Reversible Suppression of Hemostasis in Hibernation and Hypothermia

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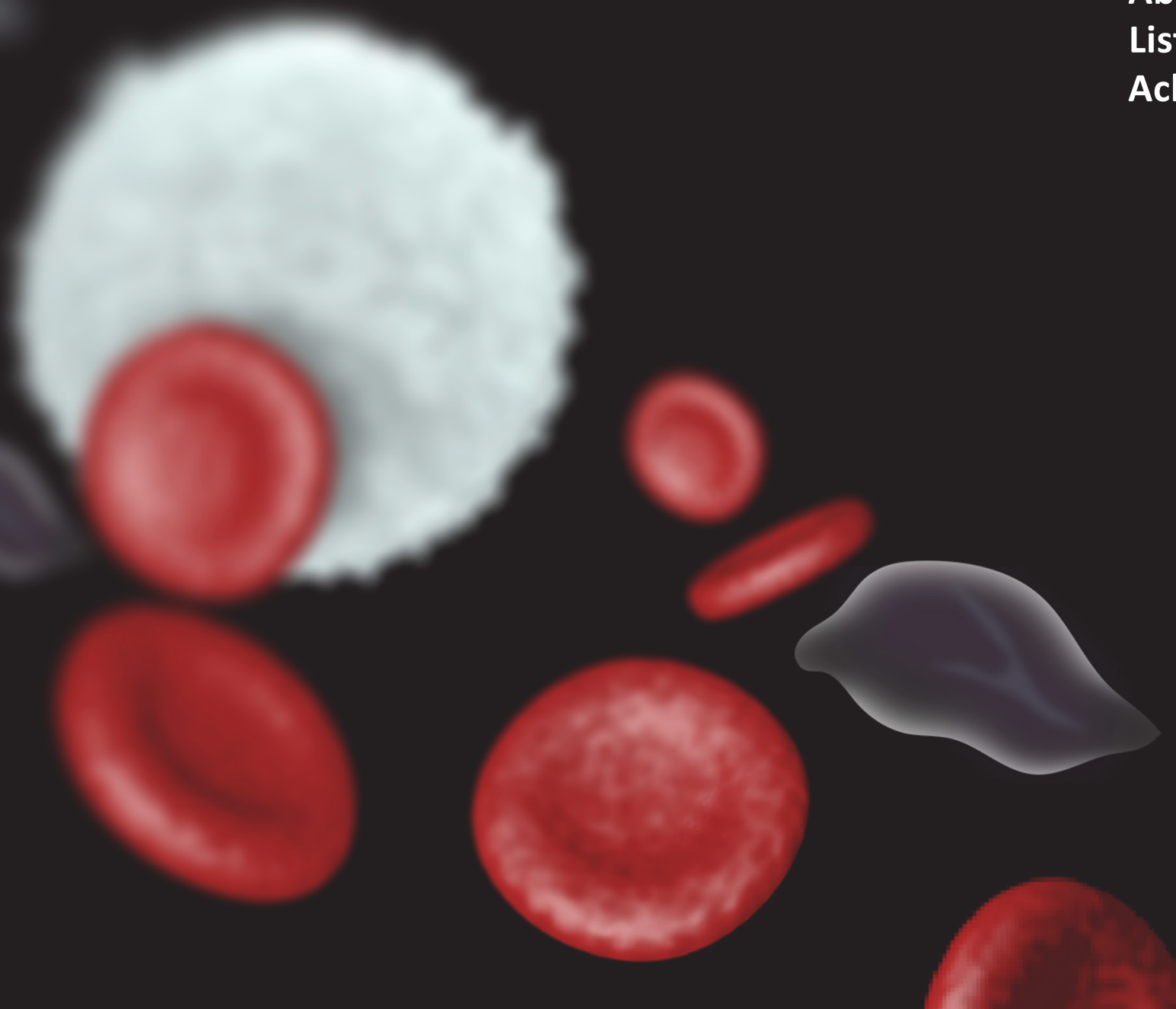
APPENDICES



NEDERLANDSE SAMENVATTING VOOR NIET-INGEWIJDEN

Inleidend

In dit hoofdstuk is het proefschrift in het Nederlands samengevat. Het proefschrift is tot stand gekomen uit een MD/PhD-traject, waarin de opleiding geneeskunde gecombineerd is met een promotietraject. Allereerst wordt nu de achtergrond van het onderzoek kort besproken, waarna één voor één de belangrijkste bevindingen per hoofdstuk worden samengevat. Biomedische terminologie wordt op enkele plekken tussenhaakjes uitgelegd. Dit hoofdstuk eindigt met een conclusie op basis van de bevindingen en interpretaties vanuit dit proefschrift.

Achtergrond en doel van het proefschrift

Trombose is de vorming van bloedstolsels door bloedplaatjes activatie en bloedstolling leidend tot afsluiting van een bloedvat en onderbreking van de bloeddoorstroming naar een weefsel of orgaan. Trombose is een belangrijke doodsoorzaak en wereldwijde ziektelast, denk hierbij aan het hartinfarct, herseninfarct, trombosebeen en longembolieën [1-6]. Zowel primaire als secundaire hemostase (het voorkomen van bloedingen door o.a. bloedstolling) zijn betrokken in veneuze (aderlijke) en arteriële (slagaderlijke) trombose. Het is te verwachten dat trombose ontstaat tijdens winterslaap vanwege verschillende factoren die in mens het trombose risico vergroten: langdurige onbeweeglijkheid [7-9], stilstand van bloed in aders en hartboezems [10], verhoogde bloed viscositeit (stroperigheid) [11-13], cycli van koeling-opwarming met relatieve hypoxie (zuurstoftekort) en herstel van zuurstof aanvoer met tekenen van endotheelschade [7, 14], en overgewicht tijdens het begin van winterslaap [15]. Ondanks deze risicofactoren tonen winterslapers geen tekenen van trombose of embolisatie (losschieten van een bloedstolsel welke vervolgens elders een bloedvat afsluit), waarschijnlijk door veranderingen aan essentiële onderdelen van hemostase tijdens de winterslaap.

Het doel van dit proefschrift was om een overzicht te genereren van veranderingen aan belangrijke componenten van hemostase tijdens winterslaap in een enkele diersoort, namelijk de Syrische (goud)hamster. Een ander doel was om aan te tonen of deze veranderingen in niet-winterslapende zoogdieren kunnen worden nagebootst via geforceerde hypothermie (onderkoeling). Dit proefschrift focusde op het krijgen van inzicht in het onderliggende mechanisme van de torpor (laag metabole fase van winterslaap) geassocieerde reversibele trombopenie (laag aantal bloedplaatjes), en van de morfologische veranderingen van bloedplaatjes, inclusief de relatieve koude resistentie van het cytoskelet (moleculaire cel-skelet) van winterslaper bloedplaatjes. Het uiteindelijke doel omhelst het identificeren van mogelijke therapeutische aangrijpingspunten voor antitrombotische medicijnen en voor lange termijn opslag van bloedplaatjes voor transfusie.

Algemene uitingen van hemostase onderdrukking in winterslaap

We bepaalden de compenten van hemostase die veranderd zijn tijdens torpor en waarschijnlijk trombose voorkomen. In **Hoofdstuk 2** onderzochten we de effecten van winterslaap en hypothermie op de dynamiek van circulerende bloedplaatjes in zowel winterslapende als niet-winterslapende zoogdieren. Ook onderzochten we het effect van een farmacologisch middel (5'-AMP) om torpor te induceren. Het verlagen van de lichaamstemperatuur is waarschijnlijk een van de belangrijkste aansturende krachten voor de reversibele daling in bloedplaatjes aantal in zowel winterslapende als niet-winterslapende diersoorten wanneer zij blootgesteld zijn aan geforceerde hypothermie. De hieropvolgende trombopenie (lage plaatjes aantal) tijdens de lage lichaamstemperatuur herstelde snel tijdens opwarming in alle onderzochte diersoorten wanneer zij weer eutherm werden, hetzij door natuurlijke arousal hetzij door geforceerd opwarmen. Door de snelheid van herstel hypothetiseerden wij dat een opslag en vrijlaat mechanisme ten grondslag ligt aan de trombopenie bij lage lichaamstemperatuur, in plaats van het onherstelbaar afbreken en vervolgen weer aanmaken van bloedplaatjes. We vonden verder dat plaatjes integriteit behouden bleef tijdens winterslaap en hypothermie in zowel winterslapende als niet-winterslapende diersoorten, omdat er geen tekenen waren van bloedplaatjes activatie gedurende de experimenten en functionaliteit van bloedplaatjes herstelde bij het bereiken van euthermie. Bovendien speelt de milt geen grote rol in de temperatuur gestuurde opslag en vrijlating van bloedplaatjes, aangezien het verwijderen van de milt alvorens de winterslaap geen effect had op de daling in bloedplaatjes aantal tijdens winterslaap. Interessant genoeg leidde farmacologische inductie van torpor door 5'-AMP injectie niet tot daling van bloedplaatjes aantal, ondanks dat de lichaamstemperatuur wel daalde. 5'-AMP interfereert daarom mogelijk met het onderliggende mechanisme van de temperatuur gestuurde bloedplaatjes dynamiek. Samenvattend is de temperatuur afhankelijke bloedplaatjes daling een grote aanpassing in het primaire hemostase systeem tijdens torpor.

In aanvulling hierop hebben we in **Hoofdstuk 3** belangrijke determinanten onderzocht van het bloedstolling systeem tijdens winterslaap in de Syrische hamster, namelijk die van primaire en secundaire hemostase als ook die van het fibrinolytische systeem. De hemostase is zeer waarschijnlijk geremd in torpor, zoals gesymboliseerd door verminderde trombine generatie met verlengde stollingstijden (PT en APTT), welke herstelden in arousal. Activatie secundaire hemostase en fibrinolyse is onwaarschijnlijk tijdens torpor gezien het plasma niveau van D-dimer laag blijft gedurende de winterslaap.

Het onderdrukken van hemostase gebeurt ogenschijnlijk door vermindering van aantal bloedplaatjes en het niveau van von Willebrand Factor (VWF), fibrinogeen, stollingsfactor V, VIII, IX, XI en door het toenemen van het plasminogeen niveau. De verminderde hemo-

stase werd gedeeltelijk tegengewerkt door matige stijging in factor II en X en een afname in antistolling factoren antithrombine, proteïne C en plasmine inhibitor. Hoe dan ook laat laat onze data zien dat tijdens torpor de hemostastische balans duidelijk helt naar remming wat gecorrigeerd wordt tijdens arousal.

Mechanismen van hemostase onderdrukking in winterslaap en hypothermie

Het onderliggende mechanisme van de bloedplaatjes dynamiek in winterslapende hamsters werd verder onderzocht in **Hoofdstuk 4**. In deze studie toonden we aan dat plaatjes opslag en vrijlating ten grondslag ligt aan de reversibele trombopenie in torpor in de hamster. Fluorescente bloedplaatjes volgden na transfusie dezelfde dynamiek in de bloedsomloop gedurende torpor-arousal cycli als de bloedplaatjes van de ontvanger. Vrijwel alle getransfundeerde bloedplaatjes herstelden zich in de bloedsomloop tijdens arousal en waren dus niet irreversibel verwijderd van de bloedsomloop. Bloedplaatjes lieten daarnaast geen tekenen zien van activatie. Verder toonden we aan dat de levensduur van bloedplaatjes met 50% is verlengd tijdens winterslaap vergeleken met niet-winterslapende hamsters. Tot slot toonden we met electronen microscopy analyse van bloedplaatjes in organen aan, dat lever sinusoiden in plaats van de milt of long de meest waarschijnlijke opslag en vrijlaat locatie is voor bloedplaatjes. Opgeslagen bloedplaatjes in lever sinusoiden waren niet gedegranuleerd. Dus, lage lichaamstemperatuur leidt tot trombopenie tijdens torpor via reversibele opslag, aannemelijk in de lever sinusoiden, wat zich herstelt tijdens opwarming tijdens arousal en gebeurt ogenschijnlijk zonder activatie en degranulatie van bloedplaatjes. Gezien de locatie van bloedplaatjes ophoping aan liver sinusoidaal endotheel, de lage bloedstroomsnelheid en de verhoogde bloed viscositeit tijdens torpor en gezien de reversibele aard van plaatjes opslag tijdens arousal, is marginatie van bloedplaatjes aan het endotheel het meest waarschijnlijke onderliggende mechanisme aan de plaatjes aantal daling.

De bevindingen in **Hoofdstuk 2** toonden aan dat de bloedplaatjes dynamiek temperatuursafhankelijk is en toepasbaar in niet-winterslapende zoogdieren. Derhalve onderzochten we verder in **Hoofdstuk 5** de opslag locatie en het mechanisme voor reversibele trombopenie in niet-winterslapende zoogdieren. Met behulp van (intravitaal) beeldvormende studies in rat en muis toonden we dat marginatie van bloedplaatjes aan lever sinusoidaal endotheel tijdens hypotherie het onderliggende mechanisme behelst van de reversibele trombopenie. Bovendien sloten we een rol uit van de milt in hypothermie geïnduceerde trombopenie door het uitvoeren van miltextirpatie alvorens en tijdens de onderkoeling, waarvan er geen effect was op de temperatuursafhankelijke bloedplaatjes dynamiek. In **Hoofdstuk 4** waren de opgeslagen bloedplaatjes in lever sinusoiden soms gezien in een speer vorm met verlengde microtubuli, wat in lijn is met eerdere bevindingen van gekoelde grondeekhoorn bloedplaatjes [16, 17]. Reddick et al. stelden voor dat

deze speer vorm in eekhoorn bloedplaatjes zou kunnen leiden tot vastlopen in de milt en dientengevolge tot de trombopenie [16]. Wij lieten echter zien dat de milt niet betrokken is bij de temperatuursafhankelijke bloedplaatjes dynamiek in hamster (**Hoofdstuk 2**), wat recentelijk ook is bevestigd in grondeekhoorn [18]. Echter, bloedplaatjes zouden alsnog kunnen vastlopen in de lever door vormverandering tijdens torpor. Of deze vormverandering van bloedplaatjes ook tijdens hypothermie gebeurt in niet-winterslapers en dus een vereiste zou kunnen zijn voor de opslag in lever sinusoiden is nog niet onderzocht. Daarom werd in **Hoofdstuk 6** de rol van veranderingen aan het cytoskelet onderzocht in vormveranderingen van bloedplaatjes tijdens winterslaap en vergeleken met vormveranderingen van bloedplaatjes van mens en andere niet-winterslapende diersoorten tijdens *ex vivo* koeling. Wij lieten zien dat in torpor met lage lichaamstemperatuur de circulerende hamster bloedplaatjes ofwel speer vormig danwel discus vormig zijn met behoud van cytoskelet structuur van tubuline. Daarentegen depolymerizeerde de tubuline in bloedplaatjes van muis, rat en mens tijdens lage *ex vivo* temperatuur, met als gevolg een bolvorm met het ontstaan van filopodia, lijkend op geactiveerde bloedplaatjes. Ondanks deze ogenschijnlijke activatie, na *ex vivo* koeling en opwarming was er geen stijging in expressie van activatie markers op bloedplaatjes uit winterslapende en niet-winterslapende dieren. We waren succesvol in het induceren van de speervorm in bloedplaatjes van muis, rat en mens na opwarming van de kou, welk mechanisme afhankelijk was van tubuline polymerizatie via de colchicine bindingsplaats op tubuline. Dus verlaging van temperatuur induceert een speervorm in bloedplaatjes van winterslapers, terwijl het weer opwarmen juist speervorming uitlokt in bloedplaatjes van niet-winterslapers. Daarom is het onwaarschijnlijk dat de speervorm een vereiste is voor bloedplaatjesopslag in lever sinusoiden tijdens hypothermie, aangezien niet-winterslapers geen speervorm maken in de kou. Winterslaper bloedplaatjes lijken ook nog eens beschermd tegen activatie door de kou en tegen het afbreken van het tubuline cytoskelet door de kou.

Conclusies

Samenvattend hebben we in dit proefschrift een aantal belangrijke elementen van primaire hemostase, secundaire hemostase en fibrinolyse geïdentificeerd die door hun aanpassing de activatie van het bloedstollingsysteem van de Syrische hamster voorkomt tijdens de winterslaap. We hebben ons gefocust op de primaire hemostase en ontraadselden de temperatuursafhankelijkheid van het mechanisme dat ten grondslag ligt aan de reversibele trombopenie in winterslapers en niet-winterslapers. We verklaarden de daling in bloedplaatjes tijdens torpor met een belangrijke rol voor de lever door opslag en later vrijlating van bloedplaatjes, resulterend in een 50% verlenging van de levensduur van winterslaper bloedplaatjes. Verder maakten we duidelijk dat lage temperaturen de

bloedplaatjes van winterslapers en niet-winterslapers niet activeerden ondanks de opvallende - doch omkeerbare - vormveranderingen. Gezamenlijk helpen deze bevindingen van dit proefschrift ons om te begrijpen waarom winterslapende zoogdieren zoals de Syrische hamster niet lijden aan tromboembolische complicaties tijdens en na de winterslaap. Bovendien bestaat de temperatuursafhankelijke onderdrukking van bloedstolling ook in niet-winterslapende zoogdieren alsook de eigenschap om omkeerbaar de vorm van bloedplaatjes te veranderen zonder hen te activeren. Deze resultaten kunnen in de toekomst leiden tot nieuwe antitrombotische strategieën en de langdurige koude opslag van bloedplaatjes voor transfusie.

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ABOUT THE AUTHOR

On 28th November 1988 I was born and hereafter raised in Stiens, Friesland, the Netherlands. At the age of 18 I finished secondary school at the Christelijk Gymnasium Beyers Naudé in Leeuwarden in 2007 and started studying Biology at the University of Groningen, since I didn't draw a place by lot for the study Medicine. This lottery for Medicine was unsuccessful the year after as well, so I continued and finished the Bachelor Degree in Biology in 2010. During my bachelor my major focused on Biomedical Sciences, e.g. via several research projects, and my minor focused on Medicine. One of my bachelor research projects studied the role of platelets in kidney transplantation, which sparked my fascination for platelets. My interest in science started to grow. By passing an entrance and selection exam I finally started studying Medicine in the same year via a fast-track (zij-instroom) program and qualified for the Bachelor of Medicine in one year to commence the master program afterwards. I realized I wanted to continue both research and Medicine and my MD/PhD proposal got accepted at the lab of prof. Henning in the Department of Clinical Pharmacy and Pharmacology. During the next years I alternated the master program with PhD research. I experienced medical internships in Groningen, Assen, Zwolle, Meppel, Harderwijk, Emmeloord and in Kumi - Uganda. My final half year of internships of choice were in Plastic, Reconstructive and Hand Surgery in the hospitals Medical Center Leeuwarden and University Medical Center Groningen. I obtained a grant to perform additional experiments which extended my PhD research, and part of the research I performed in Strasbourg, France. In 2017 I obtained my degree as Medical doctor and I started working in the Surgery department of Martini Hospital in Groningen, where I finished writing the PhD thesis.

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FameLab presentation on hibernation for spacetravel in, UKrant, 2016: https://archief.ukrant.nl/english/researchers-with-a-good-story.html

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