Effect of an ionic antineoplastic agent Cytoreg on blood chemistry in a Wistar rat model

Katiusca Villasana[1, *], William Quintero[2], Yepsys Montero[3], Cristian Pino[4], Oscar Uzcategui[4], Geizon Torres[5], Mariangel Prada[5], Lewis Pozo[6], William Bauta[7], William Jimenez[7]

1 Biochemistry Laboratories, Department of Pathology, University of the Andes, Mérida, Venezuela
2 Department of Biology, Faculty of Science, University of the Andes, Mérida, Venezuela
3 Vivarium, University of the Andes, Mérida, Venezuela
4 Department of Technical and Experimental Surgery, Faculty of Medicine, University of the Andes, Mérida,Venezuela
5 Vitalis, Clinical Laboratory, Smart Health Laboratory, Merida, Venezuela
6 Cytorex de Venezuela SA, Maracaibo, Venezuela
7 Cytorex Biosciences, Inc., Weston, FL, USA

*Correspondence to: Katiusca Villasana, MS, anastipv@gmail.com.
orcid: 0000-0002-6142-1066 (Katiusca Villasana)

Abstract

Cytoreg is an ionic therapeutic agent comprising a mixture of hydrochloric, sulfuric, phosphoric, hydrofluoric, oxalic, and citric acids. In diluted form, it has demonstrated efficacy against human cancers *in vitro* and *in vivo*. Although Cytoreg is well tolerated in mice, rats, rabbits, and dogs by oral and intravenous administration, its mechanism of action is not documented. The acidic nature of Cytoreg could potentially disrupt the pH and levels of ions and dissolved gases in the blood. Here, we report the effects of the intravenous administration of Cytoreg on the arterial pH, oxygen and carbon dioxide pressures, and bicarbonate, sodium, potassium, and chloride concentrations. Our results demonstrate that Cytoreg does not disturb the normal blood pH, ion levels, or carbon dioxide content, but increases oxygen levels in rats. These data are consistent with the excellent tolerability of intravenous Cytoreg observed in rabbits, and dogs. The study was approved by the Bioethics Committee of the University of the Andes, Venezuela (CEBIOULA) (approval No. 125) on November 3, 2019.

Key words: acid-base homeostasis; arterial blood chemistry; Cytorex; electrolyte balance; ion therapy; oxygen pressure; tissue oxygenation; Warburg effect

INTRODUCTION

In 1908, the eminent biochemist Lawrence J. Henderson defined the fundamental relationship between the acidic and basic components of living systems, which must exist in harmony (i.e., equilibrium) for proper physiological function. Because the concentration of hydrogen ions [H^+] influences all the systems of an organism, this concentration must be precisely regulated[1]; changes in [H^+] can wholly alter an organism's endocrine, hematological, and metabolic functions. A variety of factors can disturb a living system's acid-base balance, which prompts the organism to avoid acidosis or alkalosis by rebalancing pH through three lines of defense that regulate the concentration of [H^+] in physiological fluids. These include 1) acid-base buffers, which act in seconds, to counter deviations in acidity; 2) the respiratory system, which, within minutes, regulates the elimination of carbon dioxide (CO_2; by way of carbonic acid, H_2CO_3) from the extracellular fluid; and 3) the renal system, which excretes acids or bases in the urine to achieve a [H^+] level of 4×10^{-6} M or pH 7.40, as opposed to an arterial pH $\leq$ 7.35 in acidosis or pH $\geq$ 7.55 in alkalosis. The renal response, which can be measured through the concentration of HCO_3^-, reacts more slowly in comparison to the other rebalancing defenses, i.e., on the order of hours, but is nevertheless the most powerful system as a regulator of acids and bases.[2] Cytoreg is an ionic therapeutic agent consisting of low-molecular-weight organic and inorganic acids (a mixture of hydrochloric, sulfuric, phosphoric, hydrofluoric, oxalic, and citric acids). In diluted form, it has demonstrated efficacy *in vitro* against several cancer cell lines, as well as several dermatological conditions (viral, bacterial, and fungal infections). Its tolerability has been investigated *in vivo* in animal models (rats, mice, rabbits, and dogs). However, the pH of the concentrated drug is less than 1, and its principal active component is hydrofluoric acid.[3,4] Owing to its wide-ranging and attractive pharmacological effects in various studies, it is necessary to investigate whether the physicochemical properties of Cytoreg alter the acid-base equilibrium in an animal, and so infer the compatibility of the drug in human patients. Arterial blood gas analysis is one of the most useful techniques for measuring acid-base equilibria in human and animal subjects.[5,6] This respiratory monitoring technique permits the measurement of pH, partial pressures of oxygen and carbon dioxide (pO_2 and pCO_2), oxygen saturation ($SatO_2$), hemoglobin content, total carbon dioxide (TCO_2), and bicarbonate concentrations [HCO_3^-] in a sample of arterial blood.[7] Using arterial blood gas analysis, we sought to determine the acid-base equilibrium responses in male Wistar rats before and after intravenous administration of Cytoreg.

It is important to note that the pharmacodynamics and pharmacokinetic properties of an investigational drug

contribute to its safety and successful clinical outcomes.[8] An understanding of the impact of Cytoreg administration on acid-base equilibria will offer insight on its mechanism of action through its biochemical and physiological effects on arterial blood in the Wistar rat model.

MATERIALS AND METHODS

The design and protocol for the present study were evaluated and approved by the Bioethics Committee of the University of the Andes, Venezuela (CEBIOULA) (protocol number 125) on November 3, 2019.

Experimental animals

Ten male Wistar rats (weight, 350 g; 3 months of age) were obtained from the vivarium of the University of the Andes, Venezuela, and were acclimated to pathogen-free enclosures with *ad libitum* access to food and water.

During the course of the experiment, the same environmental conditions were maintained to keep results consistent. The rats were individually housed in polycarbonate Seal safe cages (Tecniplast, Xenoplus, University of Los Andes, Bioterio BIOULA, Mérida, Venezuela) for the duration of the study. The general procedures for the care and housing of animals were in accordance with the Guide for the Care and Use of Laboratory Animals.[9] Ambient temperature and humidity were maintained in accordance with the Animal Welfare Laws of the United States Department of Agriculture (USDA) with a light period of timer-controlled illumination.[10]

One flask of Cytoreg (0.105 mL/kg; Cytorex Biosciences, Inc., Weston, FL, USA; 50 mL) was used as the medication in the study. Cytoreg is a solution of mixed strong and weak acids, constituting a complex of ions with individual functions, among which hydrofluoric acid (55 mg/kg) is the active component. The test article was stored at ambient temperature in polypropylene containers and diluted in physiological saline (0.9% w/v) before intravenous administration.

Cytoreg solution (0.105 mL/kg) was administered intravenous under the Surgical Procedure protocol. Diluted Cytoreg solution was prepared by mixing concentrated Cytoreg solution (0.036 mL) with isotonic (0.9%) saline solution (0.464 mL). Once the animals weighed 350 g, the diluted Cytoreg solution was administered. Experiments were performed diurnally at $20 \pm 2°C$ at an altitude of 1690 meters above sea level; importantly, the circadian rhythms of the animals and the altitude influence the pH and pO_2 levels.[11]

Arterial blood samples from the ten animals were taken as follows. Prior to drawing the sample (time, 0 minute, T0′), each animal was anesthetized with ketamine (100 mg/kg; University of Los Andes, Bioterio BIOULA, Mérida, Venezuela)/xylazine (5 mg/kg; University of Los Andes) intraperitoneal. From the abdominal aorta, blood (0.7 mL) was removed with an insulin syringe containing heparin sodium (0.2 mL, 20%, University of Los Andes). Arterial gas analysis was immediately performed with an OPTIMedical arterial blood gas analyzer (OPTI™ CCA-TS, Bioula, Mérida, Venezuela).

The diluted Cytoreg solution (0.105 mL/kg) was then administered intravenously. Thirty minutes post-treatment (T30′), arterial blood (0.70 mL) was collected from the sedated ani-

mals and analyzed as before. After 60 minutes (T60′), the rats were again anesthetized with ketamine/xylazine (100 mg/kg), and arterial blood was collected and analyzed as before.

Surgical procedure

Each rat was weighed, and the dose of anesthesia calculated (xylazine (5 mg/kg) and ketamine (75 mg/kg). Antisepsis was applied in a supine position using betadine in alcohol (70% v/v). The abdominal wall was incised with Metzenbaum scissors (Bioula) until the abdominal viscera were exposed. The viscera were parted until the abdominal aorta was exposed and the dissection proceeded. An arterial blood sample was withdrawn from the abdominal aorta with an insulin syringe (impregnated with 0.2 mL heparin 20%) prior to treatment with Cytoreg solution. Cytoreg (0.5 mL, 0.105 mL/kg) was administered intravenously into the abdominal vena cava. At 30 minutes (T30′) post-treatment, a sample of arterial blood (0.7 mL) was collected as before, and again after 60 minutes (T60′). The visceral organs were replaced in the abdominal cavity and hemostasis was confirmed by observing the absence of bleeding or clots. The muscle layer was closed with continuous catgut 3-0 sutures, followed by the skin with simple 3-0 nylon sutures. The surgical area was treated with antiseptic (betadine in alcohol, 70% v/v). Animals were kept sedated for 1 hour during the procedure using ketamine/xylazine intraperitoneally (100 mg/kg).

Post-surgical procedure: The sedated animals were euthanized after the final sampling with an overdose of inhaled enflurane 100% (Bioula) in a closed chamber.

Statistical analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS) version 22 (IBM SPSS Inc., Chicago, IL, USA). To determine the significance of the averages between the control group (T0′), and treatment groups (T30′ and T60′) was used the repeated measurement analysis of variance for the following variables: pH, PCO_2, pO_2, $[HCO_3^-]$, TCO_2, and electrolytes (sodium, Na^+; potassium, K^+: and chloride, Cl^-). Then, Tukey's honest significant difference post hoc test was used to analyze differences between T0′ and T30′, and T0′ and T60′. The significance level was 95% and the P value was 0.05 ($P < 0.05$).

RESULTS
pH before and after Cytoreg treatment

Prior to treatment with Cytoreg, the mean arterial pH of the 10 Wistar male rats was 7.45 ± 0.046. Our data are comparable with those from other studies on the same species and strain of rat (**Table 1**).[12-16] The average pH values determined at T0′ (pre-treatment), T30′, and T60′ are 7.45 ± 0.046, 7.25 ± 0.11, and 7.22 ± 0.09, respectively (**Figure 1A**). Values which differ significantly from the initial pH (T0′) ($P < 0.05$) are still within the tolerated range for the organism, since they can be compensated by physiological buffers. However, only two rats (20% of the animals) presented low pH values, at 7.17 (T30′) and 7.16 (T60′). It is worth emphasizing that, between the average pH values at T30′ and T60′, there are no statistically significant differences ($P > 0.05$).
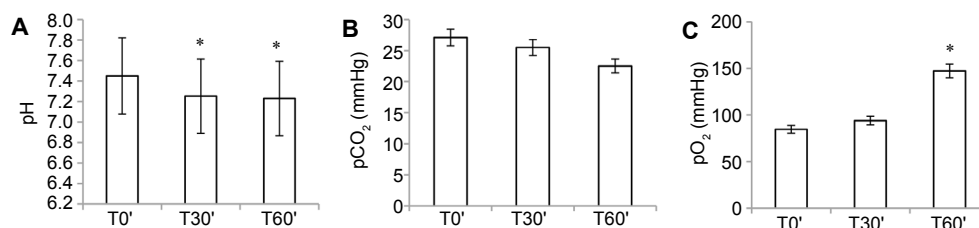
Figure 1: Effects of Cytoreg (0.105 mL/kg) on the acid-base regulatory mechanisms in abdominal arterial blood from 10 male Wistar rats at different time points.
Note: (A) Average pre-treatment (T0′) pH value of arterial abdominal blood from the 10 male Wistar rats (350 g) and comparison of arterial blood pH values over time. (B, C) Average pCO_2 (B) and pO_2 (C) values of arterial abdominal blood over time. Data are expressed as the mean ± SD ($n = 10$), and were analyzed by repeated measurement analysis of variance followed by Tukey's honest significant difference *post hoc* test. *$P < 0.05$, vs. T0′ group. pCO_2: Partial pressures of carbon dioxide pO_2: partial pressures of oxygen; T0′: Pretreatment; T30′ and T60′: 30 and 60 minutes post-treatment, respectively.

Table 1: Reported values for pH, pCO_2, and pO_2 from the arterial blood of Wistar rats

Study	pH	pCO_2 (mmHg)	pO_2 (mmHg)
Scorv et al.[16]	7.28–7.36	36.90–64.50	51.00–56.55
da Silva et al.[14]	7.37–7.38	33.06–37.68	78.02–86.36
Almeida et al.[15]	7.36–7.38	42.9–45.2	70.8–81.2
Damiani et al.[13]	7.39–7.47	36–48	73–113

Note: pCO_2: Partial pressures of carbon dioxide; pO_2: partial pressures of oxygen.

pCO_2 before and after Cytoreg treatment

The measurement of the pCO_2 in each animal before treatment afforded an average value of 27.5 ± 4.22 mmHg, which is comparable to the value of 24.97 mmHg obtained independently by Dvořáček et al.[17] and Schultz et al.[18] In the blood, carbon dioxide is present partially in the forms of bicarbonate (HCO_3^-) and carbonic acid (H_2CO_3), as well as in its free form (CO_2).[19] Given that gaseous CO_2 exerts pressure on blood vessels (i.e., the arteries, in this case), its levels can measured by the electrode in the OPTIMedical analyzer in terms of the pCO_2, which can be converted into the quantity of carbon dioxide in circulation. Comparing the pCO_2 value (**Figure 1B**) before and after Cytoreg administration reveals that it remains within the initial range (T0′) of 27.5 ± 4.22 mmHg (range 19–33 mmHg), falling post-treatment to 25.5 ± 9.22 mmHg (range 12–43 mmHg) at T30′ and 23 ± 3.37 mmHg (range 15–28 mmHg) at T60′. This is indicative of respiratory stability, or alveolar ventilation, wherein the elimination of CO_2 is realized without difficulty in an adequate length of time (60 minutes after treatment).

pO_2 and hemoglobin before and after Cytoreg treatment

The amount of oxygen found in arterial blood depends on its pressure (pO_2) in solution, as well as on the content of the oxygen transport protein, hemoglobin, and the integrity of respiratory mechanisms.[12] In our study, we obtained an average pO_2 value of 81.6 ± 7.97 mmHg before treatment with Cytoreg, with post-treatment values of 94.1 ± 19.3 and 147.3 ± 9.59 mmHg at T30′ and T60′, respectively (**Figure 1C**). Comparing these values with reported data for Wistar rats in similar studies, in which the lowest pO_2 was 51 mmHg,[16] with the ranges reported in **Table 1**, the rats present a state of normoxia at the start of our study,[14,16] and then exhibit significant increases ($P < 0.05$) at T30′ and T60′ post-treatment. Quantitation of the

hemoglobin content reveals a slight decline post-treatment, with values of 13.16 ± 2.31, 11.5 ± 2.52, and 11.6 ± 1.49 g/dL at T0′, T30′, and T60′, respectively. However, despite a decrease of ± 0.93 g/dL, we can conclude that our results are within range of the control (as observed at T0′).

[HCO_3^-] before and after Cytoreg treatment

Among the mechanisms of physiological pH adjustment, the primary action is exerted by bicarbonate ion (HCO_3^-) in equilibrium with carbonic acid, via $H_2CO_3 \rightleftarrows H^+ + HCO_3^-$.[20] In our study, the Wistar rats presented a plasma HCO_3^- concentration of 18.31 ± 2.82 mM (16.9–22.7 mM) before treatment. Upon Cytoreg administration, concentrations of 11.3 ± 4.10 mM (3.7–17.9 mM) were observed after 30 minutes and 7.94–2.92 mM (4.5–11.1 mM) after 60 minutes. Buffering action is the first of the lines of defense against a change in pH.[21] In our study, we saw a decrease in the plasma [HCO_3^-] after Cytoreg treatment, in agreement with the observed decrease in pH from its initial level (7.45) to 7.25 1 hour later. These results demonstrate the expected "bicarbonate buffer" compensation for the change in [H^+] as the first line of defense against a change in pH.[22] The values obtained were significant ($P < 0.05$) for each time point (i.e., T30′ and T60′, with respect to T0′).

TCO_2 before and after Cytoreg treatment

Total CO_2, also known as the reserve alkalinity, represents the amount of CO_2 present in the bloodstream. It comprises the sum of CO_2 contained in the forms of HCO_3^- (~98%), dissolved CO_2, and carbonic acid (H_2CO_3). Since almost all the CO_2 corresponds to plasma bicarbonate, the term "reserve alkalinity" represents the amount of CO_2 present in plasma as HCO_3^-.[23] The TCO_2 value in the Wistar rats was 19.14 ± 2.96 mM (range 12.2–23.8 mM) before treatment. After Cytoreg treatment, these values varied widely, decreasing on average to 12.06 ± 4.33 mM (4.1–19.2 mM) at T30′ and 8.54 ± 2.31 mM (5–11.9 mM) at T60′. There was a decrease in TCO_2 over time proportional to the bicarbonate buffer to compensate for the [H^+] entering the organism.

Seeing that the TCO_2 was diminished in our experiments, we inferred metabolic acidosis, by which the organism compensates for excess acid.[23] Since these data have utility if they complement the pH status and levels of potassium and chloride ion, we also measured the electrolyte ions, Na^+, K^+, and Cl^-.

Sodium is the most important cation in extracellular fluids, as it establishes the ionic pressure of interstitial liquids.[24] Its de-

termination is indispensable in the study of electrolyte balance. In our study, the Wistar rats presented stable concentrations with respect to T0′ (prior to Cytoreg administration), without significant differences ($P > 0.05$) at the different measurement times: 157.5 ± 6.51 mM at T0′, 150.4 ± 3.13 mM at T30′, and 139.6 ± 11.24 mM at T60′, with a variation of ± 9.05 mM over the duration of the experiment. The level of potassium, as the intracellular cation that predominates in striated muscle cells, together with that of sodium, is indicative of electrolyte balance, and its decrease or increase is important in electrocardiographic studies as well as in acid-base equilibrium.[24] In our study, the Wistar rats had a basal K^+ concentration (T0′) of 2.53 ± 0.51 mM, with the value rising slightly to 3.03 ± 1.09 mM at T30′ and further to 4.29 ± 1.52 mM at T60′, with significant differences ($P < 0.05$) at the different measurement times.

The chloride ion constitutes the predominant electrolytic ion in the extracellular space. Found in the organism in its free form as an ion, Cl^- maintains cellular integrity via its influence on osmotic pressure, acid-base equilibrium, and water balance.[25] Its concentration is generally found to increase in the acidosis process and diminishes in alkalosis. The concentration of Cl^- in the plasma of the Wistar rats was 119.4 mM, and remained fairly constant after treatment ($P > 0.05$, not significant) with a value of 114.2 mM.

DISCUSSION
pH
Rats are useful models for the study of acid-base mechanisms and respiratory parameters in preclinical and toxicological assays. Data considered to be normative for arterial blood gases and electrolytes help to identify healthy animals for experiments.[26] Based on our results in the Wistar rats, we found that pH decreases significantly ($P < 0.05$) after Cytoreg administration, from 7.45 to 7.25 after T30′ and 7.22 after T60′. Despite the observed changes, these results conform to the reference ranges reported in other study[16] ($7.2–7.51 \pm 0.3$). It is known that general anesthetics (phenobarbital, ketamine/xylazine, and Zoletil®) have a great influence on arterial blood pH.[16] In general, anesthetics produce a more acidic internal environment in humans and in animal models (rodents), causing respiratory system depression; affecting cardiac rhythm and locomotor function in rodents; and also affecting the mechanisms of pH regulation.[16] In our study, we used intraperitoneal ketamine/xylazine (100 mg/kg) to anesthetize the rats, and our results indicate that ionic treatment with Cytoreg does not disrupt such acid/base mechanisms.

pCO₂
All discussions on gas exchange should begin with CO_2, given that it is the only gas that affords information about ventilation, oxygenation, and acid-base equilibrium.[23] In this study, the values for pCO_2 remained constant ($P > 0.05$), or in a state of normocapnia, throughout the 1 hour treatment, indicating stable respiration or alveolar ventilation, wherein the proper elimination of CO_2 is realized without difficulty in an adequate time (T60′). As indicated previously, the second line of defense against changes in $[H^+]$, i.e., the respiratory system, acts within a few minutes to eliminate CO_2 by way

of H_2CO_3 from the organism. Our results demonstrate that Cytoreg does not affect this mitigation system.

pO₂
The values obtained before and after Cytoreg treatment for the pO_2 and hemoglobin content, which determine the saturation and transport of oxygen in experimental animals, demonstrate the physiological integrity of the respiratory mechanism. By obtaining pO_2 data, we can determine whether an individual is capturing oxygen and in what proportion; this data has great importance in all the processes by which we evaluate respiratory capacity and the transport of oxygen to the tissues. In this work, the pO_2 of the Wistar rats increases after the intravenous administration of Cytoreg, which suggests better oxygen capture in the rat tissues. As the final acceptor of electrons in the electron transport chain coupled to the production of ATP through oxidative phosphorylation, O_2 plays a critical role in cellular function at the mitochondrial level for the generation of energy, a vital process in the organism. For cells such as neurons, such functions are dependent on mitochondrial metabolism, in which oxygen has an indispensable function. Many neurodegenerative diseases involving progressive neuronal death (e.g., Parkinson's disease, Alzheimer's disease, Huntington's disease) and immunological diseases (e.g., lupus, multiple sclerosis) have their origin in a failure of mitochondrial activity.[27,28] For example, proper aerobic metabolism is very important for the vitality of the brain, in which the oxygen consumption of ~3.4 mol per day is much higher compared to other human tissues.[29] Similarly, the activation, differentiation, and production of antibodies or immunoglobulins by leukocytes, the principal cells of the immune system, depend on proper mitochondrial metabolism. The therapies for these diseases are directed to the improvement of oxygen capture through techniques such as hyperbaric chamber and coenzyme Q treatments.[30,31] Thus, the principal strategy underlying hyperbaric oxygen treatment is to increase the oxygen level in tissues. Furthermore, since a small increase in the pO_2 in ischemic areas increases the bactericidal activity of leukocytes, Cytoreg, as a therapeutic ionic agent, could play an important role in the regulation of these immune cells. Additionally, experiments have been performed on the effects of oxygen levels on cancer cells, indicating that hypoxia (insufficient oxygen to meet the metabolic demands of the cell) is a factor which favors the growth of cancer cells, referred to as the Warburg effect.[32] The role of hypoxia in metabolic pathways specific to cancer – including changes in gene expression (genes inducible by HIF) and the proteome, genomic instability, the regulation of angiogenesis (formation of blood vessels), and the evasion of apoptotic processes[33] – has driven the attention of scientists towards the creation of therapies that alter cellular metabolism such that the cell shifts from hypoxic to oxygen-rich.[16,29,32,34] On observing the effects of Cytoreg on arterial oxygen ($P < 0.05$), we see a significant increase at T30′ and T60′. The simultaneous decrease in blood pH (± 0.02) increases the liberation of oxygen from hemoglobin to the tissues, a phenomenon known as the Bohr effect,[35] without affecting the acid-base mechanisms in the organism. One of the reported therapeutic effects of Cytoreg is the inhibition of the growth and proliferation of many cancer cell lines.[36]

Our findings indicate that Cytoreg has an important effect on cellular metabolism by increasing mitochondrial activity, and suggest a mechanism for cancer cell apoptosis through the generation of reactive oxygen species, which may be considered a therapeutic mechanism of action that alters cellular metabolism from a hypoxic, oxygen-deficient state with an initial pO_2 of 84.6 ± 7.97 mmHg to a totally aerobic environment 147.3 ± 10.1 mmHg 60 minutes after administration. This mechanism of action not only influences the growth of neoplastic tissue but also affects the aforementioned illnesses, which are related to proper mitochondrial function. Our results are even more significant, given the studies on the hypoxic effects of general anesthesia on murine animal models (with hypercapnia, or increased pCO_2,[34] which significantly influence pO_2 readings. Despite the fact that the animals in this study were intraperitoneally anesthetized with ketamine/xylazine, and that arterial gas measurements were made at an altitude of 1690 m above sea level and a temperature of $20 \pm 2°C$ (low atmospheric pressure and temperature are factors that influence pO_2),[23] no hypoxia was observed upon administration of the medication, but rather, an increase in pO_2.

HCO_3^-

The acid-base equilibrium in bodily fluids depends on the concentrations of H^+ and HCO_3^- ions. In healthy and awake mammals, including humans, there are compensatory mechanisms to maintain the necessary acid-base equilibrium for normal enzymatic activity, electrolyte diffusion, hemoglobin saturation, and coronary contraction, all of which contribute to the normal functioning of vital organs.[11] Plasma bicarbonate is an important factor in directing the metabolic equilibrium in metabolic states of acidosis or alkalosis. Our data show a difference in $[HCO_3^-]$ before (18.33 ± 2.82 mM) and after treatment (7.94 ± 2.22 mM). We infer a state of metabolic acidosis (pH 7.24 ± 0.09), and in this setting, bicarbonate functions as the primary buffer to re-establish the normal acid-base equilibrium. At the human clinical level, these variations are physiologically corrected via the renal system without compromising the proper functioning of the organism. The observed decrease in bicarbonate concentration in our study demonstrates its role in compensating for the $[H^+]$ introduced into the organism. This is likewise observed in humans, where the TCO_2 is found to decrease during metabolic acidosis to compensate for excess acid in the organism, a fact which is useful if complemented with information about pH and K^+ and Cl^- levels.

Electrolytes

The electrolytes Na^+, K^+, and Cl^- play important roles in acid-base equilibrium. Although arterial pH is the principal factor that determines the secretion of acid, its excretion also depends on the concentrations of K^+, Cl^-, and aldosterone. The intracellular concentration of K^+ and the secretion of H^+ are related in a reciprocal fashion: the depletion of K^+ increases the secretion of H^+ and, as a consequence, alters acid-base homeostasis. When the extracellular pH varies, changes in the acid-base equilibrium influence the plasma concentration of K^+. During metabolic acidosis, as provoked by other causes besides the accumulation of organic acids (as from renal insufficiency), 60% or more of the excess H^+ is absorbed in the interior of cells. Electroneutrality is maintained through the movement of K^+ and Na^+.[25] Observing the effect of Cytoreg on electrolytes (Na^+ and Cl^-) in the blood, we see that the concentrations are constant relative to their pre-treatment levels (T0′), indicating a stable acid-base system. Given that Cytoreg contains hydrochloric acid (HCl) as part of its chemical composition[37]; our data suggests the capture of Cl^- by a specific tissue, which could possibly explain its therapeutic effects. We know that, in our experiment, the increase in K^+ ($P < 0.05$) from 2.56 ± 0.51 mM (T0′) to 4.26 ± 1.52 mM (T60′) might be a physiological response to a change or decrease in $[H^+]$ (and likewise a decrease in $[HCO_3^-]$) in the blood, where its concentration is primarily mediated by renal excretion, which takes hours or days to exercise its effect despite being most powerful acid-base regulatory system.[38] We observed the effect of Cytoreg on the acid-base equilibrium one hour post-treatment, and for this reason, it would be interesting to monitor the effect over several days of constant-dose administration, and thus deduce the effects of the renal regulatory system and the concentrations of electrolytes in the urine.

We consider that the only limitations we had were regarding the availability of a greater number of animals to include in the study. We had in our plan to include a greater number of animals, but the surgical procedure to be performed on each animal in the operating room was limiting due to the unavailability in the country to provide those resources to the research and development center of the Universidad de Los Andes in the city of Merida, where we performed the surgical activity. This single limitation meant that we could not include a greater number of animals in the study. This limited us to only 10 animals.

● Cytoreg is an ionic antineoplastic agent which, despite its physicochemical characteristic of low pH owing to its organic and inorganic acid composition, does not affect the acid-base equilibrium in blood because of the immediate compensatory response by plasma buffers which do not permit significant variation of pH in the blood. Our study confirmed that the principal buffer, HCO_3^-, behaves in the same manner in the rat model as it does in humans.

● We may conclude from this study using the Wistar rat model that the partial pressures of CO_2 (pCO_2), the principle gas that affords information on the levels of ventilation, oxygenation, and acid-base equilibrium, remain constant. This behavior of pCO_2 levels shows that the ionic therapeutic Cytoreg does not alter respiratory stability or alveolar ventilation, such that the adequate elimination of CO_2 is realized in an appropriate time without difficulty.

● The results also demonstrate that the ionic therapeutic Cytoreg does not compromise the physiological integrity of the organs (lung and kidney) responsible for maintaining acid-base equilibrium.

● With respect to electrolyte behavior, we conclude that treatment with Cytoreg maintains hydration and the electrolyte balance in Wistar rats.

● This study also demonstrates that the pO_2 increases after the administration of Cytoreg. This could result in improved oxygen capture by tissues and have a beneficial therapeutic effect in diseases characterized by hypoxia.

REFERENCES

1. Hamm LL, Nakhoul N, Hering-Smith KS. Acid-Base Homeostasis. *Clin J Am Soc Nephrol*. 2015;10:2232-2242.
2. Iacobelli S, Guignard JP. Renal aspects of metabolic acid-base disorders in neonates. *Pediatr Nephrol*. 2020;35:221-228.
3. Acra AM, Sakagami H, Matsuta T, et al. Effect of three fluoride compounds on the growth of oral normal and tumor cells. *In Vivo*. 2012;26:657-664.
4. Jesus RD, Fernández NV, Morales Y, et al. Chronic toxicity in wistar rats of cytoreg, an ionic antineoplastic therapeutic mix of strong and weak acids. *Adv Clin Toxicol*. 2019;4:000170.
5. Chen X, Li L, Hu J, et al. Anti-inflammatory effect of dexmedetomidine combined with hypothermia on acute respiratory distress syndrome in rats. *J Surg Res*. 2017;216:179-184.
6. Rodríguez-Villar S, Do Vale BM, Fletcher HM. The arterial blood gas algorithm: proposal of a systematic approach to analysis of acid-base disorders. *Rev Esp Anestesiol Reanim (Engl Ed)*. 2020;67:20-34.
7. Berend K, Duits AJ. The role of the clinical laboratory in diagnosing acid-base disorders. *Crit Rev Clin Lab Sci*. 2019;56:147-169.
8. Loisios-Konstantinidis I, Paraiso RLM, Fotaki N, McAllister M, Cristofoletti R, Dressman J. Application of the relationship between pharmacokinetics and pharmacodynamics in drug development and therapeutic equivalence: a PEARRL review. *J Pharm Pharmacol*. 2019;71:699-723.
9. National Research Council Institute for Laboratory Animal R. Washington (DC): National Academies Press (US). 1996.
10. van Zutphen LF, Baumans V, Beynen AC. Principles of laboratory animal science, Revised Edition. *Elsevier*. 2001.
11. Svorc P. Chronobiology of acid-base balance under general anesthesia in rat model. In: El-Esawi MA, ed. *Circadian rhythm - cellular and molecular mechanisms*. IntechOpen; 2018.
12. Gao W, Meng QM, Cui XG. Budesonide instillation immediately after reperfusion ameliorates ischemia/reperfusion-induced injury in the transplanted lung of rat. *Exp Lung Res*. 2017;43:439-446.
13. Damiani E, Casarotta E, Orlando F, et al. Effects of normoxia, hyperoxia, and mild hypoxia on macro-hemodynamics and the skeletal muscle microcirculation in anesthetised rats. *Front Med (Lausanne)*. 2021;8:672257.
14. da Silva ACL, de Matos NA, de Souza ABF, et al. Sigh maneuver protects healthy lungs during mechanical ventilation in adult Wistar rats. *Exp Biol Med (Maywood)*. 2020;245:1404-1413.
15. Almeida MR, Horta J, de Matos NA, et al. The effects of different ventilatory modes in female adult rats submitted to mechanical ventilation. *Respir Physiol Neurobiol*. 2021;284:103583.
16. Svorc P, Petrášová D, Svorc P, Jr. Arterial pH and blood gas values in rats under three types of general anesthesia: a chronobiological study. *Physiol Res*. 2018;67:721-728.
17. Dvořáček I, Hess L, Svobodník J. Anesthesia of laboratory animals. *Prague: Avicenum*. 1998.
18. Schultz JJ, Hsu AK, Gross GJ. Ischemic preconditioning and morphine-induced cardioprotection involve the delta (delta)-opioid receptor in the intact rat heart. *J Mol Cell Cardiol*. 1997;29:2187-2195.
19. Castro D, Patil SM, Keenaghan M. *Arterial blood gas*. StatPearls. Treasure Island (FL): StatPearls Publishing; 2021.
20. Romero MF, Rossano AJ. Acid-base basics. *Semin Nephrol*. 2019;39:316-327.
21. Burggren W, Bautista N. Invited review: development of acid-base regulation in vertebrates. *Comp Biochem Physiol A Mol Integr Physiol*. 2019;236:110518.
22. Ayers P, Warrington L. Diagnosis and treatment of simple acid-base disorders. *Nutr Clin Pract*. 2008;23:122-127.
23. Mejía GÁ, Ramelli MÁ. *Interpretacion clinica de laboratorio/clinical laboratory interpretation*. Editorial Médica Panamericana S.A. 2006.
24. Shrimanker I, Bhattarai S. *Electrolytes*. StatPearls. Treasure Island (FL): StatPearls Publishing; 2021.
25. Seifter JL, Chang HY. Extracellular acid-base balance and ion transport between body fluid compartments. *Physiology (Bethesda)*. 2017;32:367-379.
26. Subramanian RK, Sidharthan A, Maneksh D, et al. Normative data for arterial blood gas and electrolytes in anesthetized rats. *Indian J Pharmacol*. 2013;45:103-104.
27. Harbauer AB, Zahedi RP, Sickmann A, Pfanner N, Meisinger C. The protein import machinery of mitochondria-a regulatory hub in metabolism, stress, and disease. *Cell Metab*. 2014;19:357-372.
28. Ranganathan A, Owiredu S, Jang DH, Eckmann DM. Prophylaxis of mitochondrial dysfunction caused by cellular decompression from hyperbaric exposure. *Mitochondrion*. 2020;52:8-19.
29. Rink C, Khanna S. Significance of brain tissue oxygenation and the arachidonic acid cascade in stroke. *Antioxid Redox Signal*. 2011;14:1889-1903.
30. Liu W, Khatibi N, Sridharan A, Zhang JH. Application of medical gases in the field of neurobiology. *Med Gas Res*. 2011;1:13.
31. Harch PG, Fogarty EF. Hyperbaric oxygen therapy for Alzheimer's dementia with positron emission tomography imaging: a case report. *Med Gas Res*. 2018;8:181-184.
32. Yan R, Sun L, Zhang H. Metabolic reprogramming and tumor immunity under hypoxic microenvironment. *Curr Opin Physiol*. 2019;7:53-59.
33. Calzada MJ, del Peso L. Hypoxia-inducible factors and cancer. *Clin Transl Oncol*. 2007;9:278-289.
34. Valenza F, Pizzocri M, Salice V, et al. Sodium bicarbonate treatment during transient or sustained lactic acidemia in normoxic and normotensive rats. *PLoS One*. 2012;7:e46035.
35. Lozano JA, Galindo JD, Garcia-Borron JC, Martinez-Liarte JH, Penafiel R, Solano R. *Bioquimica y biologia molecular para ciencias de la salud*. 2nd ed. Madrid: Mcgraw Hill/Interamericana de España, S.A. 2005.
36. Johnson MM, Kumi-Diaka KJ, Zoeller R, et al. Therapeutic efficacy of Genistein-Cytoreg® combination in breast cancer cells. *Funct Foods Health Dis*. 2012;2:137-150.
37. Kumi-Diaka J, Hassanhi M, Brown J, Merchant K, Garcia C, Jimenez W. CytoregR inhibits growth and proliferation of human adenocarcinoma cells via induction of apoptosis. *J Carcinog*. 2006;5:1.
38. Guyton AC, Hall JE. *Tratado de fisiología médica: novena edición*. Madrid: McGraw-Hill/Interamericana de España. 1996.