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Research Article

The Role of Calcium in Augmenting the Efficacy of Primary Homeostasis after Hemorrhagic Shock

Thacker JM, Ledgerwood AM, and Lucas CE*

Department of Surgery, Wayne State University School of Medicine, USA

***Corresponding author:** **Charles E Lucas**
Department of Surgery, Wayne State University School of Medicine, 4201 St. Antoine, Detroit, MI, 48201, USA

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Abstract

Background: Traumatic Hemorrhagic Shock (THS) causes hypocalcemia (Ca). This study assesses how low Ca affects platelet (Plt) function.

Study Design: A research registry was queried for 555 studies on 341 patients (pts) with THS requiring 14.2 units RBC, 8.4 units FFP, 3.6 units Plt, and 18.9 mEq Ca by end of O.R. (7.3 hours). Factors analyzed included Plt count, Ca, Bleeding Time (BT), Plt aggregation with ADP (PAadp) and collagen (PAcol), including Max PA, T1/2 PA, and rate PA, plus platelet release factors, beta thromboglobulin (BTG) and Plt PF-4. Studies were made in O.R. (26 pts) during fluid uptake phase II at 29 hours (216 pts), during fluid mobilization phase III at 56 hours (283 pts), and as outpatients (30 pts).

Results: THS caused low Plts (1x10 SD) in O.R (107 ± 43), in phase II (96 ± 35), and in phase III (105 ± 59) and low Ca in O.R. (1.6 ± 0.3), in phase II (1.8 ± 0.2), and in phase III (2.1 ± 0.2). Low Plt and Ca correlated (p=<0.05) with PAadp and PAcol in O.R., phase II, and phase III. Ca correlated directly with Plt count, aggregation, BTG and PF-4, and inversely with BT. All outpatient studies were normal.

Conclusion: THS causes Ca which leads to low Plt and impaired function. Routine Ca supplementation is recommended for THS.

Keywords: Hypocalcemia; Hemorrhagic Shock; Platelet Dysfunction

Introduction

The severely injured patient with Hemorrhagic Shock (HS) requiring Massive Transfusion (MT) presents multiple challenges to successful treatment. Following the control of airway and assurance of adequate breathing, the prime focus rests with stopping hemorrhage and restoring circulation. The traditional restoration of circulation incorporates the balanced replacement of depleted blood, plasma and interstitial fluid with Packed Red Blood Cells (PRBC), plasma (FFP), Platelets (Plt), and Balanced Electrolyte Solution (BES) [1]. Recent studies suggest that a more aggressive use of FFP as part of a balanced FFP/PRBC resuscitation ratio may reduce serum ionized calcium as the increased plasma proteins, especially albumin, bind with free calcium, which is vital for primary hemostasis (formation of a platelet plug) and secondary hemostasis (formation of a fibrin plug) [2,3]. This study looks at the effects of HS requiring MT on calcium levels and the association of calcium levels on primary hemostasis.

Calcium is the most abundant element within the body, with the vast majority located within the skeletal system [4]. Most (88%) non-skeletal calcium is in the serum where it is measured as total serum calcium and includes bound calcium (50%) and ionized calcium (38%); the remaining extra-skeletal calcium is in soft tissues. Circulating ionized calcium has many functions and binds at about 30 sites to different protein molecules and is altered by pH; all ionized Ca measurements, herein, are corrected for pH [4].

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Methods

The analyses reported herein were derived from a prospectively compiled trauma registry in which all patient data were de-identified [1]. Entrance into this registry was limited to those patients who had severe injury causing HS, which required a minimum of 8 PRBC transfusions by the end of immediate operation if the patient's Systolic Blood Pressure (SBP) never went below 80 torr, or a minimum of 6 PRBC transfusions if the SBP was below 80 torr during the emergency department resuscitation or immediate operation (phase I) [1]. During the period of data collection, the average EMS run time was seven minutes and these severely injured patients were typically in the operating room within 30 minutes of injury. Data stored on these patients included admitting SBP, Pulse Rate (PR), shock time (minutes that SBP was below 80 torr), and amount of PRBC, FFP, Plt, BES, and Ca given during phase I, during the subsequent fluid uptake period (phase II), and during the first four days of the later fluid mobilization period (phase III) [1].

Based upon prior measurements of electrolytes and coagulation parameters, a guideline for a balanced resuscitation regimen was created. This included 2 units FFP and 13.7 mEq calcium for every 5 units PRBC and 5 units' platelets for every 10 units PRBC. The actual balanced resuscitation regimen administered to these 341 patients included 2.7 units FFP and 7.4 mEq calcium for every 5 units PRBC and 2.3 unit's platelets for every 10 units PRBC (Table 2).

From 1972 through 1983, 341 patients met the criteria for evaluation and underwent a total of 555 assessments during phase I, phase II, phase III, and/or convalescence (Table 1). The dedicated surgical research team worked closely with the Wayne State University Department of Physiology Coagulation Division. All blood specimens were measured immediately after collected. The times of study were designed to monitor sequential changes in the three physiological phases during and after resuscitation from HS.

The average time from arrival until end of operation (phase I) for control of bleeding was 7.3 ± 3 hours, during which the 341 patients were in shock for an average of 34 ± 36 minutes and received an average of 14.2 ± 18 PRBC transfusions (Table 1). Twenty-six of these patients died from their HS insult, usually in the OR or the ICU within the first 12 hours. These 26 patients received an average of 29PRBCs in phase I and had an average shock time of 77 minutes. There were 15 additional deaths that occurred well after the HS insult, including 11 who died of traumatic brain injury at an average of 12 days and four patients who died of sepsis at an average of 27 days after injury. The clinical research team made the judgment, concurrently, as to the cause of death.

During the postoperative period, the patients were monitored closely in order to determine the length of the obligatory postoperative fluid uptake phase II and the beginning of the subsequent mobilization phase III [1]. The combination of serial vital signs, urine output, and fluid needs almost always allowed these estimates of this transition from phase II to phase III to be within four hours and usually within two hours [1].

The studies of primary hemostasis (formation of a platelet plug) included Pltcount, Bleeding Times (BT), platelet aggregation following adenosine diphosphate (PAadp) and collagen (PAcol) stimulation, and both plasma and urine platelet release factors, including Betathromboglobulin (BTG) and Platelet Factor 4 (PF-4) [5]. The platelet counts were obtained by phase contrast microscopy, using a 20 micro liter blood collection pipette. A normal platelet count by this technique is 200,000-400,000/mm^5. Platelet aggregation was performed on a sample of platelets containing 200,000 platelets/mm^3 obtained by combining patient Platelet Rich Plasma (PRP) and Platelet Poor Plasma (PPP) [6,7]. This precluded the actual level of platelets from being a factor in the extent of platelet response to stimulated aggregation [5].

Both PAadp and PAcol were used to stimulate platelet aggregation. A stock of sodium adenosine-5-diphosphate (ADP) was prepared by adding 1 mg of ADP in 2 mL saline, which was further diluted to a final concentration of 10 micrograms (mcg) [6,7]. The stock collagen solution was diluted with saline to a final collagen concentration of 2.5 mg/mL and kept refrigerated until the aggregation studies were performed [6,7]. The extent of aggregation was measured on a CHRONO-LOG Aggregometer, using optical density to monitor changes when the aggregating agent is added to the platelet sample. Once baseline measurements have been obtained, 50 lambda (0.05 mL) of either collagen (125 mcg) or ADP (0.5 mcg) was added to the sample and the change in optical density recorded [6,7]. Specific aspects of aggregation that were measured included Maximal Aggregation (MA), the Rate of Aggregation (RA), and the time elapsed to reach half maximal aggregation (T1/2). In each of the studies, the RA and the T1/2 correlated closely with the MA [6,7]. The normal values for all aggregation studies were derived from human volunteers measurements in samples with platelet counts of 200,000/mm^3. Serum and urine samples of Betathromboglobulin (BTG) and platelet factor (PF-4) were used to monitor the platelet release products [8]. Normal plasma BTG and PF-4 are 25 mg/mL (9-41 mg/mL) and 2.9 mg/mL (1.8-6.7 mg/mL); urine BTG and PF-4 are not measureable unless serum levels are elevated [8].

Table 1: Patients Assessed For Calcium Influence on Platelet Function (555 Studies in 341 Patients).

Clinical Parameters	Mean ± SD	Median
Age (yrs)	34 ± 13	30
Phase I Time (hrs)*	7.5 ± 3.3	7
Shock Time (min)	36 ± 40	25
Phase I PRBC (units)	14.2 ± 10.1	13
Phase I FFP (units)	7.5 ± 8	6.7
Phase I Plt (units)	3.6 ± 2.7	3.4
Phase I Crystalloid (L)	10.7 ± 5.9	10.5
Calcium Given (mEq)	20.6 ± 22	23.2
Phase II Time (hrs)*	31.6 ± 21	26
Phase II PRBC (units)	3.0 ± 4.1	2
Phase II FFP (units)	2.1 ± 0.9	3.7
Phase II Crystalloid (L)	9.4 ± 6.8	8.1
Phase II Calcium (mEq)	4.1 ± 0.9	3.4
Phase III Time (days)*	6.8 ± 5.7	5
Phase III PRBC (units/first 4 days)	1.6 ± 1.8	1
Phase III FFP (units/first 4 days)	0.4 ± 1.5	1
Phase III Crystalloid (L/first 4 days)	12.4 ± 5.0	11.1
Phase III Calcium (mEq/first 4 days)	2.4 ± 0.7	0.6

*Phase I time measured from injury to end of operation; Phase II is time of obligatory fluid sequestration; Phase III is time of subsequent fluid mobilization. Resuscitation values for phase III reflect the therapy given in the first four days of the mobilization period.

The Bleeding Times (BT) were performed by the Simplate Bleeding Time device using a standardized regimen [9]. The normal BT with this technique is 2-9 minutes [9,10]. When oozing persisted beyond 15 minutes, the test was terminated and a value of 16 minutes was recorded. All of the above measurements were made immediately after collection.

Four different calcium parameters were measured including total serum calcium, total serum calcium corrected for serum albumin concentration, ionized calcium, and ionized calcium corrected for simultaneously measured arterial pH (CIC) [4]. Multiple correlations were made between the four different calcium measurements and the many parameters of platelet function; these multiple measurements demonstrated that the CIC gave the best correlations, so that CIC was used throughout the manuscript (Table 2).

Table 2: Total Serum Calcium (Tsc) And Corrected Ionized Calcium (Cic) Correlations with Platelet Level and Platelet Function.

Platelet Count	TSC 0.009	CIC 0.001
Maximal Aggregation (ADP)	0.3	0.004
Maximal Aggregation (Col)	0.4	0.04
Serum BTG	0.02	0.001
Serum PF-4	0.09	0.02
Bleeding Time*	-0.4	-0.05

*The bleeding time in many patients equaled 16 seconds, which is the maximal time recorded in the registry.

Table 3: Sequential Platelet and Calcium Levels and Platelet Function.

Parameters (Normal ± SD)	Phase I 26 Studies	Phase II 216 Studies	Phase III 283 Studies	Convalescence 30 Studies
Phase Duration (hrs)	7.3 ± 3.3	31.6 ± 21	148 ± 136	26 days
Study Time After Admit	4.8 ± 1.5 hrs	29 ± 13 hrs	56 ± 26 hrs	30 ± 13 days
Platelet Count (300 ± 50 x 10^4)	107,000 ± 43	96,000 ± 35	105,000 ± 59***	461,000 ± 272***
Bleeding Time (5 ± 2 min)	14.1 ± 2	14.1 ± 1	13.4 ± 3**	7.2 ± 3***
CIC (2.22 ± 0.3 mmo/L)	1.62 ± 0.3	1.85 ± 0.2***	1.99 ± 0.2***	2.3 ± 0.3***
Max aggregation ADP (%) (87 ± 11%)	41 ± 16	56 ± 17***	60.7 ± 19*	79 ± 12***
Max aggregation Collagen (%) (86 ± 18%)	72 ± 14	81 ± 9*	84 ± 8	89 ± 8.6**
Rate aggregation ADP (54 ± 26 sec/min)	26 ± 14	36 ± 13***	37 ± 18**	67 ± 18***
Rate aggregation Collagen (44±8%/min)	18 ± 9	19 ± 7	24 ± 14***	35 ±13***
T1/2 ADP (27 ± 4 sec)	17 ± 8	30 ± 12***	37 ± 11***	35 ± 13
T1/2 Collagen (81 ± 34 sec)	149 ± 48	163 ± 43	151 ± 56	98 ± 22***
Serum BTG (mg/ml) (25 ± 15 mg/ml)	415 ± 241	130 ± 71***	54 ± 46***	136 ±1 02***
Serum PF-4 (mg/ml) (2.9 ± 2.6 mg/ml)	36 ± 16	26 ± 18***	12 ± 9***	17 ± 7**

*=<0.05; **=<0.005; ***=<0.0005 by unpaired T-test when compared to prior study.

Discussion

The platelet is a complex structure which has what is referred to as a dense tubular system, a separate microtubular system, and a cytoskeleton. Contained within the cytoplasm are α-granules, glycogen granules, and dense granules [21]. Primary hemostasis includes the process by which platelets release different cytoplasmic granules, which encourage the clumping of platelets or the formation of a platelet plug to close off a vascular injury [21]. During this process, ADP helps additional platelets to adhere to the injury site, thus expanding the platelet plug. Meanwhile, serotonin is released and helps maintain vasoconstriction [21,24]. Prostaglandins and phospholipids are also involved in these processes. During this process, calcium allows prothrombin activator to form prothrombin which, of course, leads to thrombin. This is part of the process called secondary hemostasis, which results in the formation of a fibrin clot.

Dense granules contain a range of small molecules, including ADP, ATP, GDP, 5-HT, pyrophosphate, magnesium and calcium [16]. Historically their release from dense granules was described as "fast," but recent studies suggest that the release of serotonin occurs more rapidly than PF-4 release from the α-granules or β-hexosaminidase from lysosomes, regardless of agonist used to stimulate platelet release [22]. Consequently, the concept of a primary platelet release reaction and a secondary platelet release reaction is probably more historical than accurate, as it relates to the combination of platelet release factors occurring rapidly but at slightly different times during the process of platelet stimulation toward platelet aggregation [22].

Stalker and co-workers showed that there are two distinct populations of platelets in a growing thrombus, including a "core" of more stable P-selectant expressing platelets, and a more porous "shell" containing less activated and P-selectant negative platelets [23]. Platelets are involved in more than the process of primary hemostasis, but at the later stages after injury, are involved in immune cell recruitment, inflammation, wound healing, angiogenesis, and remodeling [24,25]. This partially explains why the platelet release factors, BTG and PF-4, remain elevated well beyond the point when hemostasis was achieved by the platelets [25]. Platelets are also involved in the antimicrobial response to infectious insults [26]. From the data herein, it is clear that platelets are involved in many things beyond hemostasis [27]. The continued rise in platelet level and platelet release factors through convalescence would reflect these other critical functions of recovery after the hemorrhagic shock insult has been controlled.

These data demonstrate a strong relationship of CIC in the physiologic response to thrombocytopenia following the HS insult. Megakaryocytes (Mks) are derived from hematopoietic stem cells and produce platelets; this process is known as megakaryopoiesis [11].

During HS, phospholipase C facilitates a rise in cytosolic calcium from the intracellular endoplasmic reticulum and activates the calcium plasma membrane channel, permitting the entrance of extracellular calcium which, in conjunction with ADP, increases platelet production [11-13]. This increase in intracellular calcium is essential for platelet production and activation [14]. Mks develop cytoplasmic filaments called proplatelets; which, under the influence of various enzymes, produce platelets [15]. This process is stimulated by the release of Adenosine Diphosphate (ADP) from the dense bodies, further augmenting platelet formation [16]. This process is closely linked to the increase in platelet cytosolic calcium concentration, which explains the highly significant direct correlation between the CIC and the platelet level [13]. Thus, calcium signaling is an important fundamental regulator of human Mks functions [11,13]. Through a complex process of signaling, sometimes referred to as intracellular calcium communication, the increase in intracellular calcium leads to increased platelet activation; STIM1, Orai 1, and hTRPC1 which are important for thrombin- and ADP-induced aggregation in human platelets [18].

DiBuduo and co-workers reported that when ADP is applied to human Mks in the absence of extracellular calcium, the initial platelet response remained unchanged while the plateau phase totally disappeared; thus, intracellular calcium release plays an important role in the subsequent platelet formation and release reactions, which are responsible for the subsequent platelet aggregation [11]. These authors also demonstrated that ADP promotes calcium release from intracellular stores, which in turn is responsible for the regulation of proplatelet formation and subsequent enhancement of platelet function [11]. This ADP-dependent platelet activation relies on the increase in cytosolic calcium concentrations [13,15]. Their studies confirmed that pharmacologic blockade of the increase in intracellular calcium prevented the natural platelet response to ADP, thus interfering with the release of platelet factors which augment platelet aggregation [11]. These effects of calcium on platelet function have been documented both in-vivo and ex-vivo after severe injury [19].

Mathay and co-authors described how ionized calcium increased platelet activation, aggregation, and clot strength after injury [19]. The findings reported herein support the concept that the external supplementation of calcium during resuscitation for HS facilitates the processes of both platelet formation and the resultant increase in platelet aggregation. This is evidenced herein by the increase in platelet count, ADP and collagen stimulated platelet aggregation, and the marked increase in both serum and urine platelet release factors, BTG and PF-4.

The ability of platelets to form stable adhesive contacts with other activated platelets (platelet cohesion or aggregation) at sites of vascular injury is essential for hemostasis and thrombosis [20,21]. Intracellular cytosolic calcium flux is critical during the development of platelet-platelet adhesion [20]. There is an important role regarding the intracellular fluxes of calcium and the intercellular calcium communication, which facilitates adhesiveness (aggregation). The data presented herein strongly suggests that the exogenous supplementation of calcium during the treatment of HS will facilitate this critical platelet function which helps control bleeding.

During the primary platelet release reaction, the platelets change shape as they become less discoid and more elongated with cytoplasmic extensions, thus increasing surface area [22,23]. The primary release reaction (degranulation) results in the platelets releasing the pre-formed cytoplasmic granules, which include the α-granules and the dense (Ω-bodies) [21,22]. The activated platelets release vasoactive compounds, cytokines, and growth factors. When the platelets release their granules upon activation, they interact with other platelets to help form the platelet plug. When injury occurs, nearby platelets are stimulated to release prothrombin activator.

These data identify clearly that calcium is critical in the initiation of platelet production and function; therefore, calcium should be included as part of the resuscitation regimen. During the years when these patients were treated, the guideline was that patients with severe hemorrhagic shock would receive 13.7 mEqCaCl for every five transfusions in order to compensate for the effect of citrate on calcium levels as part of blood donation. Despite this recommended regimen, the patients did not receive an average of 13.7 mEq calcium per each five transfusions, but rather received about half of that amount. Based upon the results of this amount of calcium replacement, one could recommend that all patients with severe hemorrhagic shock be started off with a regimen to provide 13.7 mEq (1 ampule) of calcium chloride with the plan to repeat this level of infusion for every five blood transfusions; the ionized calcium levels should be measured during operation to make sure that the calcium levels approach the low level of normal. Likewise, the calcium should be given as a continuous infusion rather than as a bolus, since calcium which is given as a bolus rapidly dissipates into the total extracellular fluid volume with the result that the beneficial effects of the calcium may be rapidly lost [28].

During hemorrhagic shock, the amount of calcium which relocates from the interstices into megakaryocytes and platelets to facilitate platelet function is unknown. Consequently, the authors recommend that postoperative calcium be monitored and calcium supplementation be provided in those patients who are hypocalcemic. This regimen will likely facilitate the continued production of platelets by the megakaryocytes and enhance platelet function.

The resuscitation regimen, per se, may also affect the calcium level. Prior reports have demonstrated that the addition of human serum albumin to a standard resuscitation regimen will cause an increase in the total serum calcium but a reduction in the ionized calcium, as the albumin combines with the free calcium [1,29,30]. The same phenomenon has been observed in patients treated with a 1:1:1 regimen, leading to a much greater amount of plasma proteins being infused. The albumin in the increased plasma infusion binds with calcium, thus increasing total calcium while decreasing ionized calcium [2,3]. Furthermore, the analysis of the data herein show that the ionized calcium level correlated significantly ($p<0.001$), in an inverse manner, with the FFP/RBC resuscitation ratio used in these patients. These observations make it even more imperative that the serum ionized calcium levels be monitored closely during operation and in the early postoperative period, especially in patients receiving more plasma during resuscitation [31,32].

There are a number of limitations to this study. First, this was an observational study and not a prospective randomized study, with not all patients receiving the same amount of calcium supplementation during resuscitation for severe hemorrhagic shock. The number of patients in the study and the clear correlation with calcium and platelet function, however, seem to reflect a true physiologic response to calcium replacement. Second, all of the patients were treated by one surgical team, so that it is not known whether the specifics of treatment af-

fected outcome; thus, the same correlations might not be seen when patients are treated by other regimens. This needs further study. Third, the effect of the HS insult alone on Ca++ levels could not be determined since most patients received calcium in phase I. Control studies are needed to study the effects of HS with and without a balanced resuscitation on calcium levels and with and without calcium replacement. Fourth, the amount of calcium that enters the interstitial space and the intracellular space after HS is unknown. Relocation of calcium from the plasma would affect the recommended amount of calcium to be infused during resuscitation. Fifth, the patients did not receive a 1:1 FFP/PRBC resuscitation ratio. Actually prophylactic FFP and platelet supplementation of PRBC therapy was initiated by the authors in the 1960's, and the recommended guideline to supplement 2 units FFP/5 units PRBC was begun in the 1970's; the actual ratio that these 341 patients received was 2.52 FFP/5 units PRBC which is slightly more FFP than was given in the only prospective randomized trial of injured civilians being resuscitated for HS by the Administration of Massive Transfusions [33,34].

Conclusions

In summary, this report shows a very strong relationship between serumionized calcium levels in both platelet levels and platelet function in patients treated for severe hemorrhagic shock. The inclusion of calcium supplementation was associated with increased platelet numbers and platelet function. Based upon these findings, the authors recommend that calcium routinely be supplemented in patients being resuscitated for severe hemorrhagic shock and that the calcium, more specifically the ionized calcium corrected for pH, be carefully monitored during operation and in the initial first two or three postoperative days [32]. Pending further observations, the recommendation is that patients receiving massive transfusions for hemorrhagic shock should be supplemented with one ampule (13.7 mEq) of calcium chloride for every five transfusions as part of the early resuscitation and that further calcium supplementation should be provided if patients develop decreased levels of ionized calcium.

Author Contribution

All authors were actively involved in the drafting (literature search, study design, data collection, data analysis, data interpretation, writing, critical revision, etc) of the manuscript and have provided final approval of this version.

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