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ORIGINAL ARTICLE

Can Albumin Trap Salicylate? An In Vitro Exploration of Salicylate Overdose Scenarios

Ryan Feldman[1,2,3,4] · **David Gummin**[5] · **Mark Hawi**[2] · **John Lyneis**[2] · **Ehab Abourashed**[2]

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Background Salicylate toxicity remains a significant cause of morbidity and mortality. At therapeutic levels, most salicylic acid (SA) is albumin-bound, but in overdose, the free fraction rises, driving toxicity. Albumin supplementation has been hypothesized as a strategy to reduce free SA, yet quantitative data are limited.
Methods An in vitro model was developed to assess albumin's binding capacity for salicylic acid across overdose-relevant concentrations. Solutions of SA (SA30 ≈30 mg/dL, SA50 ≈50 mg/dL, SA100 ≈100 mg/dL, SA120 ≈120 mg/dL) were combined with albumin ranging from 1–8 g/dL, representing subphysiologic to supraphysiologic concentrations. One solution (SA120-A) was prepared at pH 7.0 to evaluate binding in acidic environments. Free SA was quantified in each sample.
Results Increasing albumin reduced free salicylate under all conditions. From 1–8 g/dL, free SA decreased by 38.56 mg/dL (95% CI [37.62, 39.49] at SA50, 38.49 mg/dL (95% CI [37.77, 39.20] at SA100, 38.83 mg/dL (95% CI [37.85, 39.81] at SA120 (pH 7.4), and 52.77 mg/dL (95% CI [51.54, 54.01] at SA120 (pH 7.0). Correction of hypoalbuminemia (1–4 g/dL) reduced free SA by 11.33 mg/dL (95% CI [11.14, 11.53] at SA30, 21.07 mg/dL (95% CI [20.19, 21.94] at SA50, 18.40 mg/dL (95% CI [18.12, 18.68] at SA100, 18.27 mg/dL (95% CI [16.93, 19.61] at SA120 (pH 7.4), and 21.40 mg/dL (95% CI [21.00, 21.80] at SA120-A (pH 7.0). Increasing albumin further to 8 g/dL reduced free SA by an additional 17.49 mg/dL (95% CI [17.42, 17.56] at SA50, 20.09 mg/dL (95% CI [19.62, 20.55] at SA100, 20.57 mg/dL (95% CI [20.18, 20.95] at SA120 (pH 7.4), and 31.37 mg/dL (95% CI [30.12, 32.62] at pH 7.0. SA30 fell below quantification beyond 5 g/dL.
Conclusion Albumin addition reduced free salicylate, supporting its potential as a "protein sink" in salicylate toxicity. Further research is needed to determine the clinical relevance and safety of this theoretical intervention.

Keywords Salicylate poisoning · Albumin binding · Free salicylate · Overdose · In vitro study

Introduction

Salicylate (also known as salicylic acid) toxicity is a significant source of morbidity and mortality in the United States. In 2022, 14,984 single-substance aspirin exposures were reported to Americas Poison Centers. Of these cases, 177 experienced major life-threatening effects and 20 patients died [1]. Salicylic acid has multiple mechanisms of toxicity including uncoupling of mitochondrial oxidative phosphorylation, shifting to glycolysis for energy production, depletion of glycogen, and accumulation of carbon dioxide and organic anions [2].This toxicity impacts nearly all organ systems, and with no specific antidote, treatment is directed at supportive care and enhancing elimination of the toxicant via urinary alkalinization or dialysis.

At therapeutic concentrations in healthy adults, the majority of salicylic acid in the blood is bound by albumin, with only 20% of free salicylic acid able to enter tissue and cause toxicity.

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✉ Ehab Abourashed
eabourashed@mcw.edu

Ryan Feldman
rfeldman@mcw.edu

[1] The Wisconsin Poison Center, Milwaukee, WI, USA

[2] The Medical College of Wisconsin School of Pharmacy, Milwaukee, WI, USA

[3] The Medical College of Wisconsin Department of Emergency Medicine, Milwaukee, WI, USA

[4] Department of Pharmacy, Froedtert Hospital, Milwaukee, WI, USA

[5] Department of Emergency Medicine, University of New Mexico, Albuquerque, NM, USA

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However, in overdose, free salicylic acid may increase to 70% [3, 4]. Albumin is a readily available drug product in most hospitals within the United States. In animal models, exogenous administration of albumin binds free serum salicylic acid and reduces brain tissue concentrations [5]. This suggests that in salicylate-poisoned patients, increasing albumin concentration may bind excess salicylic acid, sequestering it from tissue sites where toxicity could manifest.

Though its clinical role has not been explored, several use cases can be proposed based on the mechanism and wide spread availability: correcting hypoalbuminemia as a low-risk intervention in high-risk patients and administering exogenous albumin regardless of baseline levels as a temporary bridge to hemodialysis in critically ill patients, or as an adjunct to alkalinization. While prior studies in porcine models have evaluated this intervention, they examined only a fixed dose and did not assess reductions achieved by targeting specific concentrations. Moreover, porcine albumin may differ slightly in binding properties, creating a different equilibrium between infused and endogenous albumin, and results may not fully translate to humans [6, 7]. Finally, albumin demonstrates increased binding capacity for salicylic acid at higher concentrations, and the effects of acidosis were not measured [8].

It is not known to what degree increasing albumin may reduce free salicylic acid or how conditions in overdose impact salicylic acid binding to albumin. The purpose of this study is to assess the potential of albumin as a "protein sink" to bind free salicylic acid during acute overdose. We characterize the incremental impact of increasing albumin to various concentrations and decreasing pH on free salicylic acid at various concentrations seen in overdose [9].

Methods

A solution of 25% albumin (Flexbumin 25%, Takeda Pharmaceuticals, Bannockburn IL) was diluted with phosphate-buffered saline to create eight albumin solutions with concentrations of 1–8 g/dL. The ranges were chosen to reflect incremental increases and to capture both the correction of hypoalbuminemia (1–4 g/dL) and the induction of hyperalbuminemia (4–8 g/dL). As harms have been reported in patients with a serum albumin as high as 6.6 g/dL, we considered this to represent the extreme of a clinically relevant range that might reasonably be targeted [10]. This was therefore selected as the upper boundary. A stock solution of 1.6% salicylic acid (Thermo Fisher Scientific, Heysham UK) was used to create solutions of salicylic acid in phosphate-buffered saline (PBS) with goal concentrations of 30 mg/dL, 50 mg/dL, 100 mg/dL, and 120 mg/dL. These were chosen as reflective of toxic and potentially lethal concentrations seen in overdose [9, 11]. After dilution and pH normalization, the concentrations were measured six times and actual concentrations of free salicylic acid achieved were median 31.05 mg/dL [IQR 5.63] (SA30), 48.25 mg/dL [IQR 0.93] (SA50), 98.85 mg/dL [IQR 2.00] (SA100), and 116.15 mg/dL [IQR 7.8] (SA120). For ease of labeling, samples were labeled with their target concentration in mg/dL (e.g. SA30 for target 30 mg/dL). All solutions were brought to a pH of 7.4. A fifth solution was also created with a pH of 7.0 with a target concentration of 120 mg/dL. The achieved concentration was 116.25 mg/dL [IQR 2.53] (SA120-A).

Each albumin concentration was mixed with each salicylic acid concentration to create an albumin-salicylic acid test solution. After combining albumin and salicylic acid, each solution was run through an Amicon® ultra-4 centrifugal filter (Merck KGaA, Darmstadt DE), which filtered out albumin-bound salicylate, leaving only free salicylic acid. The free salicylic acid was then diluted with PBS to fit a calibration curve and measured via high-performance liquid chromatography (HPLC). The HPLC system, a Prominence LC-2030 (Shimadzu, Japan) equipped with a photodiode array (PDA) UV/vis detector, autosampler, quaternary pump, and a HyPurity® C18 column (150 × 4.6 mm, 3 µm, Phenomenex), was used to analyze free salicylate under gradient elution conditions optimized to run for 11 min after the injection of 10 µL of sample solution. Salicylic acid was detected at 302 nm. For quantitation, a 5-point calibration curve was generated in the range of 20–160 mg/dL salicylic acid and validated for accuracy according to FDA guidelines. The lowest concentration was defined as the limit of quantitation (LOQ) for the analytical method. All analyses were performed in triplicate with the exception of pH 7.0 analysis at the concentrations of 5–8 g/dL, which was performed six times, as this was the first solution run at this pH, so additional test was run to confirm findings).

Statistical Methods

Data were tested for normality with a Shapiro-Wilk test. All data were normally distributed except the pH 7.0 analysis; median and interquartile range were presented as summary measures for all samples for consistency in presentation, and due to the lack of statistical power to reliably assess normality in these small groups. Comparisons to assess if the net change from baseline in the pH 7.0 solution was different than the net change in the pH 7.4 solution were done using a non-parametric test (Wilcoxon rank sum) due to inability to confirm normality. Free salicylic acid levels were summarized at each target SA and albumin level by the median and interquartile range. A post hoc analysis was

done to determine if the differences in each integer increase were significant using a linear regression to model free salicylic acid including target SA group, albumin level, and their interaction as fixed covariates. Although linear regression assumes normally distributed residuals, it is robust to minor deviations from normality. Given that only small deviations from normality were observed and that regression modeling offers greater statistical efficiency by using a pooled standard error across all observations, this approach was deemed appropriate. No measured confounding variables could be identified amongst authors to include within the regression analysis. The model was fit using generalized estimating equations with an exchangeable working correlation structure to account for correlation within each batch of samples. A Bonferroni p-value correction was applied to all post-hoc comparisons to adjust for multiple testing.

All statistical analyses were performed using R version 4.4.1 (2024–06–14 ucrt) (R Foundation for Statistical Computing, http://www.R-project.org). All tests were two-sided and $p<0.05$ was considered statistically significant. We visually assessed model assumptions by creating a histogram and a quantile–quantile plot of the residuals. The histogram of residuals was symmetric around zero with an approximately bell-shaped distribution (supplemental). Data may be shared upon reasonable request.

Results

As albumin concentration increased from 1 g/dL to 8 g/dL, free salicylic acid concentrations showed a corresponding decline. Individual changes at each integer albumin level are summarized in Table 1 and illustrated in Fig. 1. The only exception occurred with the SA120 solution between 1 g/dL and 2 g/dL albumin, where the concentration slightly increased, but it was not statistically significant. The change from 5 g/dL to 6 g/dL albumin in this solution also did not produce a significant reduction in salicylic acid, all other changes in free salicylic acid concentrations were statistically significant (Table 2, Fig. 2).

Impact of Correcting Hypoalbuminemia

Increasing albumin from sub-physiologic levels (1 g/dL) to physiologic levels (up to 4 g/dL) reflects the clinically relevant scenario of correcting hypoalbuminemia to normal values in a salicylate-poisoned patient. At SA30 the net reduction (regression estimated mean salicylic acid at 1 g/dL- regression estimated mean salicylic acid at 4 g/dL, Table 2) was 11.33 mg/dL (95% CI [11.1, 11.53]), at SA50 21.07 mg/dL (95% CI [20.19, 21.94]), at SA100 18.40 mg/dL (95% CI [18.12, 18.68]), and at SA120 18.27 mg/dL

Table 1 Free salicylic acid levels (mg/dL) in increasing albumin concentrations (%) at pH 7.4 and 7.0.

Free salicylic acid (mg/dL), median (IQR) [Q1-Q3]					
pH 7.4					pH 7.0
	SA30	SA50	SA100	SA120	SA120-A
PBS	31.05 (5.63) [28.43–34.05]	48.25 (0.93) [47.45–48.38]	98.85 (2.00) [97.70–99.70]	116.15 (7.80) [112.55–120.35]	116.15 (2.53) [114.78–117.30]
Albumin 1 g/dL	31.70 (0.60) [31.25–31.85]	52.50 (1.10) [51.90–53.00]	84.80 (0.35) [84.80–85.15]	99.90 (0.80) [99.50–100.30]	108.40 (0.45) [108.40–108.85]
Albumin 2 g/dL	28.80 (0.70) [28.35–29.05]	40.20 (1.15) [39.55–40.70]	83.70 (0.55) [83.50–84.05]	100.10 (0.65) [99.90–100.55]	97.20 (0.40) [97.20–97.60]
Albumin 3 g/dL	23.60 (0.45) [23.35–23.80]	38.20 (1.10) [37.70–38.80]	71.00 (0.30) [70.80–71.10]	89.80 (1.00) [89.20–90.20]	91.00 (0.00) [91.00–91.00]
Albumin 4 g/dL	20.30 (0.40) [20.00–20.40]	31.40 (0.15) [31.30–31.45]	66.70 (0.20) [66.55–66.75]	81.60 (0.65) [81.30–81.95]	87.50 (0.50) [87.10–87.60]
Albumin 5 g/dL	9.45 (0.02) [9.44–9.46]	21.94 (0.11) [21.87–21.98]	62.71 (0.26) [62.64–62.90]	73.80 (0.40) [73.40–73.80]	73.82 (11.03) [68.37–79.40]
Albumin 6 g/dL	7.45 (0.12) [7.38–7.49]	20.84 (0.08) [20.81–20.89]	55.66 (0.12) [55.56–55.68]	73.20 (0.20) [73.00–73.20]	63.13 (2.98) [61.67–64.64]
Albumin 7 g/dL	5.65 (0.01) [5.65–5.66]	15.82 (0.03) [15.79–15.82]	48.39 (0.34) [48.21–48.55]	65.60 (0.30) [65.30–65.60]	59.08 (6.21) [55.97–62.18]
Albumin 8 g/dL	5.80 (0.02) [5.78–5.80]	13.86 (0.09) [13.83–13.92]	46.63 (0.37) [46.38–46.75]	60.90 (0.25) [60.90–61.15]	55.84 (2.61) [54.57–57.18]
Reduction in free salicylic acid from 1 g/dL to 4 g/dL (median 1 g/dL – median 4 g/dL)					
Albumin 1 g/dL to 4 g/dL	11.4 mg/dL	21.1 mg/dL	17.7 mg/dL	18.3 mg/dL	20.9 mg/dL
Reduction in free salicylic acid from 4 g/dL to 8 g/dL (median 4 g/dL – median 8 g/dL)					
Albumin 4 g/dL to 8 g/dL	N/A	17.54 mg/dL	20.07 mg/dL	20.7 mg/dL	31.66 mg/dL
Reduction in free salicylic acid from 1 g/dL to 8 g/dL (median 1 g/dL – median 8 g/dL)					
Albumin 1 g/dL to 8 g/dL	N/A	38.64 mg/dL	37.77 mg/dL	39.00 mg/dL	52.56 mg/dL

PBS Phosphate buffered saline

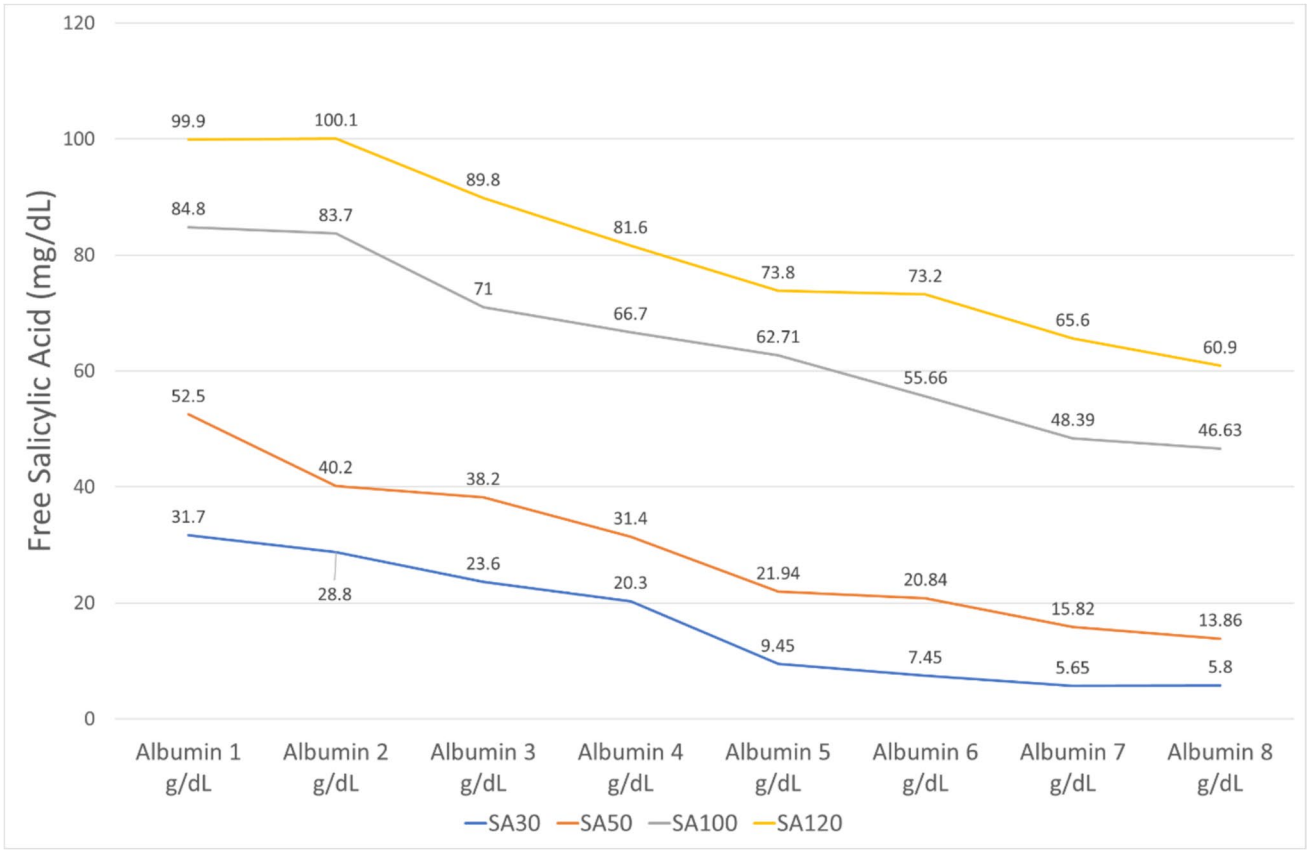

Fig. 1 Median free salicylic acid (mg/dL) at increasing human serum albumin concentrations.

(95% CI [16.93, 19.61]) under normal pH. A slightly greater net decrease was seen at SA120 when pH was 7.0 (pH 7.0, 21.40 mg/dL (95% CI [21.00, 21.80]) vs pH 7.4, 18.27 mg/dL (95% CI [16.93, 19.61]).

At a normal pH of 7.4, the largest reductions in free salicylic acid occurred when albumin increased from 2 g/dL to 3 g/dL for the SA30, SA100, and SA120 conditions, whereas SA50 showed the greatest reduction with an increase from 1 g/dL to 2 g/dL. At a more acidic pH of 7.0, the greatest decrease in free salicylic acid occurred when albumin increased from 1 g/dL to 2 g/dL. In each case, a single albumin increment (1 g/dL) accounted for 45% to 70% of the total free salicylic acid reduction observed when albumin was raised from 1 g/dL to 4 g/dL.

Impact of Increasing Albumin to Supraphysiologic Concentrations

Increasing albumin from physiologic levels (4 g/dL) to supraphysiologic levels (up to 8 g/dL) represents the addition of albumin to a patient who already has a normal serum albumin concentration. Notably, in the SA30 solution, free salicylic acid concentrations dropped below the lower limit of quantification (10 mg/dL) beginning at 5 g/dL albumin, preventing assessment of trends beyond this point, though results are reported for completeness. Increasing albumin from 4 g/dL to 8 g/dL reduced free salicylic acid by 17.49 mg/dL (95% CI [17.42, 17.56]) at SA50, 20.09 mg/dL (95% CI [19.62, 20.55]) at SA100, and 20.57 mg/dL (95% CI [20.18, 20.95]) at SA120 under normal pH, with a larger reduction of 31.37 mg/dL (95% CI [30.12, 32.62]) at SA120 when pH was 7.0. At both normal pH (7.4) and acidic pH (7.0), all solutions except SA100 showed the greatest reduction in free salicylic acid when albumin was increased from 4 g/dL to 5 g/dL. The SA100 solution demonstrated its largest reduction when albumin increased from 5 g/dL to 6 g/dL, with an equivalent decrease observed from 6 g/dL to 7 g/dL. For these solutions, a single albumin increment accounted for 37% to 64% of the total reduction in free salicylic acid observed over the entire 4 g/dL to 8 g/dL range.

Overall Trends in Salicylate Reduction

Increasing albumin from 1 g/dL to 8 g/dL reduced free salicylate by 38.56 mg/dL (95% CI [37.62, 39.49]) at SA50, 38.49 mg/dL (95% CI [37.77, 39.20]) at SA100, 38.83 mg/dL (95% CI [37.85, 39.81]) at SA120 (pH 7.4), and 52.77 mg/

Table 2 Statistical comparisons and linear regression estimated mean.

SA	Comparison	Difference in free SA (mg/dL)	95% CI	*p*-value
		Net Reduction		
SA30				
	HSA 1 g/dL—HSA 4 g/dL	11.33	[11.14, 11.53]	<0.001
	HSA 1 g/dL—HSA 8 g/dL	25.71	[25.12, 26.30]	<0.001
	HSA 4 g/dL—HSA 8 g/dL	14.38	[13.98, 14.77]	<0.001
SA50				
	HSA 1 g/dL—HSA 4 g/dL	21.07	[20.19, 21.94]	<0.001
	HSA 1 g/dL—HSA 8 g/dL	38.56	[37.62, 39.49]	<0.001
	HSA 4 g/dL—HSA 8 g/dL	17.49	[17.42, 17.56]	<0.001
SA100				
	HSA 1 g/dL—HSA 4 g/dL	18.40	[18.12, 18.68]	<0.001
	HSA 1 g/dL—HSA 8 g/dL	38.49	[37.77, 39.20]	<0.001
	HSA 4 g/dL—HSA 8 g/dL	20.09	[19.62, 20.55]	<0.001
SA120				
	HSA 1 g/dL—HSA 4 g/dL	18.27	[16.93, 19.61]	<0.001
	HSA 1 g/dL—HSA 8 g/dL	38.83	[37.85, 39.81]	<0.001
	HSA 4 g/dL—HSA 8 g/dL	20.57	[20.18, 20.95]	<0.001
SA120 (pH 7.0)				
	HSA 1 g/dL—HSA 4 g/dL	21.40	[21.00, 21.80]	<0.001
	HSA 1 g/dL—HSA 8 g/dL	52.77	[51.54, 54.01]	<0.001
	HSA 4 g/dL—HSA 8 g/dL	31.37	[30.12, 32.62]	<0.001
		Integer Reduction		
SA30				
	HSA 1 g/dL—HSA 2 g/dL	2.83	[2.73, 2.94]	<0.001
	HSA 2 g/dL—HSA 3 g/dL	5.10	[4.86, 5.34]	<0.001
	HSA 3 g/dL—HSA 4 g/dL	3.40	[3.31, 3.49]	<0.001
	HSA 4 g/dL—HSA 5 g/dL	10.71	[10.34, 11.09]	<0.001
	HSA 5 g/dL—HSA 6 g/dL	2.03	[1.92, 2.13]	<0.001
	HSA 6 g/dL—HSA 7 g/dL	1.78	[1.68, 1.88]	<0.001
	HSA 7 g/dL—HSA 8 g/dL	−0.14	[−0.17, −0.11]	<0.001
SA50				
	HSA 1 g/dL—HSA 2 g/dL	21.07	[20.19, 21.94]	<0.001
	HSA 2 g/dL—HSA 3 g/dL	−8.73	[−9.66, −7.81]	<0.001
	HSA 3 g/dL—HSA 4 g/dL	1.83	[1.69, 1.97]	<0.001
	HSA 4 g/dL—HSA 5 g/dL	16.35	[15.36, 17.33]	<0.001
	HSA 5 g/dL—HSA 6 g/dL	1.07	[0.93, 1.21]	<0.001
	HSA 6 g/dL—HSA 7 g/dL	5.05	[4.97, 5.12]	<0.001
	HSA 7 g/dL—HSA 8 g/dL	1.93	[1.84, 2.01]	<0.001
SA100				
	HSA 1 g/dL—HSA 2 g/dL	1.23	[1.02, 1.45]	<0.001
	HSA 2 g/dL—HSA 3 g/dL	12.87	[12.41, 13.32]	<0.001
	HSA 3 g/dL—HSA 4 g/dL	4.30	[4.21, 4.39]	<0.001
	HSA 4 g/dL—HSA 5 g/dL	3.84	[3.71, 3.97]	<0.001
	HSA 5 g/dL—HSA 6 g/dL	7.19	[6.93, 7.45]	<0.001
	HSA 6 g/dL—HSA 7 g/dL	7.22	[6.87, 7.58]	<0.001
	HSA 7 g/dL—HSA 8 g/dL	1.83	[1.23, 2.44]	<0.001
SA120				
	HSA 1 g/dL—HSA 2 g/dL	−0.37	[−0.92, 0.19]	1.000
	HSA 2 g/dL—HSA 3 g/dL	10.60	[9.15, 12.05]	<0.001
	HSA 3 g/dL—HSA 4 g/dL	8.03	[7.68, 8.38]	<0.001
	HSA 4 g/dL—HSA 5 g/dL	8.10	[7.77, 8.43]	<0.001
	HSA 5 g/dL—HSA 6 g/dL	0.47	[−0.10, 1.03]	1.000
	HSA 6 g/dL—HSA 7 g/dL	7.67	[7.20, 8.13]	<0.001
	HSA 7 g/dL—HSA 8 g/dL	4.33	[4.04, 4.63]	<0.001
SA120 (pH 7.0)				

Table 2 (continued)

SA	Comparison	Difference in free SA (mg/dL)	95% CI	*p*-value
	HSA 1 g/dL—HSA 2 g/dL	11.23	[11.18, 11.29]	<0.001
	HSA 2 g/dL—HSA 3 g/dL	6.47	[6.04, 6.89]	<0.001
	HSA 3 g/dL—HSA 4 g/dL	3.70	[3.21, 4.19]	<0.001
	HSA 4 g/dL—HSA 5 g/dL	13.39	[8.85, 17.92]	<0.001
	HSA 5 g/dL—HSA 6 g/dL	10.75	[5.01, 16.50]	0.009
	HSA 6 g/dL—HSA 7 g/dL	4.00	[2.68, 5.33]	<0.001
	HSA 7 g/dL—HSA 8 g/dL	3.23	[1.74, 4.72]	<0.001

HSA Human Serum Albumin

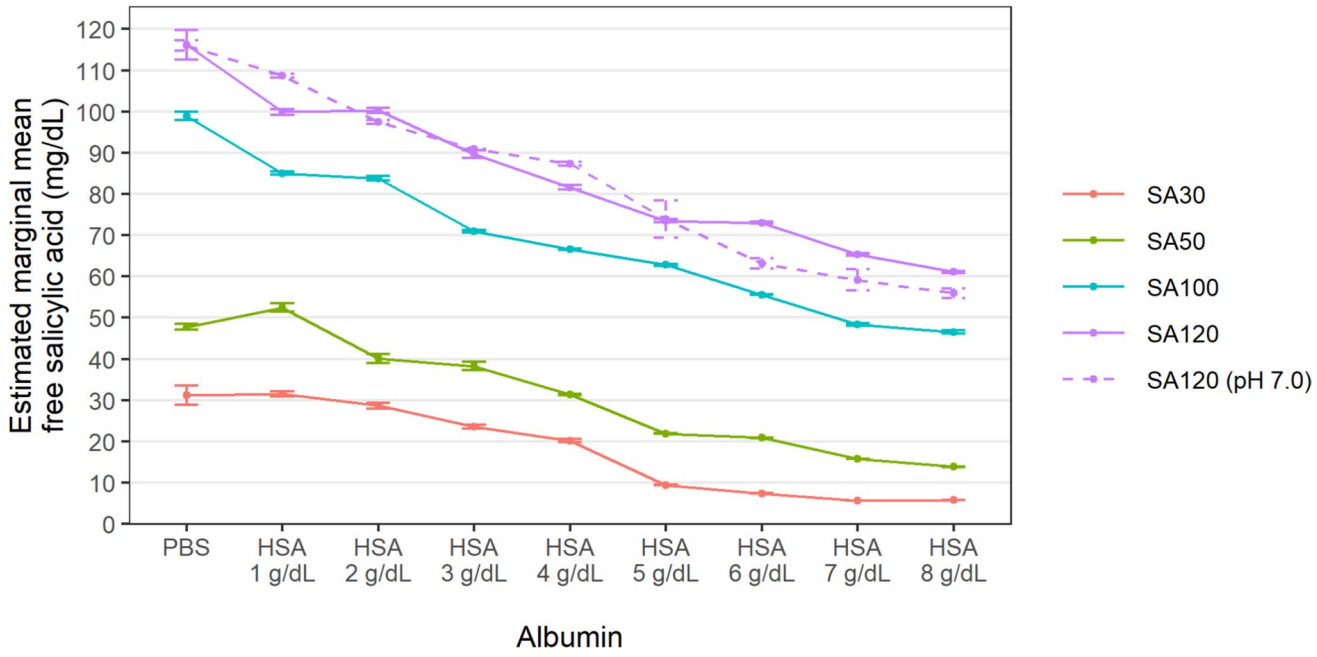

Fig. 2 Plot of estimated marginal mean free SA from regression model.

dL (95% CI [51.54, 54.01]) at SA120 (pH 7.0). At SA30, concentrations fell below the limit of quantification at 5 g/dL albumin; results are shown in Table 1 for completeness, but the total reduction could not be calculated. The largest total reduction in free salicylic acid when increasing albumin from 1 g/dL to 8 g/dL was observed in the SA120-A solution at pH 7.0, with a decrease of 52.77 mg/dL. This solution also demonstrated the greatest reductions in both the 1–4 g/dL range (21.40 mg/dL) and the 4–8 g/dL range (31.37 mg/dL) compared to all other conditions. At pH 7.4, the largest overall reduction was 38.49 mg/dL, observed in the SA120 solution (18.40 mg/dL from 1–4 g/dL and 20.09 mg/dL from 4–8 g/dL). The greatest reduction in free salicylic acid from any single integer albumin increase occurred in the SA120-A group at pH 7.0, where increasing albumin from 4 g/dL to 5 g/dL resulted in a 13.39 mg/dL (95% CI [8.85, 17.92]) decrease. The highest single-step reduction observed at normal pH (7.4) was 12.87 (95% CI [12.41, 13.32]).

Impact of Increasing Acidity

Overall, the net reduction in salicylic acid from baseline was greater in the pH 7.0 solution (Table 3, Fig. 3) compared to the reduction seen in the similar salicylic acid concentration solution at pH 7.4 (pH 7.0 median total reduction 60.14 mg/dL vs pH 50.73 mg/dL, $p = 0.024$). The reduction in baseline became significantly greater at an albumin of 6 g/dL. Prior to this, at 1 g/dL and 4 g/dL, the pH 7.0 solution had numerically lower changes from baseline compared to the pH 7.4 solution, though these were not significant (Table 3).

Discussion

Our study demonstrated that increasing albumin concentration consistently reduces free salicylic acid across a range of salicylate concentrations typically seen in acute

Table 3 Statistical difference in free salicylic acid change from baseline concentration between pH 7.0 and pH 7.4 solution at each albumin integer increase

Group		SA120	SA120 (pH 7.0)	*p*-value[1]
PBS – Albumin 1 g/dL				0.100
	Mean (SD)	11.90 (0.85)	5.83 (1.08)	
	Median (IQR)	11.80 (0.85)	6.30 (1.00)	
PBS—Albumin 2 g/dL				0.100
	Mean (SD)	11.53 (1.45)	17.07 (1.02)	
	Median (IQR)	11.50 (1.45)	17.50 (0.95)	
PBS – Albumin 3 g/dL				0.507
	Mean (SD)	22.13 (2.25)	23.53 (0.57)	
	Median (IQR)	22.90 (2.15)	23.70 (0.55)	
PBS – Albumin 4 g/dL				0.200
	Mean (SD)	30.17 (1.97)	27.23 (0.93)	
	Median (IQR)	31.10 (1.80)	27.50 (0.90)	
PBS – Albumin 5 g/dL				0.548
	Mean (SD)	38.27 (1.64)	42.15 (4.49)	
	Median (IQR)	38.90 (1.55)	42.04 (8.06)	
PBS—Albumin 6 g/dL				0.024
	Mean (SD)	38.73 (1.53)	52.91 (3.42)	
	Median (IQR)	39.30 (1.45)	52.99 (5.59)	
PBS – Albumin 7 g/dL				0.024
	Mean (SD)	46.40 (1.57)	56.91 (5.23)	
	Median (IQR)	47.10 (1.45)	57.09 (8.78)	
	[Q1, Q3]	[45.85, 47.30]	[52.59, 61.38]	
PBS – Albumin 8 g/dL				0.024
	Mean (SD)	50.73 (1.68)	60.14 (3.21)	
	Median (IQR)	51.60 (1.50)	60.25 (5.47)	
	[Q1, Q3]	[50.20, 51.70]	[57.28, 62.75]	

[1]Wilcoxon rank-sum test

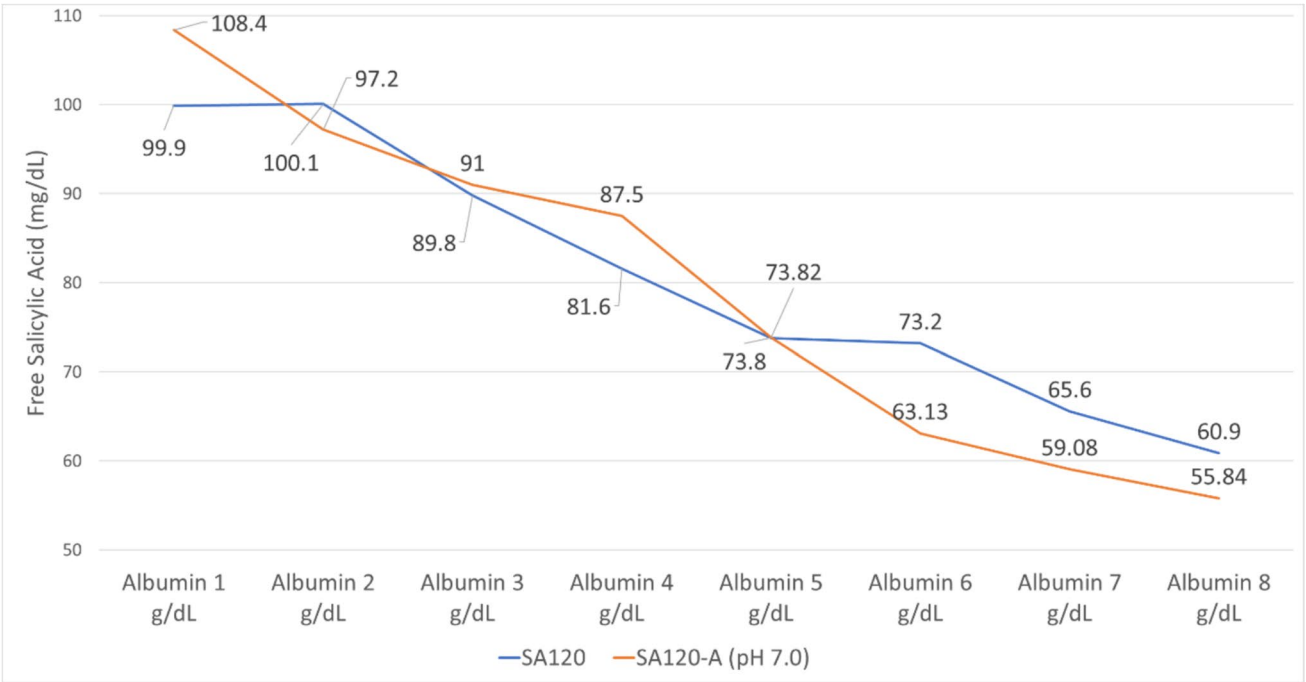

Fig. 3 Median free salicylic acid concentration (mg/dL) at increasing human serum albumin in acidic (pH 7.0) and neutral (pH 7.4) conditions.

overdose. This concept has been previously explored in an animal model, where swine poisoned with salicylic acid received a single dose of albumin (1.25 g/kg) and demonstrated numerically lower brain salicylate levels, reduced free salicylic acid, and increased clearance compared to saline-treated controls [4]. Although these findings were not statistically significant, they align with the proposed benefit of albumin therapy. Our study expands on this by characterizing the reduction in free salicylic acid over a broader range of albumin concentrations and assessing the modifying effect of pH.

Across all conditions tested, free salicylic acid decreased consistently with rising albumin concentrations from 1 g/dL to 8 g/dL, although the degree of reduction varied with both salicylate concentration and pH. At pH 7.0, which simulates the metabolic acidosis seen in severe salicylate toxicity, albumin binding capacity was overall enhanced at higher albumin concentrations. The net reduction in free salicylic acid was greater in the pH 7.0 solution when albumin increased from 1–4 g/dL and from 4–8 g/dL compared to reductions observed at pH 7.4. However, at several low albumin concentrations (1 g/dL, 3 g/dL, 4 g/dL), free salicylic acid was numerically higher at pH 7.0 than at pH 7.4, suggesting a possible decreased binding affinity under acidic conditions when albumin is scarce. These findings are hypothesis-generating, as the mechanism underlying this duality of binding at different albumin concentrations is unclear. These results may have been due to sampling, however prior work has shown a reduction in stoichiometric binding capacity at albumin concentrations below 3.33 g/dL, which may potentially be exacerbated by acidosis [8].

The net effect observed of increased binding affinity of albumin for salicylate at lower pH is generally consistent and plausible. The primary drug binding domains for human albumin are labeled Sudlow I and Sudlow II [8]. Drug binding is reversible and forms via weak chemical bonds, such as hydrogen bonds or van der Waals forces. Salicylic acid primarily binds in Sudlow I, with a smaller amount binding at site II [11]. The phenyl moiety of salicylic acid interacts with hydrophobic moieties in the Sudlow site, and this is energetically favorable, causing binding [12]. At lower pH, the carboxylic acid moiety of salicylic acid is protonated, which may favor more hydrophobic interaction at this site and increase binding. Other studies demonstrate increased binding within the Sudlow II site at lower pH [13].

Substantial reductions in salicylic acid occurred between 1 g/dL and 4 g/dL, simulating the correction of hypoalbuminemia, a common and clinically relevant scenario in critically ill patients. This represents an important therapeutic opportunity, as prior work shows a disproportionate reduction in binding capacity at subphysiologic albumin concentrations and higher free salicylate to albumin ratios have been associated with increased end organ damage in studies of salicylate toxicity [8, 14, 15]. Our findings support the hypothesis that restoring physiologic albumin levels can significantly lower free salicylate. Clinical correlation is needed, as well as safety data, but this could represent a low risk intervention in critically ill patients to aid in mitigating toxicity.

Free salicylic acid continued to decrease across all groups as albumin concentrations increased from 4 g/dL to 8 g/dL, though much of the benefit was seen when increasing from 5 g/dL to 6 g/dL in most groups (SA100, SA120, pH7 SA120) and 6 g/dL in all. These findings suggest that supraphysiologic albumin levels may offer additional binding, but the benefit of excessive concentration is not clear. While albumin replacement for hypoalbuminemia is common and generally well tolerated, the safety and clinical utility of administering supraphysiologic would require significant exploration prior to implementation.

In at least one case report, a patient developed acute kidney injury after achieving a serum albumin of 6.6 g/dL, though they received 1800 g of albumin over 3 days and reached a peak concentration 11.8 g/dL [10]. Other studies have found association of increased risk of renal injury with hypoalbuminemia, and cell studies confirm proximal tubule toxicity can be caused by hyperalbuminemia [16, 17].

Additionally, the exact amount of volume needed for correction of hypoalbuminemia, or to provide supraphysiologic albumin is not known. Theoretical dosing frameworks for change in albumin include desired albumin (g/dL)- actual albumin/estimated plasma volume (volume of distribution) [18]. However this fails to account for albumin catabolism, and individual volumes of distribution as well as daily catabolism may vary [19]. Empiric dosing where a set amount is given and the resultant change is often utilized. If excess volumes are needed, harms associated with hypervolemia may occur, and some studies have found albumin > 5 g/dL to be associated with major adverse cardiovascular events compared to those with < 5 g/dL [20].

The impact that increased drug protein binding may have on hemodialysis is also not well characterized in this setting, but it may theoretically reduce available dialyzable toxin and is a consideration that may add equipoise to an intervention which may be aimed at temporizing a patient awaiting dialysis. Finally, the degree to which binding remains persistent has not been characterized, and redistribution may be of theoretical risk. These limitations should be considered when interpreting the potential clinical application of our findings.

This study is subject to several limitations. Experiments were run at room temperature; however explicit temperature controls were not in place which may impact salicylate concentrations in solution. Our in vitro model used

pharmaceutical-grade albumin and lacked endogenous ligands or competing drugs. As a result, it likely reflects the maximum amount of salicylate that could be bound by exogenously administered albumin. This point is particularly salient given that our study evaluated normal and acidic pH conditions. In clinical salicylate poisoning, urinary or serum alkalinization may produce an alkaline environment, the impact of which is not clear on albumin binding.

Further, in vivo competition from other ligands may reduce binding, potentially resulting in less free salicylate being bound. Finally, pharmaceutical albumin has been shown to have lower binding affinity than native human serum albumin, which could lead to an overestimation of free salicylate at any given total salicylate and albumin concentration [21]. Due to lack of endogenous competition, and different binding capacity of pharmaceutical and human albumin, this model may not accurately represent the free salicylate levels expected in vivo for a given total salicylate and human albumin concentration. However our findings are consistent with past studies of salicylic acid-spiked human plasma showing approximately 80% protein binding at concentrations < 100 mg/dL and only 30% protein binding at > 120 mg/dL [3]. In our study at 4 g/dL albumin, only 25–30% of salicylic acid was protein bound.

The clinical relevance of these exploratory findings remains uncertain. Even when extrapolated to a clinical context, where our results represent the maximum expected reduction in free salicylate, the overall impact on patient outcomes is unknown. The threshold for salicylate toxicity is generally considered to be a total (free and bound) salicylic acid concentration of greater than 30 mg/dL [2]. The SA100 and SA120 concentrations had free salicylic acid concentrations higher than 30 mg/dL even at 8 g/dL albumin. Not only does that concentration remain potentially toxic, but the safety and feasibility of administering such high doses of albumin in clinical practice are unknown.

Future work should explore the prognostic value of free salicylic acid concentrations, the safety of exogenous supratherapeutic albumin, and also explore the binding capacity of native human serum albumin, such as that found in plasma, to better understand the therapeutic potential of albumin-based interventions in salicylate poisoning.

Conclusion

This study demonstrates that increasing albumin concentration reduces free salicylic acid in vitro. These data characterized the potential role of exogenous albumin as a therapeutic adjunct in acute salicylate toxicity, however significant clinical exploration of the safety and utility of this intervention would be needed. Both correction of hypoalbuminemia and elevation to supraphysiologic levels reduced free salicylate, though in some cases, the greatest reductions occurred with physiologic correction alone. Acidic conditions further enhanced albumin binding. Future research is needed to determine whether lowering free salicylate improves clinical outcomes, to assess the safety of supraphysiologic albumin concentrations, and the binding capacity of human serum albumin compared to pharmaceutical grade.

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Data availability Parts of these data were previously presented at the American Academy of Clinical Toxicology North American Congress of Clinical Toxicology Meeting, Denver, CO, 2024.

Declarations

Conflict of interest Ryan Feldman declares that he has no conflict of interest.
David Gummin declares that he has no conflict of interest.
Mark Hawi declares that he has no conflict of interest.
John Lyneis declares that he has no conflict of interest.
Ehab Abourashed declares that he has no conflict of interest.

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