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13:40:56 OCA PAD INITIATION - PROJECT HEADER INFORMATION 06/20/89

Active

Project #: E-19-694 Cost share #: Rev #: 0
Center # : 10/24-6-R6756-0A0 Center shr #: OCA file #:
Work type : RES
Contract#: AGMT DTD 6/8/89 Mod #: Document : AGR
Prime #: Contract entity: GTRC

Subprojects ? : N
Main project #:

Project unit: CHE Unit code: 02.010.114
Project director(s):
WICK T M CHE (404)894-8795

Sponsor/division names: AMERICAN HEART ASSOC /
Sponsor/division codes: 500 / 011

Award period: 890701 to 900630 (performance) 900801 (reports)

Sponsor amount	New this change	Total to date
Contract value	27,500.00	27,500.00
Funded	27,500.00	27,500.00
Cost sharing amount		0.00

Does subcontracting plan apply ?: N

Title: KINETICS OF UNUSUALLY LARGE VON WILLEBRAND FACTOR-MEDIATED SICKLE ERTHROCYTE

PROJECT ADMINISTRATION DATA

OCA contact: Kathleen R. Ehlinger 894-4820

Sponsor technical contact

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Security class (U,C,S,TS) : U ONR resident rep. is ACO (Y/N): N
Defense priority rating : N/A N/A supplemental sheet
Equipment title vests with: Sponsor GIT

Administrative comments -
INITITATION OF PROJECT.

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

NOTICE OF PROJECT CLOSEOUT

SR747-7

Closeout Notice Date 02/07/91

roject No. E-19-694 Center No. 10/24-6-R6756-0A0

roject Director WICK T M School/Lab CHEM ENGR

ponsor AMERICAN HEART ASSOC/

ontract/Grant No. AGMT DTD 6/8/89 Contract Entity GTRC

rime Contract No.

itle KINETICS OF UNUSUALLY LARGE VON WILLEBRAND FACTOR-MEDIATED SICKLE ERTHROC

ffective Completion Date 900630 (Performance) 900801 (Reports)

loseout Actions Required:	Y/N	Date Submitted
Final Invoice or Copy of Final Invoice	Y	
Final Report of Inventions and/or Subcontracts	N	
Government Property Inventory & Related Certificate	N	
Classified Material Certificate	N	
Release and Assignment	N	
Other	N	

Comments

ubproject Under Main Project No.

ontinues Project No.

istribution Required:

Project Director	Y
Administrative Network Representative	Y
GTRI Accounting/Grants and Contracts	Y
Procurement/Supply Services	Y
Research Property Managment	Y
Research Security Services	N
Reports Coordinator (OCA)	Y
GTRC	Y
Project File	Y
Other	N
	N

E-19-69K

8795

27 September 1990

Randall Tackett, Ph.D., Chairman
Research Peer Review Committee
American Heart Association, Georgia Affiliate
1685 Terrell Mill Road
Marietta, GA 30067

Dear Dr. Tackett:

Enclosed please find the terminal progress report for our project entitled "Kinetics of Unusually Large von Willebrand Factor-Mediated Sickle Erythrocyte Adhesion to Stimulated Endothelial Cells". I would like take this opportunity to thank the Georgia Affiliate of the American Heart Association for funding this project. I think you will agree that this research generated some exciting results.

Please contact me if additional information is required.

Sincerely yours,

Timothy M. Wick, Ph.D.
DuPont Assistant Professor

I. Investigator: Timothy M. Wick

Project Title: Kinetics of Unusually Large von Willebrand Factor-Mediated Sickle Erythrocyte Adhesion to Stimulated Endothelial Cells.

Period of Support: 1 July 1989 to 30 June 1990

II. Project Report

a). Project Summary

Sickle red blood cell (RBC) adhesion to endothelial cells is hypothesized to contribute to sickle cell vaso-occlusive crisis. It has previously been demonstrated that high molecular weight von Willebrand factor (vWF) multimers, synthesized by endothelial cells (EC) and stored in Weibel-Palade bodies, promote sickle RBC adhesion to human umbilical vein endothelial cells (HUVEC). If vWF-mediated sickle RBC adhesion to EC occurs in the microcirculation *in vivo*, subsequent red cell passage through the affected area would be delayed. Hemoglobin deoxygenation and intracapillary red cell sickling would result. These sickled cells along with the adherent cells would occlude the microcirculation, leading to vaso-occlusive crisis. In this project, sickle RBC adhesion to cultured EC was investigated under physiological flow conditions in a parallel-plate flow chamber. Adhesion assays were performed by perfusing washed RBC suspended in various media over EC monolayers at constant shear-stress (1.0 dyne/cm^2). Under post-capillary venule shear-stresses, we investigated the effect of EC activation by chemical agonists, such as thrombin and endotoxin, on the level of sickle RBC adhesion to their surfaces. We also compared the mechanism of sickle RBC adhesion to human microvascular EC (HMVEC) cultured from human foreskins and human umbilical vein EC (HUVEC).

To determine whether the kinetics of stimulated vWF release is rapid enough to induce sickle erythrocyte adhesion to EC *in vivo*, we attempted to stimulate vWF release and increase sickle RBC adhesion to EC under physiological flow conditions *in vitro*. Since vaso-occlusion is primarily a microcirculatory phenomenon, we used HMVEC for our experiments. HMVEC were perfused with 1.0 unit/ml human thrombin or 1.0 μg/ml endotoxin for 5-minutes. This was followed by perfusion of sickle RBC suspended in serum-free medium (SFM). In control experiments, HMVEC were perfused with thrombin-free and endotoxin-free SFM prior to sickle RBC perfusion. Thrombin or endotoxin led to a small (~2-fold) increase in sickle erythrocyte adhesion to HMVEC in some experiments. We attributed the limited effect of thrombin or endotoxin on adhesion to an inadequate opportunity for contact between sickle cells, secreted vWF, and HMVEC within the short duration of the (single-pass) adhesion assay. A more reasonable model for future stimulation experiments would be to use a multi-pass perfusion system to more accurately model events hypothesized to promote sickle RBC adhesion to EC *in vivo*. We are currently assembling a multi-pass adhesion assay system for future investigations of the effects of endothelial cell stimulation on vWF release and sickle erythrocyte adhesion.

We next investigated whether adhesion is common to all endothelium, or does the vascular source influence the adhesion? We have initiated experiments to investigate whether sickle red cell interactions with large vessel (umbilical vein) and microvessel endothelial cells are qualitatively and quantitatively similar.

HUVEC and HMVEC were cultured onto tissue culture plastic. Washed sickle erythrocytes were suspended in SFM, SFM containing 30% autologous plasma, or endothelial cell supernatant derived from HMVEC and containing high molecular weight vWF multimers.

Sickle red cells suspended in sickle plasma (PLASMA or PL) were up to 2-logs more adherent to HMVEC than the same red cells suspended in SFM (Table I). When sickle RBC were suspended in EC SUP adhesion was not different from zero. In 4 experiments where sickle cells were suspended in EC SUP containing 30% autologous plasma, the plasma-mediated adhesion was inhibited 34% to 87% by HMWvWF multimers in EC SUP (Table I).

To further investigate the role of plasma in sickle RBC adhesion to HUVEC, blood was obtained from sickle donors not in crisis and ABO/Rh-matched normal donors. Red cells were washed and resuspended in SFM containing 30% autologous platelet poor plasma or 30% platelet poor plasma from the matched donor. Sickle erythrocytes, whether suspended in sickle (SS/SS) or normal (SS/AA) plasma, are adhesive to HMVEC (Table II). Conversely, normal RBC suspended in normal plasma (AA/AA) were only minimally adherent to HMVEC. In 5 of 8 experiments, sickle RBC were more adhesive to HMVEC when suspended in sickle plasma (SS/SS) compared to normal plasma (SS/AA). Sickle RBC suspended in normal plasma (SS/AA) were more adhesive than normal RBC suspended in normal plasma (AA/AA). These results suggest that both sickle RBC and sickle plasma contribute to *in vitro* adhesion.

We have previously demonstrated that HMWvWF-mediated sickle RBC adhesion to HUVEC is inhibited ~90% by the integrin receptor agonist arginine-glycine-aspartic acid-serine (RGDS). To determine whether plasma-mediated adhesion to HMVEC (Table III) was integrin receptor dependent, either RBC or HMVEC were preincubated with RGDS prior to the adhesion assay. In 3 of 5 experiments, plasma-mediated adhesion decreased 39% when sickle RBC were preincubated with RGDS. When HMVEC were incubated with RGDS, plasma-mediated sickle RBC adhesion only decreased (23%) in one experiment. Thus, it appears that unoccupied integrin receptors are not required for plasma-mediated sickle RBC adhesion to HMVEC.

Finally, we have performed experiments comparing plasma-mediated adhesion of sickle RBC to HUVEC and HMVEC (Table IV). Plasma-mediated sickle RBC adhesion to HMVEC was 2- to 51-fold greater that to HUVEC. Since adhesion is hypothesized to occur in the venules *in vivo*, data indicating greater adhesion to microvascular EC are particularly relevant. Based on these data, we propose to further characterize the adhesion of sickle RBC to large vessel (HUVEC) and microvascular (HMVEC) endothelial cells.

In summary, sickle erythrocytes are capable of adhering to HUVEC and HMVEC. Apparently, adhesion to these two EC types occurs by different mechanisms. HMWvWF multimers are potent mediators of sickle RBC adhesion to HUVEC and this adhesion is integrin receptor dependent. HMWvWF appears to be less able to promote sickle RBC adhesion to HMVEC. Autologous sickle plasma on the other hand, promotes greater sickle RBC adhesion to HMVEC than to HUVEC and this adhesion does not appear to require unoccupied integrin receptors. High molecular weight vWF inhibition of plasma-mediated adhesion suggests that integrin receptors and plasma adhesion factor receptors are proximal and that high molecular weight vWF limits access of plasma adhesion factors to their receptor on sickle red cells.

b). Lay Summary

Sickle cell disease is characterized by chronic hemolytic anemia and periodic localized vaso-occlusive crises. These crises arise as a result of occlusion of the microvessel by rigid, nondeformable ('sickled') red blood cells. Recent experimental data suggest that red blood cell adhesion to blood vessel wall endothelial cells may also contribute to microcirculatory occlusion. We have investigated the role of sickle red cells, endothelial cells, and the local vascular milieu in sickle red blood cell adhesion to endothelial cells in *in vitro* adhesion assays. Our data suggest that adhesion depends upon alterations in sickle red cells (e.g. sickle red cells are more adhesive than normal red cells). Furthermore, the plasma from sickle patients promotes greater levels of red cell adhesion, suggesting that sickle patient plasma contains elevated levels of adhesive factors. Finally, the mechanism of sickle red cell adhesion is different to microvascular end large vessel endothelial cells, implying that endothelial cells from different vascular beds exhibit distinct functionality.

These finding indicate that not only are sickle cells (the obvious source of pathology in sickle cell disease) responsible for adhesion and microcirculatory delay; but the local plasma milieu also contributes to adhesion and vaso-occlusion. Increased sickle red blood cell adhesion to microvascular endothelial cells is further evidence that sickle erythrocyte adherence contributes to vaso-occlusive complications. The large interpatient variability may correlate with clinical severity. The extensive contribution of both sickle plasma and red cells to adhesion suggests that either or both may be altered to modulate the severity of vaso-occlusive crises.

III. Collaborators

a). Dr. James R. Eckman, Associate Professor of Medicine-Hematology/Oncology, Emory University School of Medicine, Atlanta, GA.

Dr. Eckman provided blood samples from sickle patients and normal donors for the adhesion studies. He has also supervised collection of additional clinical data such as frequency and duration of crises for possible correlation with *in vitro* adhesion levels.

b). Mr. Henri A. Brittain, Research Assistant, Georgia Institute of Technology, Atlanta, GA.

Mr. Brittain's primary duties included executing all cell adhesion assays for this project. In this capacity, he received extensive training in cell culture, blood handling, and electrophoresis techniques as well as experience in designing, implementing, and analyzing experiments.

IV. Publications resulting from this work.

a). Meeting Presentations

H.A. Brittain, J.R. Eckman and T.M. Wick. "Sickle Erythrocyte Adhesion to Endothelial Cells: A Potential Modulator of Microvascular Adhesion in Sickle Cell Disease", First World Congress of Biomechanics, San Diego, CA (September 1990).

H.A. Brittain, J.R. Eckman and T.M. Wick. "Sickle Erythrocyte Adhesion to Microvascular Endothelial Cells is Qualitatively Different from Adhesion to Large Vessel (Umbilical Vein) Endothelial Cells", to be presented at the 62nd Annual Meeting of The Society of Rheology, Santa Fe, NM (October 1990).

H.A. Brittain, J.R. Eckman and T.M. Wick. "Plasma-Mediated Adhesion of Sickle Red Cells is due to Both Sickle Plasma and Red Cell Factors and is Quantitatively Different to Large and Microvessel Endothelial Cells under Physiological Flow", to be presented at the 1990 Annual Meeting of the American Institute of Chemical Engineers, Chicago, IL (November 1990).

b). Other Presentations

T. M. Wick. "Sickle Erythrocyte Interactions with Cultured Endothelial Cells under Physiological Flow Conditions", Department of Neurology, School of Medicine, The Medical College of Georgia, Augusta, GA (June 1990).

c). Manuscripts

Brittain, H.A., J.R. Eckman, and T.M. Wick. "Plasma Mediated Adhesion of Sickle Erythrocytes to Human Dermal Microvascular Endothelial Cells is Dependent upon Both Sickle Plasma and Erythrocyte Factors". In preparation for December 1990 submission to American Journal Hematology.

V. Research Continuation

a). Work on this project is continuing with a near future in the following areas:

1. Differences in mechanism of sickle erythrocyte adhesion to endothelial cells from different vascular beds (umbilical vein and microvessel),
2. Identification of specific plasma factors which mediate sickle erythrocyte adhesion to microvascular endothelial cells, and
3. Correlations between patient clinical condition and level of red cell adhesion.

b). Efforts for Continued Support.

Current Support:

Hemostasis and Endothelial Cell Adhesion in HBSS Complications.
Georgia Tech/Emory University Biomedical Engineering Center
Funded 1 July 1990-30 June 1991.

Pending Support

Sickle Erythrocyte Adhesion to Activated Endothelium
National Institutes of Health
Funds Requested 1 July 1991-30 June 1996.

Proposals Planned

Based on results highlighted above, the PI plans to submit a proposal to the Georgia-Affiliate of the American Heart Association in 1990 and respond to an RFA entitled "Comprehensive Sickle Cell Center Program" in September 1991.

Table I. Effects of Plasma (PL) and Endothelial Cell Supernatant (EC SUP) on Sickle Red Cell Adhesion to HMVEC Under Controlled Flow Conditions.

		Adherent RBC/mm^2			
Expt Number	SS Patient	SFM	PLASMA	EC SUP	EC SUP + PL
1	SS1	6.9±4.4	32.1± 20.4		
2	SS2	0.88±0.75	345.9± 88.1		
3	SS3	0.0	70.7±39.4	0.28±1.0	9.2±8.4
4	SS4	0.15±0.72	35.1±21.3	0.29±0.99	
5	SS5	0.07±0.51	68.9±29.9	0.22±0.87	14.2±11.2
AVE		1.6±3.2	110±111*	0.27±0.92	11.7±10.2¶

Table II. Adhesion of Sickle and Normal RBC to Human Microvascular Endothelial Cells in the Presence of Plasma Under Controlled Flow Conditions.

Experiment	Sickle Patient	Normal Donor	Adherent RBC/mm^2			
			SS/SS	SS/AA	AA/SS	AA/AA
1	S1	A1	17.8±18.0	10.8±11.4	1.0±1.7	2.1±3.7
2	S2	A1	112.5±34.4	40.0±16.0¶	33.7±19.2†	8.6±7.3
3	S3	A2	152.7±70.0	28.9±24.1¶	58.0±51.3†	6.5±6.0
4	S4	A3	41.2±25.0	42.5±18.7	14.0±12.6	9.7±6.8
5	S6	A5	25.5±14.5	21.9±13.1	1.4±2.2	2.6±3.3
6	S8	A2	234±103	29.2±15.0¶	73.6±4.8†	3.2±4.8
7	S9	A7	19.9±18.0	4.3±3.8¶*	3.8±4.9	4.6±6.3
8	S7	A6	502±217	170±116¶	80.8±40.3	68.2±47.0

Table III. Effect of RGD-containing Peptides on the Plasma-Mediated Adhesion of Sickle RBC to Human Dermal Microvascular Endothelial Cells Under Controlled Flow Conditions.

Experiment	Sickle Patient	Adherent RBC/mm^2		
		Control	RGD-RBC	RGD-EC
1	S10	32.1±20.4	20.5±18.3†	25.5±14.7
2	S11	11.7±10.2	9.7±7.0	7.5±2.4¶
3	S5	76.5±36.6	81.2±44.8	82.7±48.7
4	S4	27.5±20.9	17.8±11.9†	24.0±22.9
5	S12	61.8±28.9	33.9±16.4†	96.2±38.4

Table IV. Comparison of Adhesion of Sickle RBC to Human Dermal Microvascular and Human Umbilical Vein Endothelial Cells in the Presence of Sickle Plasma Under Controlled Flow Conditions.

		Adherent RBC/mm^2	
Experiment	Sickle Patient	HMVEC	HUVEC
1	S4	41.2±25.0	20.5±13.2
2	S5	63.1±29.9	4.0±3.3
3	S6	25.5±14.5	4.1±2.3
4	S8	234±103	4.6±6.2
5	S9	19.9±18.0	2.3±2.9