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Original Article

Post-burn temporal dynamics of blood plasma histamine during the initial 6 days from injury

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Abstract: Background: Burn injuries can induce distinct, systemic inflammatory and immunological responses which occur acutely up to 72 hrs or chronically after 24 hrs. Previously published literature showed a dramatic increase in whole blood histamine values within 24 hrs of a thermal injury. However, the data is limited due to infrequent monitoring, resulting in statistically insignificant findings. The goal of this study was to determine localized histamine fluctuations for 6 consecutive days in a successive group of patients admitted immediately after a burn. Method: Using blood plasma from 7 patients (average total burn surface area 24.7%), we examined histamine within an average 4.1 (± 0.3) hrs from burn injury, by means of a monoclonal-based competitive binding enzyme immunoassay. Histamine values were normalized to patient baselines prior to determining overall averages. Patient vitals and electrolyte values were extracted from the electronic health record. A two-tailed student t-test was used to compare values with p-value ≤ 0.05 considered statistically significant using statistical software R. Results: The histamine values were significantly higher than patient baseline values up to 48 hrs (p-value ≤ 0.05), followed by a return to baseline values from approximately 3 days post-injury. Heart rates were within normal values up until 72 hrs. Hematocrit and hemoglobin began within normal values, dropped at 72 hrs, and reduced significantly from 96 hrs post-injury. The electrolyte calcium began within the normal range, and then was significantly less than the baseline value from 96 hrs post-injury. Conclusions: We have shown a distinct and significant increase in histamine plasma levels within 48 hrs after a moderate burn injury.

Keywords: Burn, histamine, mediator, inflammatory, immunological response

Introduction

A burn injury induces a complex pathophysiological reaction, resulting in metabolic disruption throughout the human body [1]. The trauma of a profound burn injury has been shown in both pediatric and adult burn patients to induce distinct systemic inflammatory and immunological responses [2], leading to changes in metabolism for several years post-injury [3, 4].

The reaction to a burn injury can be defined as two discrete responses [5]: (i) an initial acute phase, that lasts up to 72 hours from injury [6]; distinguished by localized increases in vascular permeability resulting in reduction of intravascular volume, and ultimately leading to edema formation [5]. Followed by, (ii) a chronic phase that commences around 24-72 hours after injury [7, 8]; which is characterized by an elevated heart rate and a return to normal vascular permeability [7].

One cell type that appears in animal models to have roles in both these phases are mast cells; of which two types of mast cells have been identified in human tissues [9]. Within mast cells are granules where histamine is both generated and stored, in addition to other cellular locations [10]. In an animal model it has been shown that a burn alone can stimulate the degranulation of mast cells, thus liberating histamine [11] into the extracellular environment.

Initially called β-iminazolylethylamine, histamine was first described in 1910 [12]. Within the immune response histamine serves two chief functions (a) vasodilation and (b) fluid secretion. In response to burn injury, the human body reacts in an immediate and complex manner by releasing stress hormones and inflammatory mediators [13]; subsequently inducing the release of histamine from mast cells [11].

However, little is known regarding the actual temporal dynamics of histamine release within the thermal injury paradigm in humans. A procedure to determine histamine pharmacologically in normal blood was first developed in 1935, with the aim to examine histamine metabolism, in particular in burn-induced shock [14]. In 1936 [15] and then corroborated in 1942 [16], 1957 [17] and 1969 [18], early twentieth century patient studies showed a dramatic increase in whole blood histamine values (relative to normal) within 24 hrs of thermal injury, with a precipitous decline between 3-5 days post-injury and a return to elevated histamine levels after approximately 5 days post-injury [16]. However, this previous work looking at patients within the initial 24 hr thermal injury window to the final discharge was performed on limited numbers with sporadic monitoring, resulting in non-statistically significant findings.

For this study there are two distinct phases of interest (i) the initial massive histamine release within the first 24 hrs from injury, (ii) the sudden drop, originally observed between approximately 3-5 days post-injury [16]. Yet, more recently a report concluded there were no significant changes over time in the urinary excretion of either histamine or methyl histamine in 8 burn patients for up to 48 hrs from the burn injury [19].

Thus, the aim was to return to the foundation of the original observations [15, 16] and examine localised histamine fluctuations immediately after a burn injury, using blood plasma with a modern biochemical assay and follow the patients for 6 consecutive days.

Methods

Subjects

This study was conducted at a tertiary hospital in SW Missouri within a specialized burn intensive care unit. Blood was drawn upon patient induction into this study and approximately every 24 hrs for 6 days. Blood plasma was isolated and plasma histamine (ng/ml) values were tested using an enzyme immunoassay (monoclonal-based competitive binding EIA) after chemical derivatization by acylation [20] (Viracor-Eurofins, Lee's Summit, MO). With this method the normal range for histamine in blood plasma is 0-1 ng/ml. This methodology facilitates the examination of active histamine present in the blood plasma, without either the stored histamine found in whole blood or histamine metabolite interference in whole blood and plasma [20], a limitation of previous approaches.

We postulated at the beginning of the study that values would return to normal from approximately day 4 (99 hrs; [17]), thus samples were compared to the last 3 timepoints as baseline (percent of control) prior to averaging between patients. Furthermore, sample time points were normalized relative to the time of injury (hours).

Two data points were excluded from this dataset; both were outside of the testing parameters/standard curve of the assay, each from different patients and at different time points. All other data points were included utilizing the following inclusion criteria: ≥ 18 years old, ≤ 6 hours from burn injury, ≤ 35% TBSA and a length of stay (LOS) within the burn unit of ≥ 6 days.

For the 6-month study period, 62 total patients had a disposition to the burn unit. Utilizing the inclusion criteria listed brings the total number who could have entered the study to n = 10. 3 patients were lost to the study. For overall patient demographics see **Tables 1** and **2**.

Statistical analysis

The descriptive statistics of the values are expressed as averages ± standard error. An assumption was made at the commencement of the study that values would return to normal from approximately day 4 (99 hrs), thus samples were compared to the last 3 timepoints for statistical purposes. A two-tailed student t-test was used to compare the true values at a 5% significant level. All test results with p-value < 0.05 are considered statistically significant. All

Table 1. Pre-hospital patient demographics

Case Number	Sex	Age	TBSA%	APACHE II Score	Mechanism of Injury	Source of Burn
1	M	40	18	28	Thermal	Gasoline
2	M	64	20	16	Thermal	Gasoline
3	M	70	21	15	Thermal	Gasoline
4	F	69	35	23	Thermal	Gasoline
5	M	25	22	14	Thermal	Gasoline
6	F	77	30	29	Thermal	Gasoline
7	M	24	27	17	Thermal	Gasoline
Average (± SEM)		52.71 (± 8.50)	24.71 (± 2.33)	20.29 (± 2.21)		

Table 2. Burn unit patient demographics

Case Number	Time first sample taken relative to Injury (Hours)	First 24-hour Fluid Resuscitation: AVG ml/Kg/TBSA%	Received Surgery during Study Timeframe?	Time to Surgery relative to Injury (Days)	Mechanical Vent?	Inhalation Injury?
1	4.5	4.53	Yes	2	Yes	No
2	5	2.24	Yes	3	No	No
3	2.8	2.87	Yes	2	Yes	No
4	4	3.51	Yes	4	Yes	No
5	4	4.71	Yes	2	Yes	Yes
6	4	3.94	Yes	4	Yes	Yes
7	4.3	2.45	Yes	3	Yes	Yes
Average (± SEM)	4.09 (± 0.25)	3.46 (± 0.34)		2.86 (± 0.32)		

statistical analyses were performed using statistical computing software R [21].

The study received approval from the Mercy Institutional Ethics Review Board.

Results

Histamine

At approximately 4 hrs from the injury, histamine values were significantly higher than baseline values (220% vs 100%; **Figure 1**). And then, there is a continuous reduction at 24 and 48 hrs (106% and then to 86% over baseline, respectively), but still significant relative to the baseline values (**Figure 1**). A return to baseline values was observed approximately 3 days post-injury, from where values appear to approximate a repetitive oscillation near the baseline until day 6 (**Figure 1**).

Patient vitals

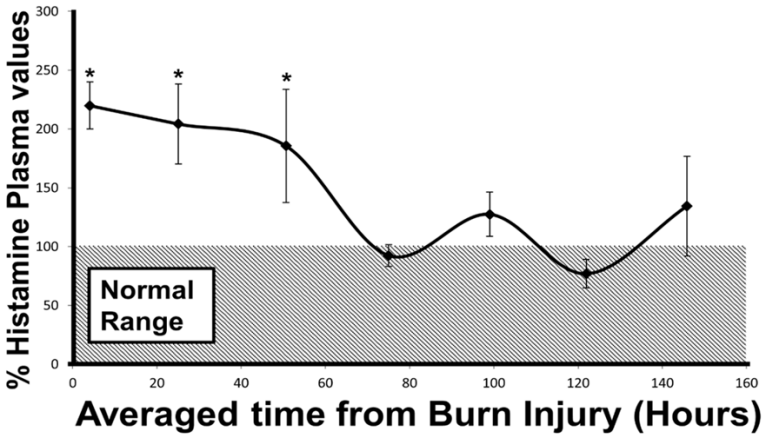
The heart rate remained relatively stable within the normal range for the first 3 days, followed by a gradual non-significant 20% increase by day 6 (**Figure 2A**). Interestingly at 48 hrs the HR was significantly different to the final 3 time points.

Both hematocrit and hemoglobin utilize a fixed volume assay and began within their normal ranges up to day 2 (**Figure 2B** and **2C**). At 72 hrs they both dropped below these normal ranges by approximately 25%, still significantly different from the final time points followed by a further 22% reduction from 96 hrs post-injury until the end of the study.

Initially, both the systolic and diastolic blood pressures were significantly higher (23% and 37%, respectively) than the later time-points. By the 48 hr time point, blood pressure returns to insignificant oscillations around the baseline values (**Figure 2D**; Systolic 120 ± SEM and Diastolic 60 ± SEM) that continues until the end of the study timeframe.

Patient electrolytes

As shown in **Figure 3A** and **3B**, sodium and potassium values remained within the normal ranges throughout the timeline of this study.



Time from Injury, Hours (± SEM)	% Histamine (± SEM)	*p*-Value
4.07 (±0.27)	219.94 (±20.00)	<0.05
25.08 (±1.43)	204.34 (±33.91)	<0.05
50 64 (±1.86)	185.69 (±48.20)	<0.05
75.00 (±1.52)	92.45 (±9.20)	0.63
99.00 (±1.52)	127.52 (±18.87)	0.13
121.92 (±1.68)	76.93 (±10.91)	0.17
145.83 (±1.64)	134.43 (±37.93)	0.47

Figure 1. Histamine Plasma Values Normalized to Burn Injury Time and Baseline Threshold Values. Average TBSA 24.7% (SEM ± 2.5), n = 7. Error bars are SEM and * denotes a p-value ≤ 0.05 that was considered significant, comparing time point to overall baseline. Basal plasma histamine concentrations up to 1 ng/ml were defined as normal.

Later, using modifications of the original histamine method [15], showed a marked increase in histamine an hour from burn injury and then a rapid noticeable histamine decrease between the third and fifth day [16]. As the edema subsided, the histamine level of the blood returned to normal or above normal [16]. This corresponds to the normalized and averaged data in **Figure 1**. From approximately 4 hrs post-injury there is an initial 120% increase from the normal range, decreasing by 14% at 25 hrs. By approximately 48 hrs the histamine levels are reduced to 86% above normal range, followed by a drop to normal values at hour 75 (approximately day 3). Additionally, this data is in line with reports that examined histamine in both burn patient blood and urine [17]. However, in a recent study [19] patients observed for an initial 48 hrs from burn injury showed no significantly elevated histamine or methylhistamine within patient urine [19].

Calcium (**Figure 3C**) began with the normal range, and then from 24 hrs post-injury dropped. At the final 3 timepoints the calcium is significantly less than the initial value.

At the beginning of the study timeline, chloride (**Figure 3D**) started within the normal range and from the 24 hr timepoint ascended to the upper edge of the normal range, however, the variations outside of the normal range were statistically insignificant.

Discussion

In 1936, a histamine elevation from the norm was described in several burn patients after hospital admission [15], that was still present 7 days post-injury; with the resultant observation that the elevation of histamine appeared to be relative to burn size.

In the original literature, HR did not significantly change during the periods of histamine elevation [15]. Our data corroborates this finding, however at 48 hrs HR was significant compared to the elevated HR observed at the end of the study (**Figure 2**). Furthermore, this HR elevation at day 6 is corroborated by recent observations [22] that saw an elevated HR at hospital discharge (average LOS = 30 days).

Another aspect to burn injuries are the resultant electrolyte disturbances that can be life-threatening when the TBSA% is above 20% [23]. Studies of burn patient electrolytes from the day of injury through to post-injury day 3 [23], showed no changes of either sodium or potassium across their timeline and our data is in agreement, with no significant changes of either sodium or potassium (**Figure 3A** and **3B**). In addition, the indication of hypocalcemia from

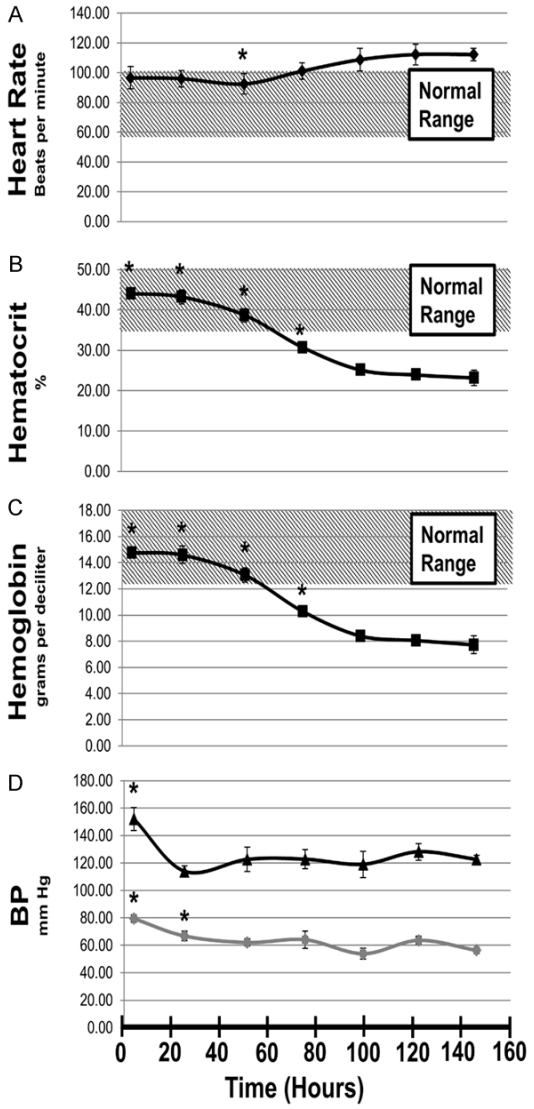


Figure 2. Averaged patient vitals across the study timeline. N = 7. Error bars are SEM and * denotes a *p*-value ≤ 0.05 that was considered significant.

24 hrs post-injury (**Figure 3C**) also follows the observations reported in the literature [23] which continued until the end of this study.

The early literature [15, 16], proposed that the rapid decline of histamine were a prelude to burn-induced secondary shock, observed at approximately 72 hrs post-burn injury, however, this was not seen in any of the patients in this study.

The discrepancy of the results from a recent study [19] is not well understood, given several studies have shown alignment between histamine studies in urine and blood plasma [17, 20]. Comparably (i) the two patient groups are similar size (n = 7 vs n = 8); (ii) with similar ages (average 53 vs 48.5) and (iii) analogous TBSA (average 24.7% vs 23.5%). The disparities include (i) a shorter time to first sample timepoint (average 4.1 hrs vs 9.07 hrs). (ii) a longer observational time frame (average 6 days vs 2 days). This extended timeframe enables use of patients' basal histamine levels as opposed to a shorter timeframe, using a separate control group to normalize values. Natural inter-patient histamine variation could negate observational histamine levels [20] if a separate control group is used for normalization. Although the authors acknowledge they did not investigate the possible localized effects of histamine in burned skin [19]. In this current study, use of a blood-based assay shows a more localized focus than the urine assay.

Thus, what is the role of histamine post-burn? The systemic change in vascular permeability sets in early after a burn and is maintained during the first 24-48 hours [24], which tracks with the elevated histamine timeline in **Figure 1**. Furthermore, the return of normal vascular permeability from approximately 72 hrs post-burn injury, is inferred with the hematocrit averaged values (**Figure 2**); dropping by over 40% (compared to normal values) which is indicative of an increase in vascular volume [25]. In animal models, histamine has been shown to increase vascular permeability mainly by nitric oxide dependent vascular dilation and subsequent blood flow increase [26]. However, in humans, a recent article [19] using a urine sourced histamine assay concluded "…findings do not support that histamine is an important mediator of the increased systemic vascular permeability seen after burn".

Interestingly, there may be another role for histamine within the burn/wound paradigm which has been shown in animal models. Utilizing a mouse knock-out model, studies show that histamine plays an enhancing role in the skin wound healing process, specifically in the release of infiltrating macrophages [27]. Further, neovascularization was controlled by the growth factor basic fibroblast growth factor (bFGF) after being triggered by histamine [27]. Thus, accelerated wound-healing activity was generated by histamine, which was most pronounced at days 3 to 5 post-injury [27]. Aspects of

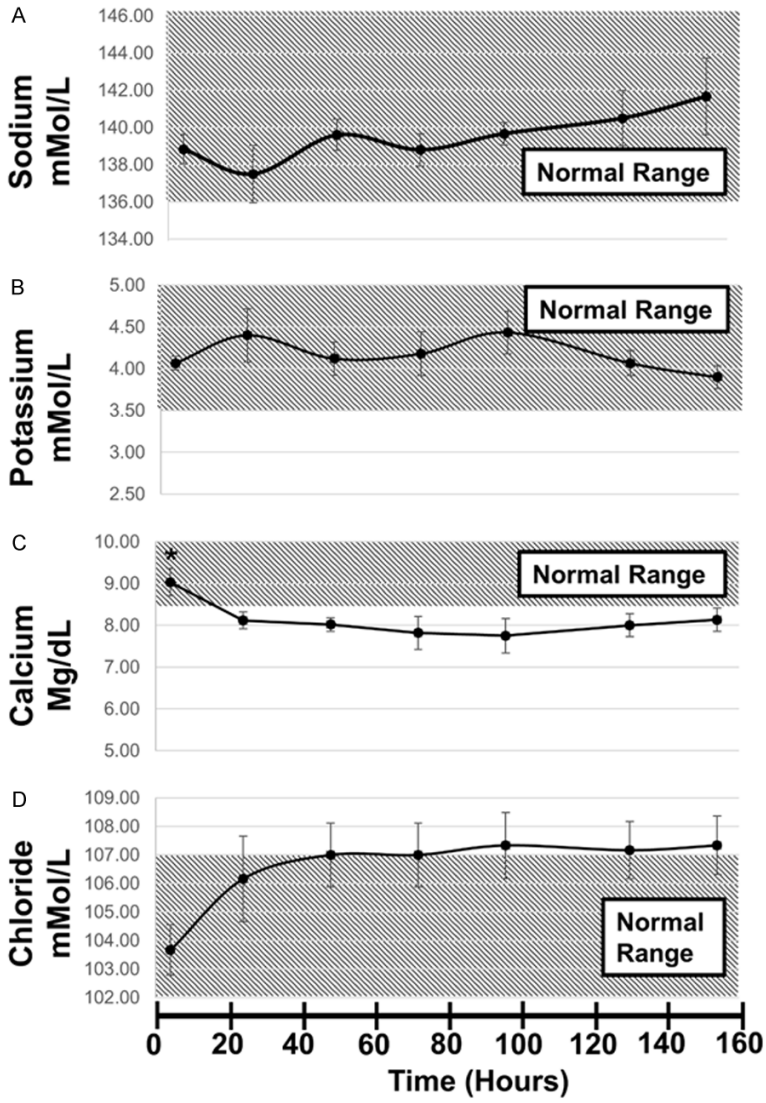


Figure 3. Averaged patient electrolytes across the study timeline. N = 7. Error bars are SEM and * denotes a *p*-value ≤ 0.05 that was considered significant.

this has been observed in human burn wounds, where treatment with bFGF showed improved cutaneous wound quality [28].

Insomnolence is common in the hospital environment with patients frequently requesting pharmacological sleep aids and the antihistamine diphenhydramine is a prevalent choice with hospital pharmacists as a temporary sleep aid [29], which is of particular consequence for burns patients.

Clinical significance

Therefore, could the use of diphenhydramine as a sleep agent impact burn patient wound healing times? Would the use of other non-antihistamine-related sleep agents reduce patient healing times and thus the patient's hospital length of stay? This data has shown that further studies are warranted to determine the role histamine holds in burn injuries.

In conclusion, previous research literature on histamine response in burns either follows singular patients or normalizes the burn patient group with a completely distinct/separate healthy population. Thus, our intent was to revisit these observations utilizing a modern technique and normalize each patient with their own baseline before subsequent averaging between patients to provide a clear, generalized overview. Our data shows a pronounced and significant increase in histamine plasma levels within the first 2 days after a moderate burn injury; by day 3 there is a return to normal range.

Limitations

Although this study is hampered by a small number of patients (n = 7), it is in line with the previous literature. The majority of burns were minor to moderate (average TBSA 24.7%) and there was some variation (see **Figure 1**) in the times that blood samples were taken from patients after the initial samples.

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Disclosure of conflict of interest

None.

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