



Supplementary Information

Alkaline Liquid Ventilation of the Membrane Lung for Extracorporeal Carbon Dioxide Removal (ECCO₂R): In Vitro Study

Luigi Vivona ^{1,†}, Michele Battistin ^{2,†}, Eleonora Carlesso ^{1,†}, Thomas Langer ^{3,4,*}, Carlo Valsecchi ⁵, Sebastiano Maria Colombo ^{1,5}, Serena Todaro ¹, Stefano Gatti ², Gaetano Florio ¹, Antonio Pesenti ^{1,5}, Giacomo Grasselli ^{1,5} and Alberto Zanella ^{1,5}

- ¹ Anesthesia and Critical Care, Department of Pathophysiology and Transplantation, University of Milan, 20122 Milan, Italy; luigi.vivona@unimi.it (L.V.); eleonora.carlesso@unimi.it (E.C.); sebastiano.colombo@gmail.com (S.M.C.); serena.todaro@outlook.it (S.T.); gaetano.florio@unimi.it (G.F.); antonio.pesenti@unimi.it (A.P.); giacomo.grasselli@unimi.it (G.G.); alberto.zanella1@unimi.it (A.Z.)
- ² Center for Preclinical Research, Fondazione IRCCS Ca' Granda-Ospedale Maggiore Policlinico, 20122 Milan, Italy; battistin.michele@gmail.com (M.B.); stefano.gatti@policlinico.mi.it (S.G.)
- ³ Department of Anesthesia and Intensive Care Medicine, Niguarda Ca' Granda, 20162 Milan, Italy
- ⁴ Department of Medicine and Surgery, University of Milan-Bicocca, 20900 Monza, Italy
- ⁵ Dipartimento di Anestesia, Rianimazione ed Emergenza Urgenza, Fondazione IRCCS Ca' Granda-Ospedale Maggiore Policlinico, 20122 Milan, Italy; carlovalsecchi5@gmail.com
- * Correspondence: thomas.langer@unimib.it; Tel.: +39-02-64448580; Fax: +39-02-55033230
- † These authors equally contributed to the study.

Citation: Vivona, L.; Battistin, M.; Carlesso, E.; Langer, T.; Valsecchi, C.; Colombo, S.M.; Todaro, S.; Gatti, S.; Florio, G.; Pesenti, A.; et al.

Alkaline Liquid Ventilation of the Membrane Lung for Extracorporeal Carbon Dioxide Removal (ECCO₂R): In Vitro Study. *Membranes* **2021**, *11*, 464.

<https://doi.org/10.3390/membranes11070464>

Academic Editor(s): Gennaro Martucci; Antonio Arcadipane; Marco Giani

Received: 28 May 2021

Accepted: 20 June 2021

Published: 22 June 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



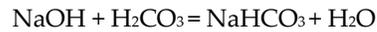
Copyright: © 2021 by the author. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).

Additional Methods:

Reaction between NaOH and CO₂

The mechanism of neutralization between NaOH and CO₂ depends on the concentration of the NaOH solution and on the pH value.

When NaOH is very diluted (pH<10) hydration of CO₂ occurs and H₂CO₃ is formed. Carbonic acid then reacts with NaOH to form sodium bicarbonate (NaHCO₃):



When NaOH solution is highly concentrated one (pH>10), carbon dioxide directly reacts with NaOH forming bicarbonate. Bicarbonate further reacts with the alkali to form sodium carbonate (Na₂CO₃):



Thus, highly concentrated NaOH solutions may absorb a conspicuous amount of CO₂ while keeping PCO₂ almost down to zero.

When the concentration of the alkali solution is quite low compared to the amount of CO₂ added, the reaction proceeds via the formation of carbonic acid and PCO₂ increase. But the acidic oxide is not completely neutralized in this case.

Mathematical Model:

To simulate the effects of CO₂ absorption by aqueous NaOH we computed the solutions of a system of equations starting from standard mass-action, mass-conservation and electroneutrality laws.

We simulated a *closed* system with aqueous NaOH at varying concentrations and flows in which we introduced a CO₂. The species involved in the equilibrium were water, NaOH and CO₂.

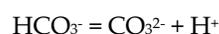
Gaseous carbon dioxide dissolves in water (**CO₂dissolved**) and it forms carbonic acid (**H₂CO₃**):



which dissociates in proton (**H⁺**) and bicarbonate (**HCO₃⁻**):



The bicarbonate may furtherly dissociate in H⁺ and carbonate ion (**CO₃²⁻**):



The dissociated plus the undissociated forms of carbonic buffers are termed total CO₂ (TCO₂).

Accordingly:

$$\frac{[HCO_3^-] \times [H^+]}{[CO_2 \text{ dissolved}]} = K_c \text{ (Equation S1)}$$

where K_c represents the carbonic dissociation constant, which, in plasma, is equal to $\approx 10^{-6.129} = 7.43E-7$ (pK_c ≈ 6.129),

$$\frac{[CO_3^{2-}] \times [H^+]}{[HCO_3^-]} = K_3 \text{ (Equation S2)}$$

Where K₃ is equal to $\approx 10^{-10.329} = 4.69E-11$ (pK₃ ≈ 10.329) and

$$[TCO_2] = [HCO_3^-] + [CO_3^{2-}] + [CO_2 \text{ dissolved}] \text{ (Equation S3)}$$

Finally CO₂dissolved is equal to the product of solubility of CO₂ ($\alpha = 0.0307 \text{ mmol} \times \text{L}^{-1} \times \text{mmHg}^{-1}$) and partial pressure of CO₂:

$$[CO_2 \text{ dissolved}] = \alpha \times PCO_2 \text{ (Equation S4)}$$

Water dissociation is characterized by the equation:

$$\frac{[H^+] \times [OH^-]}{[H_2O]} = K_w \text{ (Equation S5)}$$

where, at 37°C, K_w is equal to $4.3 \times 10E-16$, and [H₂O] is the molar concentration of water, i.e. $55.3 \text{ mol} \times \text{L}^{-1}$. As K_w is relatively small the changes in water concentration are insignificant compared to the total water concentration. Therefore, it is commonly accepted that the product K_w × [H₂O] is constant (water ionic product, K'_w). This means that:

$$[H^+] \times [OH^-] = K'_w \approx 10E-14 \text{ (equation S6)}$$

Sodium hydroxide (NaOH) is a strong base almost completely dissociated in water into sodium ions (Na⁺) and hydroxyl ions (OH⁻). This means that the dissociation constant is much higher than 1 (K $\approx 1.0000E+03$, pK ≈ -3). Analogously to weak substances, we can refer to an hypothetical undissociated form as NaOH and we can define Natot as the sum of Na⁺ and NaOH. Accordingly:

$$\frac{[Na^+] \times [OH^-]}{NaOH} = K_{NaOH} \text{ (Equation S7)}$$

where K_{NaOH} is the dissociation constant of strong electrolytes equilibrium; and

$$[Na^+] = [Natot] - [NaOH] \text{ (Equation S8)}$$

Finally, according to electroneutrality law:

$$[Na^+] + [H^+] - [HCO_3^-] - 2 \times [CO_3^{2-}] - [OH^-] \text{ (Equation S9).}$$

The final system was composed of equations S1, S2, S3, S4, S6, S7, S8 and S9 and was solved for [H⁺], [OH⁻], [HCO₃⁻], [CO₃²⁻], [CO₂dissolved], PCO₂, [Na⁺], [NaOH]. Finally, pH was computed as:

$$\text{pH} = -\log_{10}[H^+] \text{ (Equation S10).}$$

We simulated a *closed* system with aqueous NaOH at varying concentrations (from 10 to 100 by 20 mmol \times L⁻¹) in which we introduced CO₂ at different concentrations (from 0 to 100 by 5 mmol \times L⁻¹).

The system was solved iteratively substituting incrementing of 0.001 pH values from 0 to the maximal pH (i.e. pH value of the solution at the current NaOH concentration when no CO₂ was added) and minimizing the difference between added and estimated TCO₂. Estimated TCO₂ was computed according to equation S3.

Computed variables

Bicarbonate ion concentration ($[HCO_3^-]$) was calculated from pH and PCO_2 according to the Henderson-Hasselbalch equation ($pH = pK + \log_{10} \frac{[HCO_3^-]}{\alpha \times PCO_2}$) where $\alpha=0.0307 \text{ mmol} \times \text{L}^{-1} \times \text{mmHg}^{-1}$ (solubility of CO_2 in plasma) [1,2] and $pK = 6.129$ (negative logarithm of the equilibrium constant) [2–4].

$$HCO_3^- = \alpha \times PCO_2 \times 10^{pH-pK}$$

Plasma carbon dioxide content (expressed in $\text{mmol} \times \text{L}^{-1}$) from each side of the membrane was calculated according to the logarithmic form of the Henderson-Hasselbalch equation for CO_2 using the method published by Douglas et al. [5]:

$$TCO_2 = \alpha \times PCO_2 \times (1 + 10^{pH-pK})$$

Carbon dioxide transfer across the membrane, VCO_2 (expressed in $\text{mL} \times \text{min}^{-1}$), was calculated from the transmembrane TCO_2 difference:

$$VCO_2 = (TCO_{2PRE} - TCO_{2POST}) \times \text{blood flow} \times 25.45$$

TCO_{2PRE} represents CO_2 content before the membrane while TCO_{2POST} is the CO_2 content after the membrane, blood flow is measured in $\text{L} \times \text{min}^{-1}$ and the conversion factor is in $\text{mL} \times \text{mmol}^{-1}$.



Additional Table

Table 1s

	NaOH	10	30	60	90	100	<i>P</i> <i>Conc.</i>	<i>P</i> <i>PRE/</i> <i>POST</i>	<i>P</i> <i>Int.</i>
pH ^s	PRE	7.328 (7.323 – 7.333)	7.333 (7.324 – 7.336)	7.315 (7.300 – 7.322)	7.331 (7.314 – 7.346)	7.331 (7.327 – 7.334)	0.044	<0.001	0.005
	POST	7.577 (7.485 – 7.643)* #	7.732 (7.651 – 7.753)*#	7.862 (7.790 – 7.893)*	7.906 (7.870 – 7.948)*	7.913 (7.885 – 7.943)*			
	Difference	0.249 (0.152 – 0.321)°§ #	0.396 (0.325 – 0.418)§ #	0.557 (0.487 – 0.574)	0.575 (0.556 – 0.602)	0.585 (0.556 – 0.612)	<0.001		
PCO ₂ (mmHg) ^s	PRE	59.4 (58.4 – 60.4)	58.4 (57.6 – 58.8)	59.1 (58.0 – 61.4)	59.0 (57.1 – 60.9)	59.5 (58.6 – 60.7)	0.100	<0.001	0.042
	POST	27.3 (21.9 – 35.4)* #	16.2 (15.1 – 21.4)*	12.0 (11.0 – 15.0)*	11.4 (10.2 – 12.4)*	12.4 (11.3 – 13.1)*			
	Difference	-32.2 (-38.6 – -23.1)§ #	-41.4 (-43.1 – -36.8)	-47.7 (-49.5 – -44)	-47.8 (-48.6 – -47)	-48.2 (-48.4 – -46.6)	<0.001		
PO ₂ (mmHg)	PRE	157.0 (153.5 – 159.5)§#	153.5 (151.0 – 157.5)#	145.5 (145.0 – 150.5)#	147.5 (146.0 – 151.0)#	125.0 (124.5 – 125.0)	<0.001	0.676	0.006
	POST	159.0 (149.5 – 176.0)§ #	151.0 (142.0 – 170.0)§ #	136.0 (129.5 – 152.5)#	140.0 (128.0 – 156.0)#	109.2 (97.2 – 126.5)*			
	Difference	2.0 (-4.0 – 16.5)#	-5.0 (-13.0 – 16.5)	-9.5 (-16.0 – 2.5)	-7.5 (-18.0 – 5.0)	-15.3 (-27.3 – 1.5)	0.006		
K ⁺ (mEq×L ⁻¹)	PRE	4.3 (4.3 – 4.3)§#	4.4 (4.4 – 4.5)	4.5 (4.5 – 4.5)	4.4 (4.4 – 4.5)	4.5 (4.4 – 4.5)	0.005	0.005	0.366
	POST	4.3 (4.2 – 4.3)*§#	4.3 (4.3 – 4.3)*	4.4 (4.4 – 4.5)*	4.3 (4.3 – 4.4)*	4.4 (4.3 – 4.4)*			
	Difference	0.0 (-0.1 – 0.0)	-0.1 (-0.2 – -0.1)	0.0 (-0.1 – 0.0)	-0.1 (-0.1 – -0.1)	-0.1 (-0.1 – -0.1)	0.366		
Na ⁺ (mEq×L ⁻¹)	PRE	140.5 (140.0 – 141.0)	141.5 (140.5 – 143.5)	140.5 (139.5 – 141.5)	140.0 (139.0 – 142.5)	142.5 (142.0 – 143.0)	0.429	<0.001	0.263
	POST	138.5 (137.5 – 139.0)*	137.5 (137.0 – 138.5)*	137.0 (136.5 – 139.0)*	137.0 (136.0 – 139.0)*	139.5 (139.0 – 140.0)*			

	Difference	-2.0 (-3.0 – -1.5)	-4.0 (-5.5 – -3.0)	-2.5 (-4.0 – -1.5)	-3.0 (-3.5 – -3.0)	-3.0 (-3.0 – -3.0)	0.263		
Ca⁺⁺ (mEq×L⁻¹)^s	PRE	1.1 (1.1 – 1.1) ^{°§ #}	1.2 (1.2 – 1.2) ^{§ #}	1.3 (1.3 – 1.4)	1.3 (1.3 – 1.4)	1.4 (1.4 – 1.4)	<0.001	<0.001	<0.001
	POST	1.0 (1.0 – 1.0) ^{*§ #}	1.1 (1.0 – 1.1) ^{*§ #}	1.2 (1.2 – 1.2) ^{*#}	1.2 (1.1 – 1.2) ^{*#}	1.3 (1.3 – 1.3) [*]			
	Difference	-0.1 (-0.1 – -0.1) ^{°§}	-0.1 (-0.1 – -0.1)	-0.1 (-0.2 – -0.1) [#]	-0.2 (-0.2 – -0.2) [#]	-0.1 (-0.1 – -0.1)	<0.001		
Cl⁻ (mEq×L⁻¹)^s	PRE	107.0 (106.5 – 107.0) [#]	106.0 (104.5 – 106.0) [#]	108.0 (106.5 – 108.0)	108.0 (105.5 – 108.5)	110.0 (110.0 – 110.0)	0.017	<0.001	0.190
	POST	110.0 (108.5 – 111.5) ^{*#}	110.5 (109.5 – 111.0) ^{*#}	112.5 (110.5 – 113.0) [*]	112.0 (109.5 – 113.0) [*]	114.0 (114.0 – 114.0) [*]			
	Difference	3.0 (2.0 – 4.5)	5.0 (4.5 – 5.5)	4.5 (4.0 – 5.0)	4.0 (4.0 – 4.5)	4.0 (4.0 – 4.0)	0.135		
Lac (mEq×L⁻¹)^s	PRE	2.3 (2.3 – 2.4) ^{°§ #}	2.5 (2.5 – 2.6) ^{§#}	3.1 (3.1 – 3.2)	2.7 (2.6 – 2.8) [#]	4.4 (4.2 – 4.5)	<0.001	0.517	0.736
	POST	2.4 (2.3 – 2.4) ^{°§ #}	2.7 (2.4 – 2.9) ^{§#}	3.1 (3.1 – 3.2)	2.6 (2.6 – 2.7) [#]	4.3 (4.2 – 4.5)			
	Difference	0.0 (0.0 – 0.1)	0.1 (-0.1 – 0.3)	0.0 (-0.1 – 0.1)	0.0 (-0.1 – 0.0)	0.0 (-0.1 – 0.0)	0.842		
Hb (g×dL⁻¹)	PRE	13.90 (13.90 – 13.95) ^{§ #}	14.10 (14.00 – 14.20) ^{§ #}	10.40 (10.35 – 10.45) [#]	10.55 (10.50 – 10.60) [#]	9.20 (9.10 – 9.30)	<0.001	0.651	0.183
	POST	13.85 (13.80 – 13.95) ^{§ #}	14.05 (13.85 – 14.20) ^{§ #}	10.50 (10.50 – 10.50) [#]	10.55 (10.50 – 10.80) [#]	9.25 (9.15 – 9.35)			
	Difference	-0.10 (-0.10 – 0.00)	0.00 (-0.20 – 0.05)	0.10 (0.05 – 0.15)	0.00 (0.00 – 0.20)	0.05 (0.00 – 0.10)	0.183		
HCO₃⁻ (mmol×L⁻¹)	PRE	28.8 (28.7 – 29)	28.5 (28.1 – 28.7)	27.9 (27.7 – 27.9)	28.8 (28.6 – 28.9)	29.1 (28.7 – 29.5)	<0.001	<0.001	0.021
	POST	23.2 (21.9 – 24.5) ^{*°§}	19.9 (19.5 – 21.3) ^{*#}	20 (19.6 – 20.8) ^{*#}	20.7 (20.4 – 21) ^{*#}	22.7 (22.2 – 23.1) [*]			
	Difference	-5.6 (-7.1 – -4.2)	-8.6 (-9.2 – -6.8)	-7.9 (-8.3 – -6.9)	-7.9 (-8.4 – -7.7)	-6.4 (-6.6 – -6.3)	0.021		
plasma TCO₂ (mmol×L⁻¹)	PRE	30.7 (30.5 – 30.8)	30.2 (29.9 – 30.5)	29.7 (29.5 – 29.8)	30.6 (30.5 – 30.6)	30.9 (30.5 – 31.3)	<0.001	<0.001	0.019
	POST	24 (22.6 – 25.6) ^{*°§}	20.4 (19.9 – 21.9) ^{*#}	20.3 (20 – 21.3) ^{*#}	21.1 (20.8 – 21.3) ^{*#}	23.1 (22.5 – 23.5) [*]			
	Difference	-6.6 (-8.2 – -4.9)	-9.9 (-10.5 – -7.9)	-9.4 (-9.8 – -8.3)	-9.4 (-9.8 – -9.2)	-7.8 (-8.1 – -7.7)	0.019		

VCO₂ (mL×min⁻¹)		73.9 (54.3 – 91.8)	109.7 (88.3 – 117.1)	104.5 (92.8 – 108.7)	104.3 (102.4 – 109.0)	87.2 (85.8 – 89.9)	0.019		
--	--	--------------------	----------------------	----------------------	-----------------------	--------------------	-------	--	--

Table 1s: Safety and feasibility tests results. Abbreviations: PCO₂, partial pressure of carbon dioxide; PO₂, partial pressure of oxygen; Na⁺, sodium; K⁺, potassium; Ca⁺⁺, calcium; Cl⁻, chloride; Lac, Lactate; Hb, Hemoglobin; HCO₃⁻, bicarbonate, TCO₂, total CO₂ content, VCO₂, amount of carbon dioxide removed by the membrane lung. Data are expressed median (IQR); Differences were computed as POST values – PRE values. P: P values of two-way ANOVA RM or two-way ANOVA RM on ranks (§) for PRE and POST values (P PRE/POST) and NaOH concentration (P Conc.) and interaction (P int.); one-way ANOVA RM or one-way ANOVA RM on ranks were applied for difference values. Post-hoc analysis with Bonferroni or Tukey corrections: * P<0.05 vs pre; ° P<0.05 vs 30; § P<0.05 vs 60; || P<0.05 vs 90; # P<0.05 vs 100.

References

1. Austin, W.H.; Lacombe, E.; Rand, P.W.; Chatterjee, M. Solubility of Carbon Dioxide in Serum from 15 to 38 C. *J. Appl. Physiol.* **1963**, *18*, 301–304, doi:10.1152/jappl.1963.18.2.301.
2. Constable, P.D. Total Weak Acid Concentration and Effective Dissociation Constant of Nonvolatile Buffers in Human Plasma. *J. Appl. Physiol.* **2001**, *91*, 1364–1371, doi:10.1152/jappl.2001.91.3.1364.
3. Harned, H.S.; Bonner, F.T. The First Ionization of Carbonic Acid in Aqueous Solutions of Sodium Chloride. *J. Am. Chem. Soc.* **1945**, *67*, 1026–1031, doi:10.1021/ja01222a037.
4. Putnam, R.W.; Roos, A. Which Value for the First Dissociation Constant of Carbonic Acid Should Be Used in Biological Work? *Am. J. Physiol.-Cell Physiol.* **1991**, *260*, C1113–C1116, doi:10.1152/ajpcell.1991.260.5.C1113.
5. Douglas, C.G.; Haldane, J.S. (From the Physiological Laboratory, Oxford, and the Institute of General Pathology, Copenhagen.). 28.