparin did not influence the mobility of the IC-complex, either in the buffer with heparin or in heparin plasma (Figure 2A and B). However, the peak of free cTnT shifted to a higher molecular mass region in both the heparin-containing buffer and heparin plasma (Figure 2C and D). These experiments indicated that heparin interacts with cTnT but not with the IC-complex.

Heparin binding to troponin complex in plasma samples of MI patients

Heparin and citrate plasma samples collected simultaneously from patients with MI were subjected to GF, and the ternary troponin complex was detected using the Tcom8-7E7

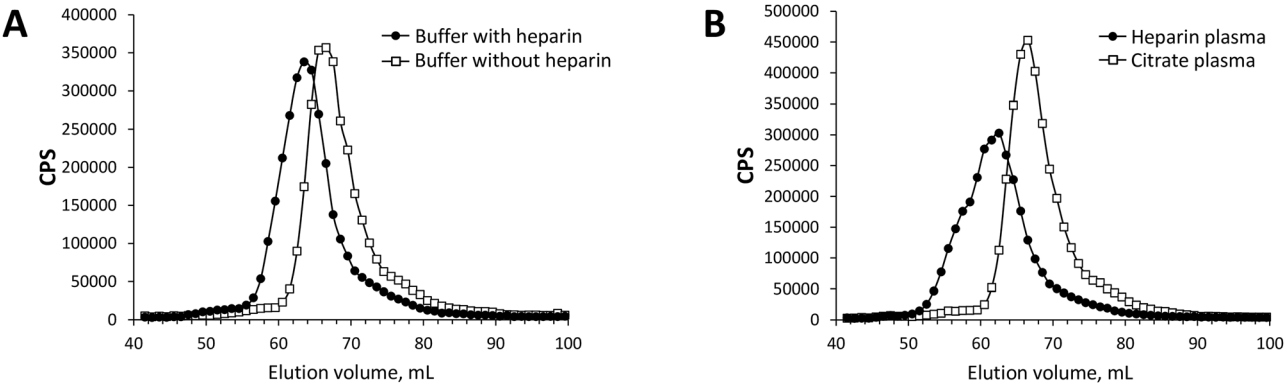

Figure 1: Gel filtration study of ITC-complex in different matrices. (A) ITC-complex in buffer with or without heparin. (B) ITC-complex in heparin and citrate plasmas. Immunoreactivity in fractions was measured by the Tcom8-7E7 assay.

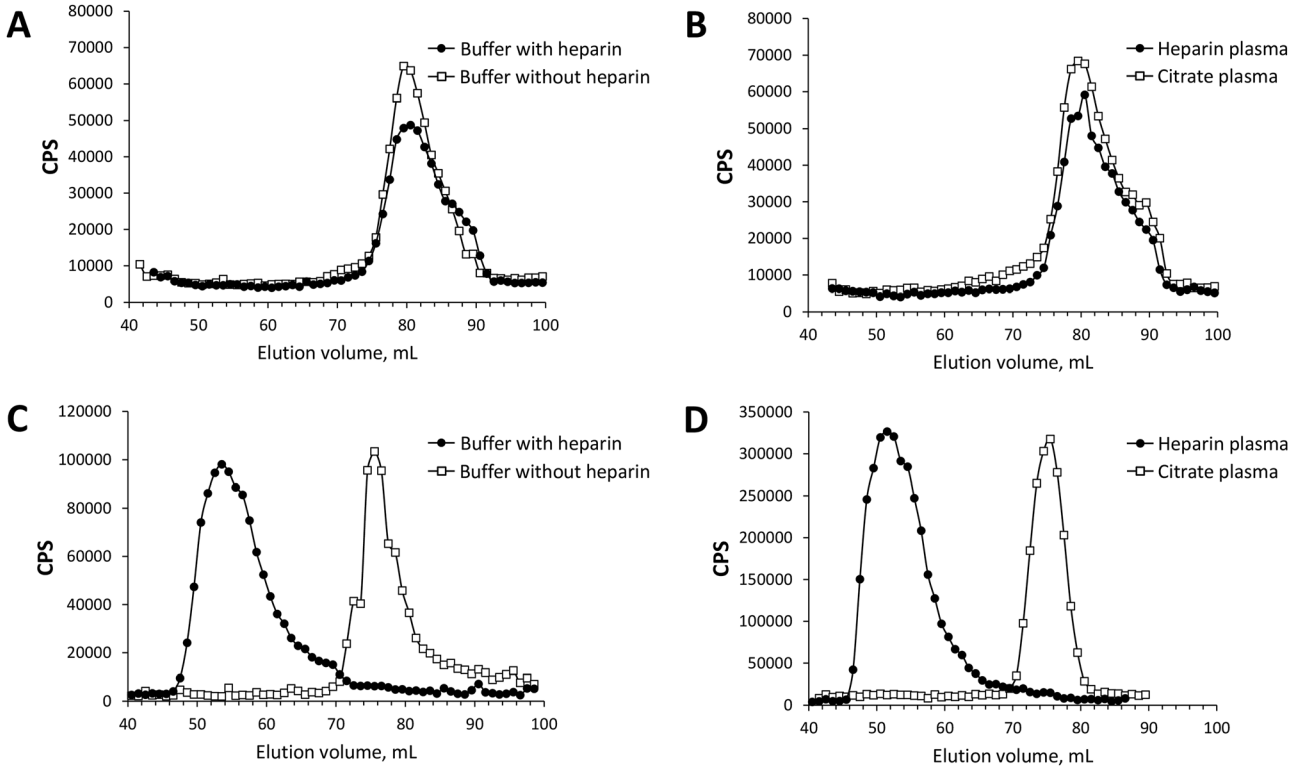

Figure 2: Gel filtration study of IC-complex and cTnT in different matrices. (A) IC-complex in buffer with or without heparin. (B) IC-complex in heparin and citrate plasmas. (C) cTnT in buffer with or without heparin. (D) cTnT in heparin and citrate plasmas. IC-complex and cTnT immunoreactivity in fractions were measured by a 19C7-7B9 and 9-1A11 assays respectively.

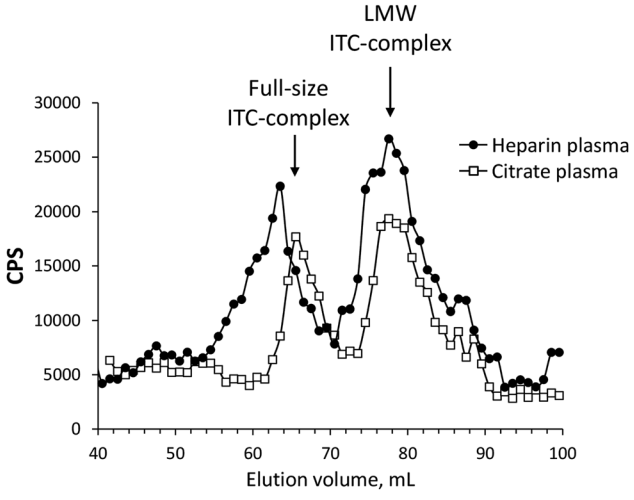

Figure 3: Gel filtration study of ITC-complex in heparin and citrate plasma samples of a representative MI patient. ITC-complexes were detected by the Tcom8-7E7 assay.

assay. In the GF profile, the ITC-complex was presented by two peaks of immunochemical activity: the first peak corresponded to a full-size ternary complex and the second peak was an LMW ternary complex [4].

In the heparin plasma sample, the full-size ternary complex had a higher molecular mass than in the citrate plasma. In contrast, the LMW ITC-complex eluted in the same volume in citrate and heparin plasmas (Figure 3).

This result indicates that heparin interacts with the full-size ITC-complex, but not with the LMW ITC-complex. Since the main difference between these two complexes is that the LMW ITC-complex contains only the C-terminal fragment (approximately 190–287 aar) of cTnT, we presume that heparin interacts with ITC-complex through the N-terminal and/or central parts of cTnT.

Identification of cTnT regions that are sensitive to heparin influence

To identify the regions of the cTnT molecule that are affected by heparin, we spiked cTnT or the ITC-complex into the buffer solution that contained or did not contain heparin or into normal citrate and heparin plasma samples and compared the recoveries of the proteins in these matrices. To detect free cTnT, we used 7G7 or 1C11 mAbs (depending on the cTnT region tested) as capture and various anti-cTnT mAbs specific

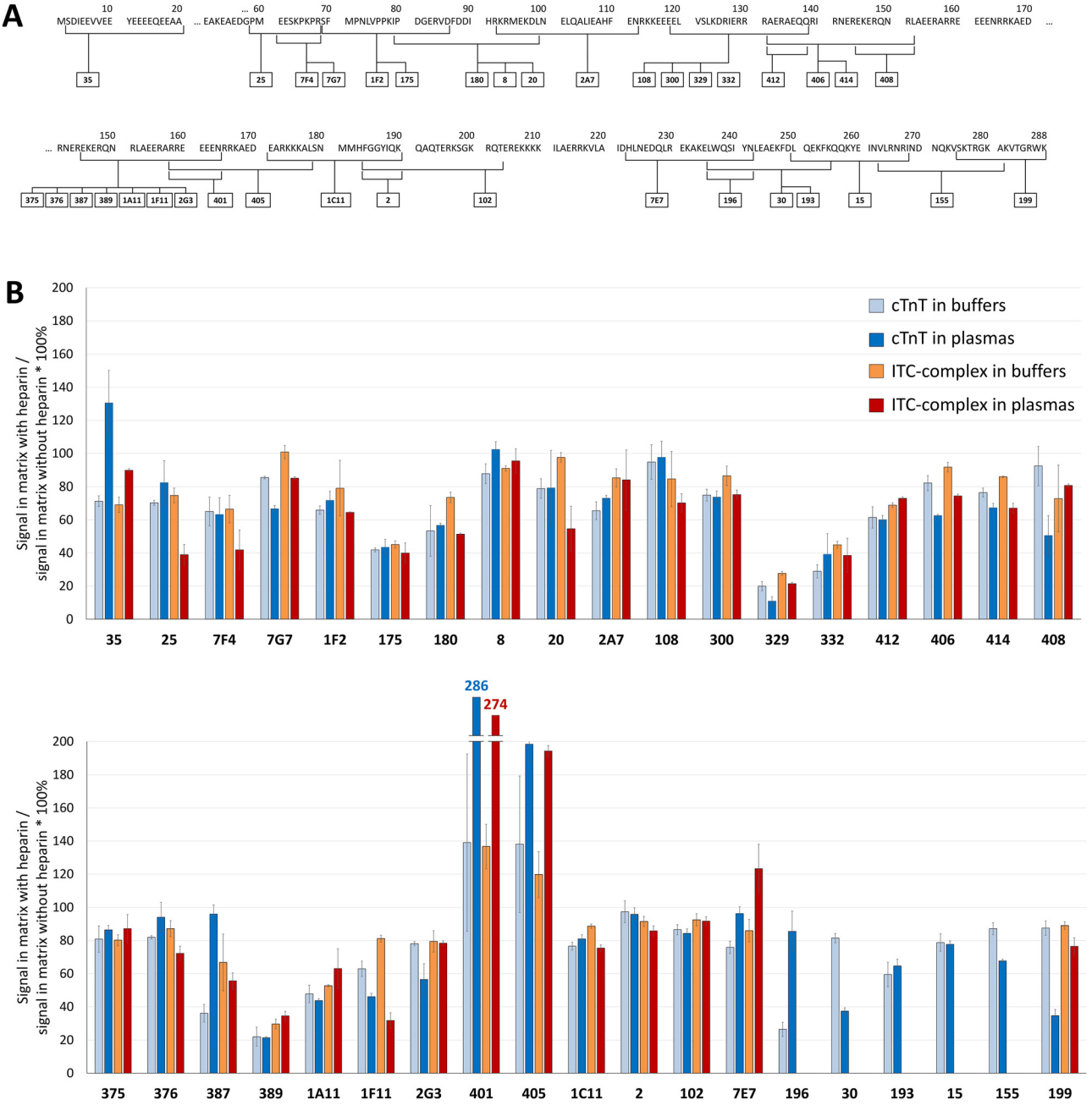

Figure 4: Detection of cTnT regions that are sensitive to heparin influence in the plasma and buffer. (A) cTnT amino acid sequence; epitopes of different antibodies are shown. (B) Recoveries of cTnT and ITC-complex in the presence or absence of heparin. MAbs 7G7 or 1C11 (for conjugates of mAbs 25, 7F4, 7G7, 175, 1F2) were used as capture antibodies. Names of the antibodies that were used for detection are stated below the graph. Please note that the cTnT epitopes of mAbs 196, 30, 193, 15 and 155 (approximately aar 236–281) are hindered in ITC-complex by cTnI and TnC. The graph shows the ratio of the signals in matrix with heparin (buffer with heparin or heparin plasma) to the signals in matrix without heparin (buffer or citrate plasma) given in % (average±SD).

to different regions of the cTnT molecule for detection (Figure 4). To minimize the influence of the capture antibody on the detection of cTnT in the ITC-complex, we used mixed sandwich assays comprising anti-cTnI mAb MF4 as the capture antibody and the same set of anti-cTnT mAbs for detection (see Supplementary Figure S1).

We discovered that the recovery of both free cTnT and ITC-complex in heparin-containing matrices (buffer with

heparin or heparin plasma) was lower by approximately 10 % than in matrices without heparin (buffer solution without heparin or citrate plasma, respectively) in most of the assays. However, in several assays, the recovery in heparin-containing matrices was lower by 35–90 %. These assays used detection antibodies that recognized the regions bordered by approximately 69–99 aar (mAbs 175, 180), 119–138 aar (mAbs 329, 332) and 145–164 aar (1A11, 1F11, 2G3, 375, 387, 389). We also observed that mAbs 401 and 405 specific to aar 158–178 gave significantly (2-3-fold) higher signals in the presence of heparin.

The recovery of free cTnT was similar to that of cTnT in the ITC-complex in most assays. However, several mAbs specific to the C-terminal fragment of free cTnT (mAbs 196 and 30, epitopes lie approximately between 236 and 255 aar) showed considerably lower signals in heparin-containing matrices. Notably, this region of cTnT is hindered in the ternary ITC-complex by cTnI and/or TnC.

Since heparin is highly negatively charged and was shown to bind with some proteins via calcium ions [27, 28], one can presume that heparin binding to cTnT may be mediated by Ca^{2+}. Although the interaction of cTnT with Ca^{2+} was not shown yet, we have checked this hypothesis and found that the absence of calcium ions does not affect the binding of cTnT with heparin (see Supplementary Figure S2).

Immunodetection of cTnI in the presence of heparin

We also studied the influence of heparin on the detection of cTnI after spiking with free cTnI, the IC-complex, and the ITC-complex in buffer solutions with or without heparin, or in citrate and heparin plasmas. Anti-cTnI mAb MF4 or anti-TnC mAb 7B9 were used for capture, and various anti-cTnI mAbs were used for detection. Due to the high non-specific binding of free cTnI, the results with this protein could not be confidently interpreted (data not shown). As in the case of cTnT, most of the signals for the IC- and ITC-complexes in heparin-containing buffer or heparin plasma were approximately ~10–20 % lower than in buffer without heparin or citrate plasma. However, we found only one anti-cTnI mAb that was considerably affected by the presence of heparin (10F4, region 33–37 aar, Figure 5). Interestingly, the influence of heparin on this region appeared only in heparin plasma and not in heparin-containing buffer A. These data are in accordance with gel filtration studies and indicate that heparin mostly affects cTnT, but not cTnI.

The influence of heparinase on the detection of cTnT in heparin plasma

To confirm that the specific decrease in cTnT recovery in heparin plasma in some assays was due to heparin, we treated normal heparin plasma with heparinase, an enzyme that specifically cleaves heparin. Normal heparin, heparinase-treated heparin, and normal citrate plasmas were spiked with cTnT, and cTnT levels were measured using several assays that previously showed a low recovery of spiked cTnT in heparin plasma samples. In heparin plasma pretreated by heparinase, the signals recovered from 19.3 ± 0.9 % to 112 ± 1.3 % in the 7G7-329 assay and from 44.7 ± 1.4 % to 115.5 ± 2.8 % in the 7G7-389 assay as compared with the signals of cTnT were measured by these assays in citrate plasma (see Supplementary Figure S3).

Influence of low molecular weight heparin on the immunodetection of cTnT

Since LMW heparin is often used to prevent blood coagulation in MI therapy, we studied its effect on the immunochemical detection of cTnT using two assays that we found to be sensitive to unfractionated heparin. In this study, we added Clexane® at different concentrations to cTnT that was dissolved in Buffer A (Figure 6).

The concentration of approximately 0.5–2 units of unfractionated heparin per mL of buffer solution was sufficient to decrease the immunoreactivity of cTnT by half in the tested assays (Figure 6A). LMW heparin also decreased the recovery of cTnT; a two-fold decrease was detected at a concentration of approximately 10 anti-Xa IU/mL in the 7G7-329 assay (Figure 6B).

Discussion

Heparin is an established anticoagulant that is widely administered intravenously to prevent clot formation in the circulatory system of patients with MI. It is also used to prepare heparin plasma samples [22]. If serum samples are taken shortly after heparin administration, they may contain sufficient heparin to prevent serum clotting [13]. Controversial data regarding the influence of heparin on the immunodetection of cTnI and cTnT have been reported for several decades. However, it is unclear whether heparin directly interacts with troponins.

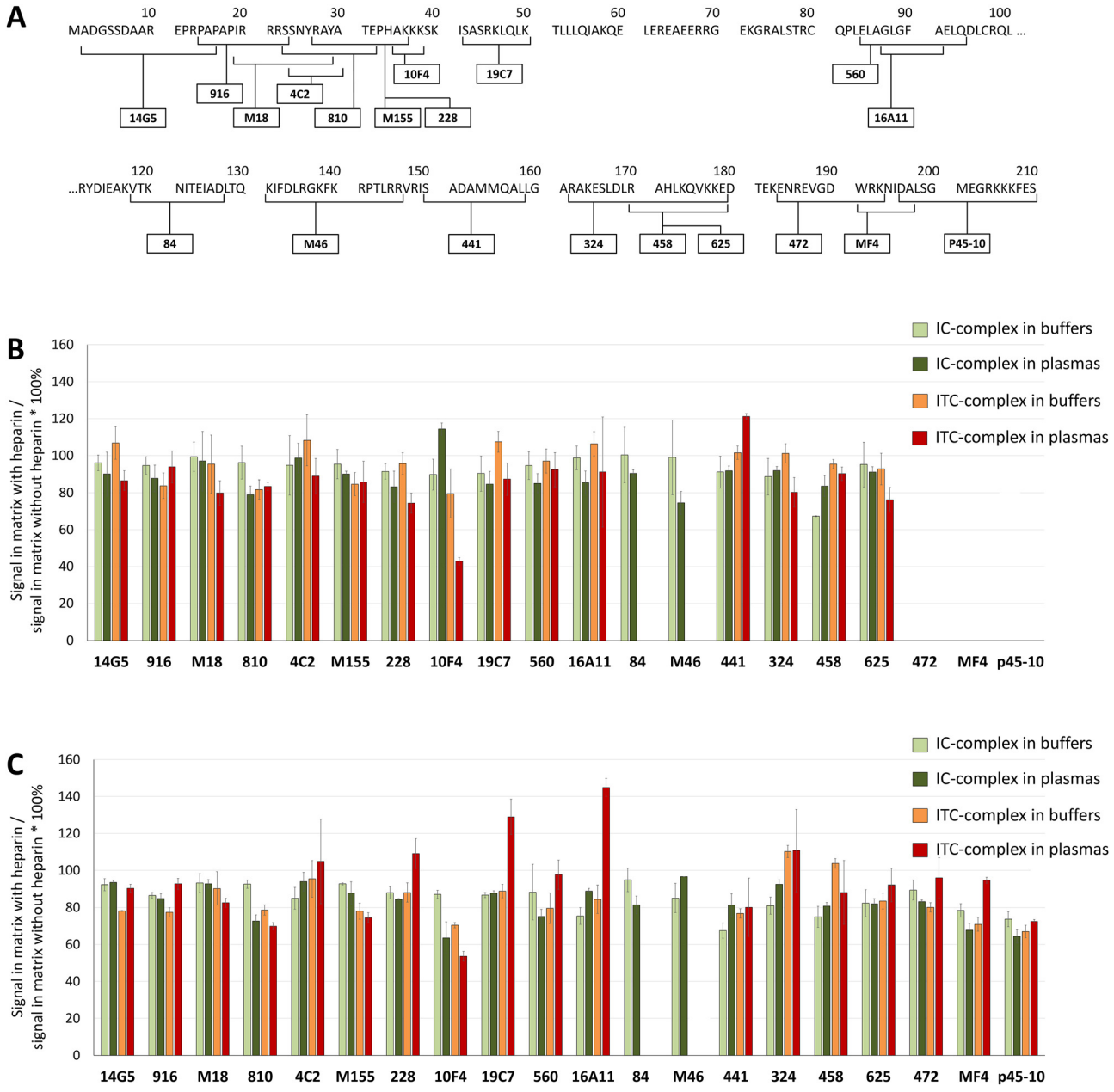

Figure 5: Detection of cTnI regions that are sensitive to heparin influence. (A) cTnI amino acid sequence; approximate epitopes of anti-cTnI antibodies used in the assays are shown. (B–C) Recoveries of IC-complex and ITC-complex in the presence or absence of heparin. (B) Anti-cTnI mAb MF4 is used as capture. Please note that mAbs 472 and p45-10 do not form pairs with mAb MF4 due to the proximity of the epitopes. (C) Anti-TnC mAb 7B9 is used as capture. MAbs specific to aar ~40–80 of cTnI were not used in this study, for this part of cTnI molecule is hindered by TnC and cTnT. Please note that a region limited by approximatly 117–145 aar (mAbs 84 and M46) is hindered by cTnT and therefore cTnI is undetectable in ITC-complex. The graph shows the ratio of the signals in matrix with heparin to the signals in matrix without heparin given in % (average±SD).

In this study, the interaction of heparin with the ITC-complex was demonstrated by a gel filtration study, in which the peak of the ITC-complex shifted to a higher apparent molecular mass region in the presence of heparin. As heparin did not change the mobility of the IC-complex but shifted the peak of free cTnT to a higher molecular mass region, we suggest that heparin interacts with the ITC-complex via the cTnT molecule. Our findings confirm the data of Speth et al. [29], where the binding of heparin to cTnT was shown by affinity chromatography of troponin from patients with MI on heparin Sepharose.

Although there are no motifs in the amino acid sequence of cTnT that are considered to be targets for binding to heparin (XBBXBX or XBBBXXBX, where B is a basic amino

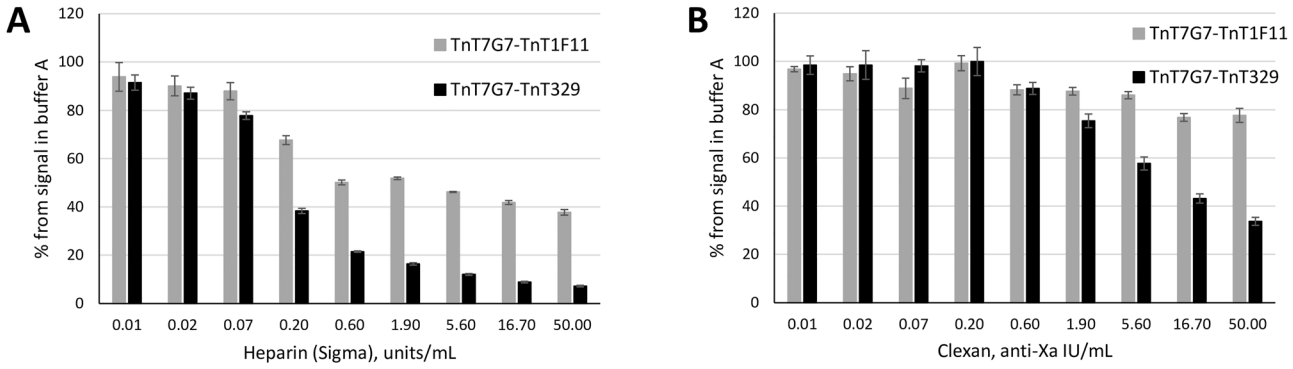

Figure 6: Recovery of cTnT in buffer with unfractionated heparin and LMW heparin. (A) Unfractionated heparin. (B) LMW heparin. Recovery of cTnT in buffer A without heparin was taken for 100 %. Anti-TnT7G7 was used as capture antibody and 1F11 and 329 as detection mAbs (average±SD).

acid and X is a hydrophobic residue [30]), many researchers assume that the prediction of binding sites by amino acid sequence is too inaccurate due to a strong influence of the spatial structure of the protein on its properties. This problem should be solved for each protein-glycosaminoglycan complex individually [31–33], especially given the possibility of both specific and non-specific binding [34].

To reveal the sites affected by heparin on the cTnT molecule, we spiked the ITC-complex into buffer solutions with or without heparin or into normal citrate and heparin plasma and compared the recognition of different cTnT epitopes by various anti-cTnT mAbs. The signals were generally lower in heparin-containing matrices than in buffers without heparin or citrate plasma by approximately 10–20 % for most of the mAbs. However, there were antibodies that were more sensitive to the presence of heparin. These mAbs were specific to 69–99 aar, 119–138 aar, and 145–164 aar that are located in the N-terminal and central parts of the cTnT molecule. This, together with the observation that the full-size native ITC-complex shifted to a higher molecular mass area upon GF of a heparin plasma sample from patients with MI, whereas the LMW ITC-complex that lacks these parts of cTnT did not shift, indicates that the N-terminal and central parts of cTnT are the main targets for heparin interaction.

It is important to note that we have observed the difference in the recovery for some antibodies specific to the same epitope, e.g. mAbs 108, 300 and 329, 332 (Figure 4). In this study the epitopes of mAbs were identified utilizing the 20 aar-long peptide library. The epitope of an antibody is usually shorter (5–10 amino acids), so the differences in the recoveries could be explained by the assumption that these mAbs are specific to the different portions of the same 20-aar peptide. It can also be assumed that some antibodies that interact with different overlapping peptides have a same or nearby epitope and thus give equally reduced signals in the presence of heparin (e.g. mAb 175, epitope 69–86 aar, and mAb 180, epitope 80–99 aar). According to that we may reduce the possible site of heparin interaction to the region comprising 80–86 aar. As there were no available mAbs specific to the 20–60 aar of cTnT, we could not investigate heparin binding to that part of the molecule. Two mAbs specific to the region bordered by aar 158–178 (401 and 405) gave significantly increased signals in heparin matrices. This effect was more pronounced in plasmas than in buffers. We presume that interaction with heparin could change the conformation of the cTnT in this area and lead to a better exposure of this epitope to the antibodies.

As free cTnT shifted in the gel filtration profile to a higher molecular mass region much further than the ITC-complex, we suggest that free cTnT had more sites available for heparin interactions than the ITC-complex. This was confirmed by the experiments that revealed an additional epitope sensitive to heparin at the C-terminus of free cTnT (Figure 4). However, in the ternary ITC-complex, most of this part of cTnT is shielded by cTnI or TnC molecules and is probably not available for heparin. Free full-sized cTnT is not detected in the blood samples [4] but regions affected by heparin are located on the surface of the ternary complex and could be present as stable fragments in patients' blood [8].

In our gel filtration study, we did not observe any interactions between heparin and the IC-complex. However, heparin bound to cTnT in the ITC-complex could affect the detection of cTnI using anti-cTnI mAbs. Therefore, we tested cTnI in the IC- and ITC-complexes for possible susceptibility to heparin utilizing a panel of anti-TnI mAbs. As we observed it in case of cTnT, all anti-cTnI assays gave 10–15 % lower signals in heparin-containing matrices, but we did not identify any specific regions where anti-cTnI antibodies gave much lower signals in buffer solution in the presence of heparin, as was found for cTnT. This may indicate that cTnI in the ITC-complex does not contain sites available for

heparin binding and that heparin does not significantly affect the detection of cTnI. However, mAb 10F4 (34–37 aar) showed a significantly lower signal in heparin plasma than in citrate plasma in the case of the ITC-complex, although we observed no difference in the recognition of cTnI by this antibody in buffer solutions with or without heparin. In this case, we assume that it is not the effect of heparin itself but the combined effect of heparin with some plasma components that influences the immunodetection of cTnI by this mAb.

We found that for some mAb pairs, unfractionated heparin decreased cTnT recovery by 50 % or more, even at concentrations as low as 0.5–2 IU/mL. This is much lower than the heparin concentration in the vacutainer tube (17 IU/mL according to the manufacturer's information) and could be close to the peak concentration of heparin in the blood after its administration to patients. LMW heparin (enoxaparin sodium, Clexan®) that is used for prevention of coagulation after MI also decreased cTnT recovery in the regions affected by unfractionated heparin. However, the effect of enoxaparin can be observed at a concentration of at least 10 anti-Xa IU/mL that exceeds Clexan® concentrations in the blood of patients, which can reach at most 1.16–1.5 anti-Xa UI/mL according to the manufacturer's information. This finding requires further investigation, but it seems that the injected enoxaparin, even at its peak concentration, would not exert a considerable effect on the immunodetection of cTnT.

Commonly used assays for cTnT detection utilize mAbs specific to the region affected by heparin, and the first assays were susceptible to heparin interference [8]. The latest generation of anti-TnT assays gives similar results in serum and heparin plasma samples, indicating that the manufacturer was able to eliminate heparin interference without changing the antibodies [11]. However, according to our data, the possible effect of heparin on the interaction of antibodies with cTnT should be taken into account when developing new immunochemical systems.

Conclusions

Heparin binds to free cTnT and the cardiac ITC-complex via cTnT. In the ITC-complex, heparin binding sites are located at the N-terminal and central parts of the cTnT molecule. In the present study, we did not observe the binding of heparin to cTnI. Heparin hinders the determination of the ITC-complex via cTnT by certain antibodies, and this effect should be considered when selecting antibodies for new generations of cTnT assays.

Research ethics: Blood samples of MI patients were collected according to the protocol approved by the Independent Ethical Committee of National Medical Research Centre of Cardiology named after academician E.I. Chazov, Moscow, Russia. All procedures were performed in accordance with the current revision of the Helsinki Declaration.

Informed consent: Informed consent was obtained from all individuals included in this study, or their legal guardians or wards.

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