Thrombosis Research xxx (xxxx) xxx

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Short communications

Differential regulation of human thrombospondin-1 upon systemic desmopressin *versus* endotoxin challenge

Andreas Scheuba [a], Branislav Zagrapan [a], Luca Martelanz [a], Vanessa Eder [a], Nahla Ibrahim [a], Sonja Bleichert [a], Viktoria Knöbl [a], Hubert Hayden [a], Sarah von Kuenheim [a], Katharina Münch [a], Nina Buchtele [b,c], Christian Schoergenhofer [d], Katarina D. Kovacevic [d], Edith Lackner [d], Christa Drucker [d], Christoph Neumayer [a], Bernd Jilma [d], Christine Brostjan [a,*]

[a] *Department of General Surgery, Division of Vascular Surgery, Medical University of Vienna, University Hospital Vienna, Austria*
[b] *Department of Medicine I, Intensive Care Unit 13i2, Medical University of Vienna, University Hospital Vienna, Austria*
[c] *Intensive Care in Hematologic and Oncologic Patients (iCHOP), Medical University of Vienna, University Hospital Vienna, Austria*
[d] *Department of Clinical Pharmacology, Medical University of Vienna, University Hospital Vienna, Austria*

ARTICLE INFO

Keywords
Thrombospondin-1
Von Willebrand factor
Endotoxin
Desmopressin

1. Introduction

Von Willebrand factor (vWF) and thrombospondin-1 (TSP-1) are glycoproteins with procoagulant and prothrombotic properties, playing additional roles in inflammation and cell adhesion [1,2]. They are stored in granules of platelets and endothelial cells (ECs) and are released upon cell activation by agonists like lipopolysaccharide (LPS) or desmopressin [1,2]. *In vivo* changes in blood concentrations of vWF have previously been studied in human models of desmopressin or low-dose LPS administration to healthy volunteers [3–5], while little is known about the time course of TSP-1 plasma concentrations in these settings [6]. Of note, systemic LPS induces acute inflammatory responses and a transient loss of leukocytes and platelets from circulation by adhesion to lung and liver endothelium [7,8]. In contrast to LPS, desmopressin is not a major activator of leukocytes or platelets but triggers vWF release from endothelial Weibel-Palade-bodies [2]. Thus, despite their functional interplay and concomitant production in platelets and ECs, a distinct control of vWF and TSP-1 release by these cellular activators seemed feasible and of interest when clinically addressing selective properties of the two molecules in inflammatory or hemostasis disorders.

To gain more insight into the *in vivo* regulation of TSP-1 and compare it to vWF release from ECs and platelets, we performed two human studies with healthy volunteers who received either 0.3 μg/kg body-weight desmopressin [9], 2 ng/kg LPS or placebo intravenously [10]. The primary objective was to establish the kinetics of circulating TSP-1 after stimulation with LPS or desmopressin. Secondary analyses included the time course of vWF, platelets and leukocytes after challenge. To address the potential role of TSP-1 in changes of circulating leukocytes and platelets during experimental endotoxemia, we further conducted studies in wildtype *versus* TSP-1 knockout mice upon administration of 0.5 mg/kg LPS. A detailed description of methods is provided in the Supplemental material. TSP-1 and vWF were determined in plasma samples by enzyme-linked immunosorbent assay, and blood cell counts were assessed by hematology analyzer.

2. Results

Data points were calculated in percent of baseline (set to 100 %) for each participant to adjust for biological variability in starting levels. Absolute plasma concentrations are specified and illustrated in Supplemental files 1 and 2.

* Corresponding author at: Department of General Surgery, Division of Vascular Surgery, Medical University of Vienna and University Hospital Vienna, Anna Spiegel Center for Translational Research, 25.05.002, Waehringer Guertel 18-20, 1090 Vienna, Austria.
E-mail address: christine.brostjan@meduniwien.ac.at (C. Brostjan).

https://doi.org/10.1016/j.thromres.2024.01.011
Received 15 September 2023; Received in revised form 28 December 2023; Accepted 12 January 2024
Available online 15 January 2024

2.1. Circulating vWF is rapidly increased upon desmopressin challenge, but shows a delayed induction by LPS

The plasma concentration of vWF antigen increased rapidly upon desmopressin infusion (Fig. 1A), *i.e.* was significantly elevated after one hour with a median increase of 262 % (IQR: 64 %, $p < 0.001$ for 1 h *vs.* baseline) and remained high at two hours before gradually dropping again to almost baseline values at 24 h.

In contrast, LPS infusion led to an initial, statistically significant decrease of median vWF antigen levels to a minimum of 74 % of baseline after 1.5 h (IQR: 11 %, $p = 0.043$; 1.5 h *vs.* baseline) which has not been reported before, as most studies did not include this time point. Yet, two hours after LPS infusion vWF antigen increased significantly and reached a maximum of 217 % (IQR: 119 %) after four hours ($p = 0.003$; 4 h *vs.* baseline) followed by a slow decline.

2.2. Thrombospondin 1 plasma levels are increased by desmopressin but not by LPS challenge

When TSP-1 plasma concentrations were determined in the same samples, induction by desmopressin was delayed and less pronounced compared to vWF changes, *i.e.* a maximum median increase of 215 % (IQR: 125 %) was reached after two hours ($p < 0.001$). TSP-1 levels declined slowly over 8 h and reached baseline values at 24 h.

Remarkably, TSP-1 plasma concentrations decreased immediately upon LPS stimulation and reached a minimum of 45 % (IQR: 23 %, $p = 0.004$) after two hours. After four hours, TSP-1 plasma concentrations had returned to baseline levels but were never significantly increased within the observation period of 24 h (Fig. 1B).

2.3. A loss of leukocytes from circulation is triggered by LPS but not by desmopressin

White blood cells (WBCs) remained at baseline values after desmopressin administration for the initial two hours and increased significantly after four hours to a maximum level of 148 % (IQR: 69 %, $p = 0.001$). The time course of blood leukocytes was substantially different upon LPS stimulation of healthy human volunteers. WBC count initially decreased to 49 % (IQR: 41 %, $p = 0.028$) of baseline after 1.5 h and increased after two hours, reaching a maximum median value of 163 % (IQR: 46 %, $p = 0.001$) at 8 h (Fig. 1D). Fig. 1E–G illustrates differences in the time course of leukocyte populations upon desmopressin or LPS challenge, with monocytes and lymphocytes experiencing a more pronounced and prolonged loss in endotoxemia than the predominant subset of neutrophils.

2.4. LPS induces a more pronounced drop in platelets than desmopressin

Platelets decreased moderately upon desmopressin administration to 93 % (IQR: 13 %, $p = 0.002$) of baseline value after one hour, and to 91 % at later time points (4–8 h). There were no significant differences between the desmopressin and placebo period at any given time point during the study duration.

However, the drop of platelets after LPS challenge was more

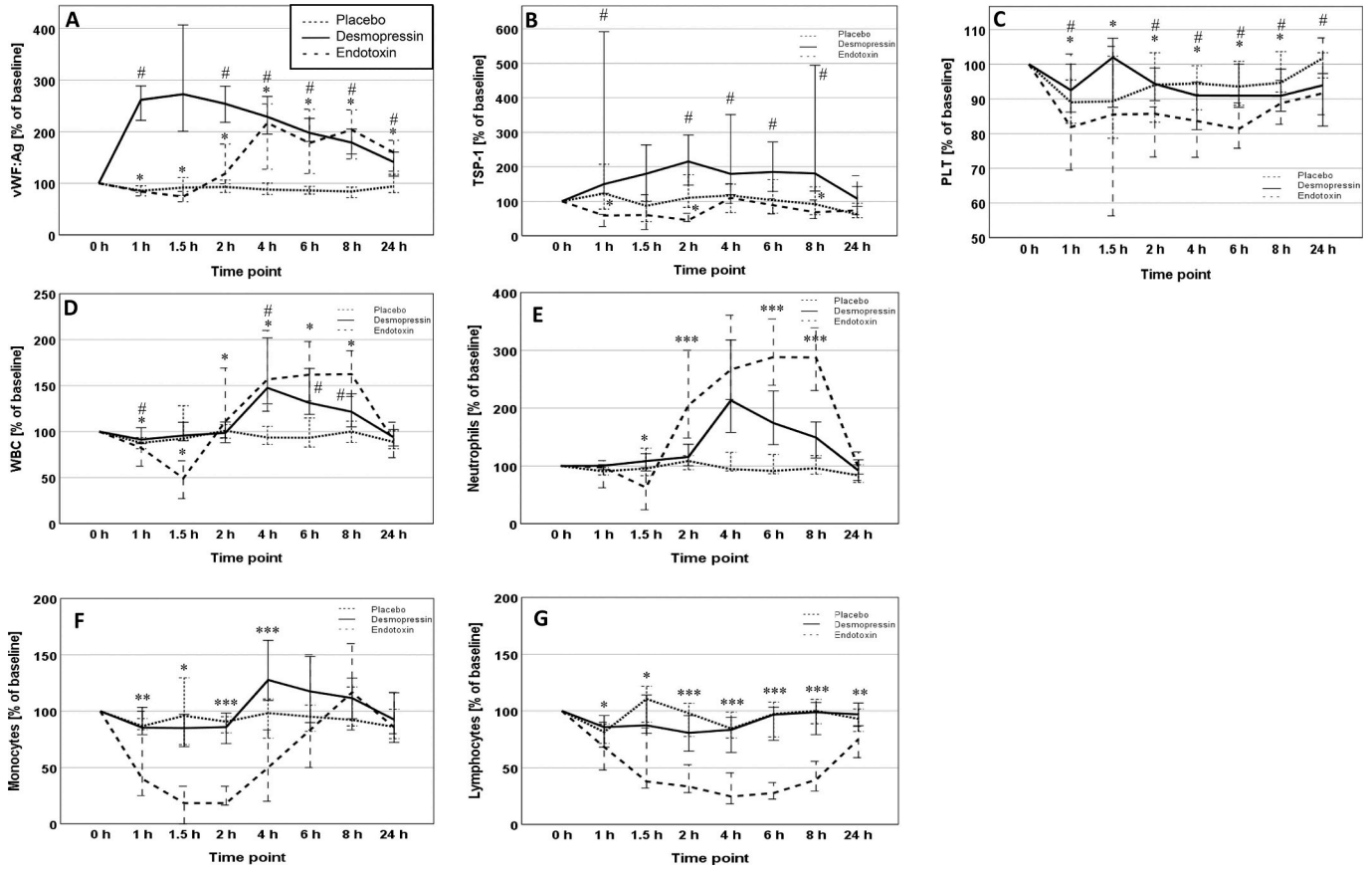

Fig. 1. Line graphs illustrating relative changes compared to baseline (set to 100 %) of A) vWF antigen (vWF:Ag), B) TSP-1, C) platelet (PLT) count, D) white blood cell (WBC) count, E) neutrophil count, F) monocyte count, and G) lymphocyte count in response to 0.3 μg/kg desmopressin ($n = 16$), placebo ($n = 12$) or 2 ng/kg body weight LPS ($n = 12$–15). Of note, a limited number ($n = 4$–6) of blood samples was retrieved at 1.5 h. Data represent median blood levels and 95 % confidence intervals. A–D) # and * indicate significant changes (Wilcoxon signed-rank test) compared to baseline for the desmopressin and LPS trial, respectively. E–G) p-values (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) refer to group differences between the LPS and desmopressin group at the indicated time points and were based on Mann-Whitney-*U* test.

pronounced than for desmopressin administration. Platelets reached a minimum of 82 % (IQR: 19 %, p = 0.028) of baseline at 1 h after LPS administration and remained decreased for 6 h (Fig. 1C).

2.5. Loss of circulating leukocytes and platelets upon LPS challenge does not differ between TSP-1 deficient and wildtype mice

As the characteristic initial drop of circulating blood cells in human endotoxemia was accompanied by a pronounced decrease of TSP-1 plasma levels which was not observed upon desmopressin challenge, we addressed the question whether TSP-1 might be causally involved in the transient loss of blood cells from circulation. Thus, mice deficient in TSP-1 were compared to wildtype animals, *i.e.* were challenged with 0.5 mg/kg LPS. Platelet (Fig. 2A) and leukocyte (Fig. 2B) counts were determined over a time frame of 8 h.

Leukocyte counts dropped rapidly within 1 h of LPS infusion and remained decreased for the entire monitoring period. Wildtype and TSP-1 ko mice did not differ significantly at any investigated time point, reaching a maximum decrease of leukocytes to 14 % (IQR: 13 %) of baseline after two hours in wildtype animals and 16 % (IQR: 7 %) after four hours in TSP-1 deficient mice. Among the three major WBC populations (Fig. 2C–E), granulocyte counts displayed the fastest recovery in circulation.

Comparably, levels of blood platelets (Fig. 2A) dropped gradually to a minimum of 55 % (IQR: 16 %) and 57 % (IQR: 30 %) at 8 h after LPS challenge in wildtype and TSP-1 deficient animals, respectively. A significant group difference (p = 0.001) was only recorded for the 6 h time point, where TSP-1 ko animals showed a faster loss of platelets from circulation (61 %; IQR: 2 %) than wildtype mice (77 %; IQR: 19 %).

3. Discussion

In this study we compared the *in vivo* kinetics of TSP-1 and vWF upon administration of desmopressin, LPS or placebo in healthy human volunteers and observed a distinct, stimulus-dependent regulation for the two molecules. In line with previous analyses, circulating vWF was potently increased by both agonists [3–5,9]. Plasma TSP-1 also increased upon desmopressin stimulation. However, TSP-1 dropped immediately after LPS administration to about half of starting level and was never elevated in experimental endotoxemia. This observation was highly unexpected, because vWF and TSP-1 are both produced in platelets and ECs and are co-stored in platelet α–granules [1,2], suggesting that systemic activation by LPS should result in their concomitant release.

The differences in TSP-1, vWF and blood cell kinetics upon LPS *versus* desmopressin administration are possibly explained by proinflammatory processes during endotoxemia that are not elicited by desmopressin challenge. LPS triggers expression of adhesion molecules like selectins on activated vascular cells. Hence, leukocytes and platelets accumulate in the microvasculature of lungs and liver which causes systemic leukocytopenia and thrombocytopenia [7,8]. TSP-1 and vWF are both glycoproteins with pro-adhesive properties in primary hemostasis that could also play a role in cell sequestration during endotoxemia. Furthermore, cellular uptake and degradation of TSP-1 is rapidly promoted by the low density lipoprotein receptor-related protein with regulatory functions in inflammation [11]. It is thus conceivable that TSP-1 might be consumed during blood cell sequestration in lungs and liver and therefore vanishes from circulation after systemic LPS challenge. Of note, TSP-1 plasma levels dropped considerably more in systemic endotoxemia when compared to vWF plasma levels.

To address this potential role of TSP-1 we compared the time course of circulating leukocytes and platelets in wildtype C57BL/6 *versus* TSP-1 knockout mice after endotoxin administration. LPS induced a rapid and pronounced loss of leukocytes from circulation in both wildtype and TSP-1 deficient mice. However, there were no significant group differences. In contrast to our hypothesis, the loss of platelets from circulation seemed to proceed slightly faster in TSP-1 knockout mice. Yet, they only differed significantly from wildtype animals at the 6 h time point, thus not substantiating the notion that TSP-1 is crucially involved in the proinflammatory sequestration of leukocytes or platelets upon LPS challenge in mice.

In conclusion, TSP-1 and vWF show a comparable increase after desmopressin challenge but a discordant regulation upon LPS infusion. While TSP-1 may bind to activated cell surfaces and has previously been reported to promote leukocyte recruitment to local sites of infection and inflammation [12], it does not seem to contribute significantly to blood cell sequestration in systemic endotoxemia. It remains to be determined whether this is due to a redundancy of adhesion molecules [7,8], the distinct blood cell composition of mice and men, or whether TSP-1 effects on blood cell infiltration are more readily detected at local sites

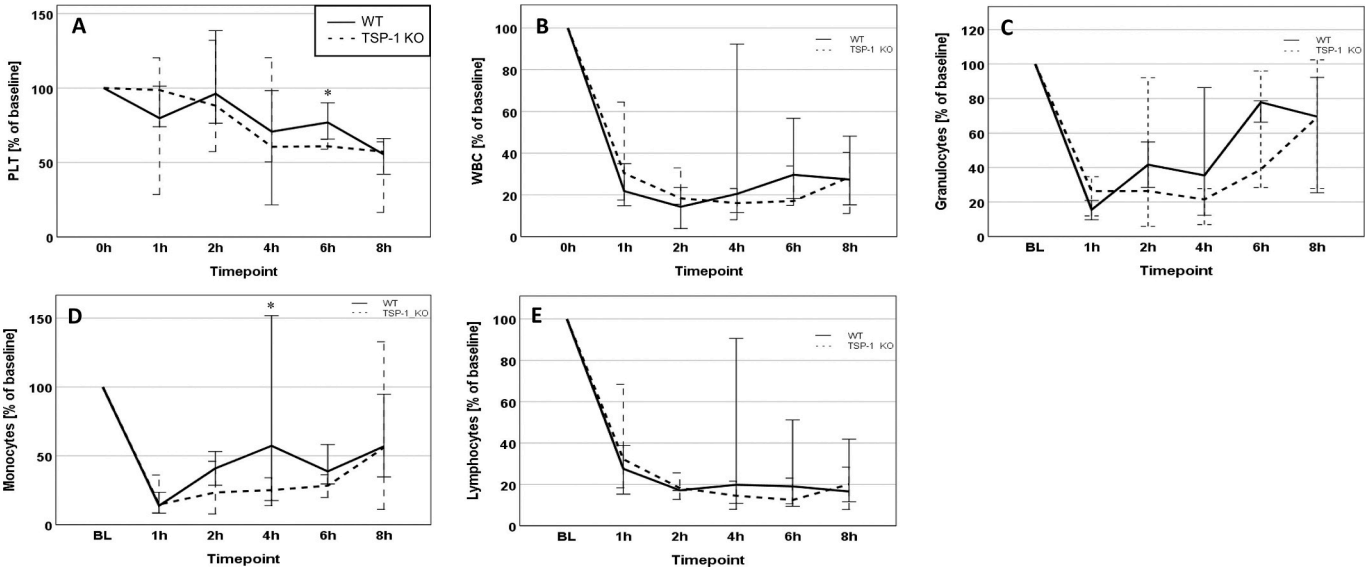

Fig. 2. Line graphs illustrating the relative changes compared to baseline (set to 100 %) of A) platelet (PLT), B) white blood cell (WBC), C) granulocyte, D) monocyte, and E) lymphocyte count upon administration of 0.5 mg LPS/kg mouse bodyweight in wildtype (WT: C57BL6/J) *versus* TSP-1 knockout (TSP-1 KO: B6.129S2-Thbs1^{tm1Hyn}/J) mice. Data represent median blood levels and 95 % confidence intervals. p-Values (*p < 0.050) refer to group differences at the indicated time points and were based on Mann-Whitney-U test.

than by loss from systemic circulation.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.thromres.2024.01.011.

Funding

This research was funded in whole by the Austrian Science Fund (FWF) [F 5409-B21 issued to C. Brostjan]. For the purpose of open access, the author has applied a CC BY public copyright license to any author-accepted manuscript version arising from this submission. The sponsors had no role in study design, the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

Declarations

The human studies were conducted at the Dept. Clinical Pharmacology, Medical University of Vienna [9,10] according to the ethical principles of the Declaration of Helsinki of 1964 and its later amendments. They were approved by the Ethics Committee of the Medical Univ. Vienna. All healthy volunteers gave written informed consent. Animal experiments were approved by the local ethics committee and the Austrian Ministry of Science (BMWFW-66.009/0048-WF/V/3b/2016), conforming to the European Directive 2010/63/EU on the protection of animals used for scientific purposes and the Austrian Animal Experiment Act 2012.

CRediT authorship contribution statement

Andreas Scheuba: Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing, Investigation. **Branislav Zagrapan:** Conceptualization, Data curation, Formal analysis, Investigation, Writing – review & editing. **Luca Martelanz:** Data curation, Investigation, Writing – review & editing. **Vanessa Eder:** Data curation, Investigation, Writing – review & editing. **Nahla Ibrahim:** Data curation, Investigation, Writing – review & editing. **Sonja Bleichert:** Data curation, Investigation, Writing – review & editing. **Viktoria Knöbl:** Data curation, Investigation, Writing – review & editing. **Hubert Hayden:** Data curation, Formal analysis, Investigation, Writing – review & editing. **Sarah von Kuenheim:** Data curation, Investigation, Writing – review & editing. **Katharina Münch:** Data curation, Investigation, Writing – review & editing. **Nina Buchtele:** Data curation, Investigation, Methodology, Writing – review & editing. **Christian Schoergenhofer:** Methodology, Writing – review & editing. **Katarina D. Kovacevic:** Methodology, Writing – review & editing. **Edith Lackner:** Methodology, Writing – review & editing. **Christa Drucker:** Methodology, Writing – review & editing. **Christoph Neumayer:** Conceptualization, Project administration, Supervision, Writing – review & editing, Funding acquisition. **Bernd Jilma:** Conceptualization, Project administration, Supervision, Writing – original draft, Writing – review & editing, Funding acquisition. **Christine Brostjan:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that support the findings of this study are available from the corresponding author, CB, upon reasonable request.

Acknowledgements

We thank Prof. Podesser's team (Dept. Biomedical Research), the Core Facility for Laboratory Animal Breeding and Husbandry, Medical Univ. Vienna, for assistance in animal experiments.

References

[1] A. Bonnefoy, R. Moura, M.F. Hoylaerts, The evolving role of thrombospondin-1 in hemostasis and vascular biology, Cell. Mol. Life Sci. 65 (5) (2008) 713–727.
[2] G.P. Luo, B. Ni, X. Yang, et al., von Willebrand factor: more than a regulator of hemostasis and thrombosis, Acta Haematol. 128 (3) (2012) 158–169.
[3] A. Borchiellini, K. Fijnvandraat, J.W. ten Cate, et al., Quantitative analysis of von Willebrand factor propeptide release in vivo: effect of experimental endotoxemia and administration of 1-deamino-8-D-arginine vasopressin in humans, Blood 88 (8) (1996) 2951–2958.
[4] P.M. Mannucci, C. Capoferri, M.T. Canciani, Plasma levels of von Willebrand factor regulate ADAMTS-13, its major cleaving protease, Br. J. Haematol. 126 (2) (2004) 213–218.
[5] R.A. Reiter, K. Varadi, P.L. Turecek, et al., Changes in ADAMTS13 (von-Willebrand-factor-cleaving protease) activity after induced release of von Willebrand factor during acute systemic inflammation, Thromb. Haemost. 93 (3) (2005) 554–558.
[6] C.V. Prowse, A. Farrugia, F.E. Boulton, et al., A comparative study using immunological and biological assay of the haemostatic responses to DDAVP infusion venous occlusion and exercise in normal men, Thromb. Haemost. 51 (1) (1984) 110–114.
[7] G. Andonegui, C.S. Bonder, F. Green, et al., Endothelium-derived Toll-like receptor-4 is the key molecule in LPS-induced neutrophil sequestration into lungs, J. Clin. Invest. 111 (7) (2003) 1011–1020.
[8] U. Derhaschnig, R. Reiter, P. Knobl, et al., Recombinant human activated protein C (rhAPC; drotrecogin alfa [activated]) has minimal effect on markers of coagulation, fibrinolysis, and inflammation in acute human endotoxemia, Blood 102 (6) (2003) 2093–2098.
[9] N. Buchtele, K.D. Kovacevic, C. Brostjan, et al., Differential osteoprotegerin kinetics after stimulation with desmopressin and lipopolysaccharides in vivo, Thromb. Haemost. 120 (7) (2020) 1108–1115.
[10] C. Schoergenhofer, P. Matzneller, M. Mussbacher, et al., Colistin dampens fibrinolysis and endothelial activation during endotoxaemia, A randomised, double blind trial, Thromb Haemost 117 (9) (2017) 1714–1721.
[11] I. Mikhailenko, D. Krylov, K.M. Argraves, et al., Cellular internalization and degradation of thrombospondin-1 is mediated by the amino-terminal heparin binding domain (HBD), High affinity interaction of dimeric HBD with the low density lipoprotein receptor-related protein, J Biol Chem 272 (10) (1997) 6784–6791.
[12] G. Martin-Manso, D.H. Navarathna, S. Galli, et al., Endogenous thrombospondin-1 regulates leukocyte recruitment and activation and accelerates death from systemic candidiasis, PLoS One 7 (11) (2012) e48775.