Defining the role of albumin infusion in cirrhosis-associated hyponatremia

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1Department of Medicine, University of California Los Angeles, Los Angeles, California; 2Department of Pathology & Laboratory Medicine, University of California Los Angeles, Los Angeles, California; and 3Brain Research Institute, University of California Los Angeles, Los Angeles, California

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Nguyen MK, Ornekian V, Kao L, Butch AW, Kurtz I. Defining the role of albumin infusion in cirrhosis-associated hyponatremia. Am J Physiol Gastrointest Liver Physiol 307: G229–G232, 2014. First published May 15, 2014; doi:10.1152/ajpgi.00424.2013.—The presence of negatively charged, impermeant proteins in the plasma space alters the distribution of diffusible ions in the plasma and interstitial fluid (ISF) compartments to preserve electroneutrality and is known as Gibbs-Donnan equilibrium. In patients with hypoalbuminemia due to underlying cirrhosis, the decrease in the plasma water albumin concentration ([Alb]pHw) would be expected to result in a decrease in the plasma water sodium concentration ([Na+]pw) due to an alteration in the distribution of Na+ between the plasma and ISF. In addition, cirrhosis-associated hyponatremia may be due to the renal diluting defect resulting from the intravascular volume depletion due to gastrointestinal losses and overdiuresis and/or decreased effective circulatory volume secondary to splanchnic vasodilatation. Therefore, albumin infusion may result in correction of the hyponatremia in cirrhotic patients either by modulating the Gibbs-Donnan effect due to hypoalbuminemia or by restoring intravascular volume in patients with intravascular volume depletion due to gastrointestinal losses and overdiuresis. However, the differential role of albumin infusion in modulating the [Na+]pw in these patients has not previously been analyzed quantitatively. In the present study, we developed an in vitro assay system to examine for the first time the quantitative effect of changes in albumin concentration on the distribution of Na+ between two compartments separated by a membrane that allows the free diffusion of Na+. Our findings demonstrated that changes in [Alb]pHw are linearly related to changes in [Na+]pw as predicted by Gibbs-Donnan equilibrium. However, based on our findings, we predict that the improvement in cirrhosis-associated hyponatremia due to intravascular volume depletion results predominantly from the restoration of intravascular volume rather than alterations in Gibbs-Donnan equilibrium.

MATERIALS AND METHODS

Salt-free albumin was generated using a mixed cation and anion exchange resin (MBD-10-LTCC; ResinTech, West Berlin, NJ) that exchanges Na+ for H+ and Cl− for OH−. Human albumin (126654; Calbiochem, EMD Biosciences, Darmstadt, Germany) was dissolved in ultrapure water (1 g/5 ml) and was subsequently loaded on a column containing an equal volume of the exchange resin equilibrated with ultrapure water at a flow rate of 10 ml/h. The effluent solution was reloaded and run on the column five separate times. The salt-free albumin solution was lyophilized using a Savant Speed Vac concentrator (Savant Instruments, Hailbrook, NY).

We examined the role of varying albumin concentrations on the distribution of Na+ between two compartments in vitro separated by a semipermeable dialysis membrane (Spectra/Por Dispodialyzer, 15,000 mol wt cutoff; Spectrum Laboratories) (Fig. 1). To simulate the in vivo distribution of H2O between the plasma and ISF, 2 ml of a standard 127 mM NaCl solution were added to one compartment, and 9.3 ml were added to the other compartment since the plasma volume is known to be approximately one-fifth of the extracellular volume (plasma 3 l, ISF 14 l) (9). A standard 127 mM NaCl solution was chosen to obtain [Na+] in the protein-containing compartment in the 129–140 range. Salt-free albumin was dissolved in the 2 ml NaCl solution in various amounts to vary the albumin concentration. Electrochemical equilibrium between the two compartments was then allowed to occur for 24 h.

Once electrochemical equilibrium was complete, duplicate measurements of the [Na+] were measured in the two compartments, and the mean of the two measurements of the [Na+] was determined. The [Na+] in each compartment was measured by an Olympus AU5400 analyzer (Olympus America, Melville, NY). Because salt-free albumin was added to vary the albumin concentration from 1 to 7.4 g/dl, the [Na+] must be expressed in units of millimoles per liter of H2O (mmol/l H2O) since Na+ is only present in the aqueous phase. In other words, the problem of a factitiously low [Na+] that is due to an increased percentage of protein or a factitiously high [Na+] that is due to a decreased percentage of protein can be avoided by expressing the [Na+] in units of millimoles per liter of H2O. To express the [Na+] in units of millimoles per liter of H2O, the water content was determined gravimetrically for each sample using a Mettler AG204 balance (Mettler-Toledo, Columbus, OH) as follows. A sample was initially weighed and then lyophilized to evaporate the water content followed by repeat weighing. The water component of each sample was then calculated as the difference between the pre- and postlyophilization weights. To ensure complete evaporation of the water component in each sample, lyophilization was continued until there was no further change in the mass of the lyophilized sample. The water studies that have investigated the quantitative relationship between changes in the albumin concentration ([Alb]) and the sodium concentration ([Na+]) that would allow clinicians to estimate the magnitude of the contribution of the Gibbs-Donnan effect to the hyponatremia in patients with cirrhosis. The purpose of this study was to differentiate quantitatively the contribution of Gibbs-Donnan effect from the renal diluting defect due to either intravascular volume depletion or diminished effective circulatory volume in the generation of hyponatremia in cirrhotic patients.

HYponatremIA is a common electrolyte disorder in patients with cirrhosis. The hyponatremia in patients with cirrhosis is attributed to the renal diluting defect due to either intravascular volume depletion or diminished effective circulatory volume resulting from splanchnic vasodilatation (2, 4, 5, 11). The intravascular volume depletion is due to the lactulose-induced osmotic diarrhea and overly aggressive diuresis in these patients. It is also well known that the presence of negatively charged, impermeant albumin in the plasma space alters the distribution of diffusible Na+ in the plasma and interstitial fluid (ISF) compartments to preserve electroneutrality (8). Therefore, hypoalbuminemia would be expected to result directly in a decrease in the plasma water sodium concentration ([Na+]pw) due to an alteration in the distribution of Na+ between the plasma and ISF. Unfortunately, there are no prior

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content was then determined by dividing the mass of the water component of each sample by the volume of the sample being lyophilized. The measured [Na+] was then expressed in millimole per liter H2O by correcting for the water content in each sample. The [Alb−] is also measured on each sample using an Olympus AU5400 automated chemistry analyzer.

Statistics. Linear regression analysis was performed to correlate alterations in the [Na+] and changes in the [Alb−], the relationship between [Alb−] and the electrical potential (Em), and the relationship between [Alb−] and the Gibbs-Donnan ratio for sodium distribution.

RESULTS

It is well known that Gibbs-Donnan equilibrium is established when the altered distribution of Na+ results in electrochemical equilibrium (10). At Gibbs-Donnan equilibrium, the chemical gradient is equal in magnitude and opposite in direction to the electrical gradient as depicted by the following equation (10):

\[ F_{E_m} = -RT \ln \left( \frac{[Na^+]_1}{[Na^+]_2} \right) \]  

where \( F \) is Faraday’s constant; \( R \) is ideal gas constant; \( T \) is absolute temperature; \([Na^+]_1\) is the sodium concentration in the protein-containing compartment; and \([Na^+]_2\) is the sodium concentration in the non-protein-containing compartment.

As expected, the [Na+] was greater in the albumin-containing compartment due to Gibbs-Donnan equilibrium (Table 1). As the [Alb−] decreased from 7.4 to 1 g/dl, the [Na+] decreased from 136 to 129 mmol/l, respectively, in the protein-containing compartment. The relationship between the [Na+] and the [Alb−] was analyzed by linear regression, and results are shown in Fig. 2. The [Na+] was related to the [Alb−] according to the following equation:

\[ [Na^+] = 0.982 [Alb^-] + 127.65 \]  

Based on Eq. 1, the \( E_m \) imposed by negatively charged albumin can be calculated based on the ratio of sodium concentration in the protein-containing compartment and sodium concentration in the non-protein-containing compartment, \([Na^+]_1/[Na^+]_2\) (Table 2):

\[ E_m = -\frac{RT}{F} \ln \left( \frac{[Na^+]_1}{[Na^+]_2} \right) \]  

The relationship between [Alb−] and \( E_m \) was analyzed by linear regression as shown in Fig. 3. [Alb−] was related to \( E_m \) according to following equation:

\[ E_m = -0.303 - 0.181 [Alb^-] \]  

Additionally, since the albumin concentration determines the distribution of Na+ between the two compartments, the relationship between [Alb−] and the Gibbs-Donnan ratio for sodium distribution \([Na^+]_1/[Na^+]_2\) was analyzed. As shown in Fig. 4, [Alb−] defines \([Na^+]_1/[Na^+]_2\) by the following equation:

\[ \frac{[Na^+]_1}{[Na^+]_2} = 1.0111 + 0.00736 [Alb^-] \]  

DISCUSSION

Hyponatremia commonly occurs in patients with advanced cirrhosis. In cirrhosis, the pathogenesis is thought to be related to the hemodynamic changes and secondary neurohumoral adaptations that occur in these patients (2, 4, 5, 11). Patients

<table>
<thead>
<tr>
<th>Albumin-Containing Compartment</th>
<th>Albumin-Free Compartment</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Na+], mmoll H2O</td>
<td>[Alb−], g/dl</td>
</tr>
<tr>
<td>129</td>
<td>1.0</td>
</tr>
<tr>
<td>130</td>
<td>1.6</td>
</tr>
<tr>
<td>130</td>
<td>2.2</td>
</tr>
<tr>
<td>129</td>
<td>3.0</td>
</tr>
<tr>
<td>133</td>
<td>4.8</td>
</tr>
<tr>
<td>132</td>
<td>4.9</td>
</tr>
<tr>
<td>134</td>
<td>6.0</td>
</tr>
<tr>
<td>133</td>
<td>6.7</td>
</tr>
<tr>
<td>136</td>
<td>7.4</td>
</tr>
</tbody>
</table>

[Na+], sodium concentration; [Alb−], albumin concentration.

\[ [Na^+] = 0.982 [Alb^-] + 127.65 \]  

\[ E_m = -\frac{RT}{F} \ln \left( \frac{[Na^+]_1}{[Na^+]_2} \right) \]  

\[ E_m = -0.303 - 0.181 [Alb^-] \]  

\[ \frac{[Na^+]_1}{[Na^+]_2} = 1.0111 + 0.00736 [Alb^-] \]  

Fig. 1. Spectra/Por Dispodialyzer.

Fig. 2. Linear regression analysis depicting changes in the albumin concentration ([Alb−]) and alterations in sodium concentration ([Na+]) due to Gibbs-Donnan equilibrium: [Na+] = 0.982 [Alb−] + 127.65 (r = 0.91).
Table 2. Relationship between albumin concentration, membrane potential, and Gibbs-Donnan ratio for Na⁺ distribution

<table>
<thead>
<tr>
<th>[Alb⁻], g/dl</th>
<th>Eᵣm, mV</th>
<th>[Na⁺]/[Na⁺]₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>−0.809</td>
<td>1.032</td>
</tr>
<tr>
<td>1.6</td>
<td>−0.600</td>
<td>1.024</td>
</tr>
<tr>
<td>2.2</td>
<td>−0.600</td>
<td>1.024</td>
</tr>
<tr>
<td>3</td>
<td>−0.605</td>
<td>1.024</td>
</tr>
<tr>
<td>4.8</td>
<td>−1.186</td>
<td>1.047</td>
</tr>
<tr>
<td>4.9</td>
<td>−0.992</td>
<td>1.039</td>
</tr>
<tr>
<td>6</td>
<td>−1.378</td>
<td>1.055</td>
</tr>
<tr>
<td>6.7</td>
<td>−1.389</td>
<td>1.056</td>
</tr>
<tr>
<td>7.4</td>
<td>−1.962</td>
<td>1.079</td>
</tr>
</tbody>
</table>

Eᵣm, electrical potential; [Na⁺]/[Na⁺]₂, Gibbs-Donnan ratio for sodium distribution.

with cirrhosis usually have a marked reduction in systemic vascular resistance and mean arterial pressure due to splanchnic vasodilatation. The reduction in pressure at the carotid and renal baroreceptors induced by splanchnic vasodilatation leads to activation of the sodium-retaining neurohumoral mechanisms in an attempt to restore perfusion pressure to normal. These include the renin-angiotensin system, the sympathetic nervous system, and antidiuretic hormone (ADH). Of these three neurohumoral mechanisms, increased ADH secretion plays a central role in the pathogenesis of hyponatremia in patients with cirrhosis since suppression of ADH release is required to excrete the daily water intake. In addition, in patients with significant lactulose-induced osmotic diarrhea and overly aggressive diuresis of the ascites and peripheral edema, intravascular volume depletion can result in disruption in the mechanisms underlying urinary free water excretion. The normal physiology of urinary dilution is determined by numerous factors such as normal glomerular filtration rate, adequate delivery of fluid to the diluting segments of the nephron mediated by inhibition of proximal Na⁺ and H₂O absorption, intact Na⁺ and Cl⁻ reabsorption at both the thick ascending limb of the loop of Henle and distal convoluted tubules, and the inhibition of H₂O absorption in the collecting duct resulting from the suppression of ADH secretion. The renal diluting defect associated with intravascular volume depletion results from the decreased glomerular filtration rate, avid proximal sodium and fluid reabsorption with resultant diminished delivery of fluid to the diluting segments of the nephron, inhibition of tubular Na⁺ and Cl⁻ reabsorption by diuretics, and hypovolemia-induced increased secretion of ADH. In these cirrhotic patients with intravascular volume depletion, albumin infusion may ameliorate the hyponatremia by expanding the intravascular volume (1, 3, 6, 7). Indeed, two studies have suggested that the administration of albumin, which aids the expansion of plasma volume, may improve plasma sodium concentration in patients with cirrhosis (6, 7).

It is also well known that the [Na⁺]pw and ISF [Na⁺] differ despite the high permeability of Na⁺ across the capillary membrane that separates these two fluid compartments (8, 9). This difference in ionic concentrations between the plasma and the ISF is due to the much higher concentration of proteins in the plasma compared with the ISF. Proteins are large-molecular-weight substances and therefore do not pass the capillary membrane easily. This lack of protein permeability across capillary membranes is responsible for causing ionic concentration differences between the plasma and ISF (8, 9). Because proteins are negatively charged nonpermeant molecules present predominantly in the plasma space, their presence will attract positively charged Na⁺. The distribution of Na⁺ is altered to preserve electroneutrality in the plasma and ISF. As a result, the [Na⁺]pw is typically greater than the ISF [Na⁺] (8, 9).

Due to alterations in Gibbs-Donnan equilibrium, hypoalbuminemia in the setting of cirrhosis would be expected to result directly in a decrease in [Na⁺]pw by modulating the distribution of Na⁺ between the plasma and ISF. We therefore hypothesized that albumin infusion, by inducing changes in the plasma water albumin concentration ([Alb⁻]pw), can directly lead to amelioration of the hyponatremia due to the direct effect of Gibbs-Donnan equilibrium that is independent of its effect on plasma volume expansion. To test this hypothesis, we examined the role of varying albumin concentrations on the distribution of Na⁺ between two compartments in vitro. Because hyponatremia in cirrhosis may be caused by the defect in urinary free water excretion resulting from intravascular volume depletion and/or decreased effective circulatory volume, the role of the renal diluting defect must be distinguished clinically from the direct effect of changes in the [Alb⁻]pw on alterations in the [Na⁺]pw due to Gibbs-Donnan equilibrium. Therefore, by evaluating the role of varying albumin
concentrations on the distribution of Na\(^+\) between two compartments in vitro, one can directly for the first time determine quantitatively the contribution of Gibbs-Donnan equilibrium to the development of cirrhosis-associated hyponatremia. As expected, the [Na\(^+\)] is greater in the albumin-containing compartment due to Gibbs-Donnan equilibrium as shown in Table 1. In addition, as the [Alb\(^-\)] decreased from 7.4 to 1 g/dl, the [Na\(^+\)] decreased from 136 to 129 mmol/l, respectively, in the protein-containing compartment. Importantly, alterations in the [Na\(^+\)] in the protein-containing compartment resulting from changes in the [Alb\(^-\)] are quantitatively defined by the following equation:

\[
[\text{Na}^+] = 0.982 [\text{Alb}^-] + 127.65 \quad (r = 0.91)
\]

Furthermore, according to Gibbs-Donnan equilibrium as defined by Eq. 1, the Em exerted by the nonpermeant albumin can be calculated based on [Na\(^+\)]/[Na\(^+\)]. Specifically, the greater the Gibbs-Donnan ratio for sodium distribution, the greater is the chemical gradient and therefore the Em imposed by the negatively charged albumin. As shown in Fig. 3, the Em increases proportionally with incremental changes in the albumin concentration according to the following linear regression equation:

\[
\text{Em} = -0.303 - 0.181 [\text{Alb}^-] \quad (r = 0.9).
\]

Additionally, because [Alb\(^-\)] determines [Na\(^+\)]/[Na\(^+\)], the relationship between [Alb\(^-\)] and [Na\(^+\)]/[Na\(^+\)] can be determined as shown in Fig. 4 where:

\[
[\text{Na}^+] /[\text{Na}^+] = 1.011 + 0.00736 [\text{Alb}^-] \quad (r = 0.9).
\]

According to this equation, the ratio of the sodium concentration in the protein-containing compartment and sodium concentration in the non-protein-containing compartment is 1.04 at an albumin concentration of 4 g/dl. This finding is consistent with the in vivo Gibbs-Donnan ratio for the distribution of univalent cations between the plasma and ISF given that the Gibbs-Donnan ratio for the distribution of Na\(^+\) between the plasma and ISF is 1.05 (8).

In terms of clinical implications, based on Eq. 2, as the plasma [Alb\(^-\)] increases from 2 to 4 g/dl, the plasma [Na\(^+\)] is expected to increase by 2 mmol/l. Therefore, within the physiological range of alterations in the plasma [Alb\(^-\)], improvement in cirrhosis-associated hyponatremia resulting from albumin infusion is due predominantly to plasma volume expansion rather than alterations in Gibbs-Donnan equilibrium. In other words, in patients with cirrhosis-associated hyponatremia and hypoalbuminemia, albumin infusion was expected to significantly improve the hyponatremia in patients with clinical evidence of true intravascular volume depletion (i.e., low central venous pressure). In contrast, albumin infusion would not be expected to significantly improve the hyponatremia in patients with cirrhosis-associated hyponatremia and hypoalbuminemia who have clinical evidence consistent with intravascular hypervolemia (i.e., elevated central venous pressure).

**Limitations.** A limitation of our in vitro study is that the in vitro semipermeable membrane used in our experiment does not have active transporters like the cell membrane. Indeed, the Na\(^-\)K\(^+\)-ATPase in the cell membrane actively pumps Na\(^+\) out of the cell. However, the subsequent distribution of Na\(^+\) between the plasma and ISF will be determined by the Gibbs-Donnan effect due to impermeant plasma proteins. Consistent with this fact, the in vitro Gibbs-Donnan ratio of the sodium concentration in the protein-containing compartment and sodium concentration in the non-protein-containing compartment of 1.04 at an albumin concentration of 4 g/dl is similar to the in vivo Gibbs-Donnan ratio for the distribution of Na\(^+\) between the plasma and ISF of 1.05.

It is well known that the diffusion of middle- to large-molecular-weight solutes across semipermeable membranes is dependent on the effective pore size of the membrane. The effective pore size of the in vitro dialysis membrane (15,000 mol wt cutoff) used in this study may differ from that of the cell membrane in vivo. However, this is not a concern since, in our study, the diffusion of small ions such as Na\(^+\) (atomic weight 23) was unlikely affected by the pore size of the semipermeable membrane used.

In conclusion, we have demonstrated for the first time that changes in the [Alb\(^-\)] can directly lead to changes in the [Na\(^+\)] due to the effect of Gibbs-Donnan equilibrium. Our findings demonstrated that, for each 1 g/dl increase in the plasma [Alb\(^-\)], plasma [Na\(^+\)] is expected to increase by 1 mmol/l due to the Gibbs-Donnan effect. Therefore, within the physiological range of alterations in the plasma [Alb\(^-\)], correction of cirrhosis-associated hyponatremia due to intravascular volume depletion by albumin infusion results predominantly from plasma volume expansion rather than alterations in Gibbs-Donnan equilibrium.

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**DISCLOSURES**
The authors declare that they have no conflict of interest.

**AUTHOR CONTRIBUTIONS**
M.K.N. conception and design of research; M.K.N. analyzed data; M.K.N. interpreted results of experiments; M.K.N. drafted manuscript; M.K.N. and I.K. edited and revised manuscript; M.K.N. approved final version of manuscript; V.O., A.W.B., and I.K. performed experiments; L.K. prepared figures.

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