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Lung Water Assessment in Isolated Lung Perfusion Model via Reactance Monitoring

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Abstract— **The aim of this work was to build up a new monitoring technique for the lung preservation. The medical aside problem is to measure the integrity and functionality of the lung tissue, specifically at cellular preservation level in order to improve the survival time until it is grafted. The Impedance monitoring technique for diagnosis edema development is the key in this new technique. The hypothesis was that lung edema formation is highly correlated with the reactance changes so that a rat lung perfusion model was considered as a good model to produce edema in vitro. To prove that pulmonary edema can be induced increasing the venous pressure and the perfusion time, the reactance and hemodynamic parameters were recorder in 16 pulmonary blocks of Wistar rats as methodology. Results showed statistical changes in each pulmonary block weight as a consequence to apply 7.5±1.2 and 10.2±1.7 mmHg venous pressure (multiple samples, Anova, $p<0.05$). These edema weights were correlated with the reactance changes giving 0.6 ($p<0.05$, Pearson). Also, data analysis showed significant differences in reactance with the time of perfusion at 16, 30, and 50 min when venous pressure level were intermittent switched from 7.5 to 10.2 mmHg. The conclusion was this preliminary evidence sustains that reactance measurement is a good technique for monitoring the lung edema level in rats. However, more research should be continuing in bigger animal models in order to prove the validity and application of this monitoring technique in human lungs.**

I. Introduction

A. Preservation and edema formation

THE aim of lung preservation is to maintain the integrity and functionality of it, specifically at the level of cellular membranes in both inter o intracellular gaps. Despite the state of the art in harvesting preservation and transportation, the lung still as the organ with less time of preservation due to the ischemia- reperfusion phenomenon [1], [2]. As a result, edema formation is the outcome of ischemia-reperfusion injury [3]. Thus, water pulmonary content (WPC) in the form of edema and cellular membranes gap are the main observation targets. Thus, the objective is to asses ischemia levels in order to improve the preservation and transportation time [1]-[4].

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B. Pulmonary edema assessment

There are several methods to diagnose pulmonary edema using both direct and indirect forms so that only few have been well established in research and clinical application. For instance, the direct medical imaging techniques like chest X-Ray, Computerized Tomography, and Magnetic Resonance Image are standard techniques used in many high cost health centers. However, for the purpose of this work, they show tremendous disadvantages like high levels of radiation, not portability and without enough diagnostic information in real time, as well [5], [6]. There are other methods based in gravimetric assessment in order to obtain weight indexes (wet weight of lung/body weight index or wet weight/ dry weight index), which are indirectly related to edema development. These methods are easier to implement but require complex set-ups with high and many of them are only used experimentally [7]. Therefore, authors' premise is both WPC and condition of cellular membrane could be obtained by reactance monitoring in order to edema diagnosis.

C. Reactance in biological Tissue

The tissue impedance as been widely studied offering a feasible monitoring technique to WPC measurement. Specifically, impedance spectroscopy can give reliable data for tissue structure, when it is separated in both resistance and reactance [8]. Resistance reflects basically the properties of the electric electrolytes conductivity, whereas reactance component reflects the intra and extra cellular membrane dielectric characteristics. Hence, changes in the cell's membrane permeability, because of the ionic pump dysfunction (ischemia) or interruption of the ionic channels is possible to observe through the tissue reactance. Hence resistance is more susceptible to the size and geometry of electrodes while reactance measurements are immune to this drawback giving more reliable tissue information [9]-[11].

Reactance monitoring offers important features like its minor invasivity, high portability and real time measurement that are good characteristics that are essential during organ preservation. Hence, the main objective of this work To analyze reactance changes through WPC modification in an isolated lung perfusion model , so that measuring reactance

changes versus lung block weight changes could provide indirectly the pulmonary edema (PE) level.

II. Methodology

A. *Isolated Lung Perfusion Model*

The PE is commonly generated as a consequence of water slow flux imbalance between vessels and interstice. This relationship is described by the Starling equation, where different edema types can be established, one of them is edema produced via hydrostatic pressure increment, which it is often induced by a pulmonary venous pressure increment, therefore, in this paper pulmonary edema was induced by venous pressure rise at two levels: 7.5± 1.7 mmHg and 10.2 ± 1.2 mmHg. This maneuver was used in this work in controlled way at different times as a manner of stimuli in rat lungs. The levels were applied at 10, 20 and 40 minutes with 3 minutes of duration each one as it is shown in Fig 2. The venous pressure basal value (Vp= 1.9 ± 0.85) was established at the time of the set up pulmonary block extraction. Each lung block was placed into an isolator organ glass set-up (Kent Scientific, Inc.) where catheters were connected to the pulmonary artery (Pa) and to the left auricle (La). The general isolated rat lung perfusion model diagram is shown in Fig. 1.

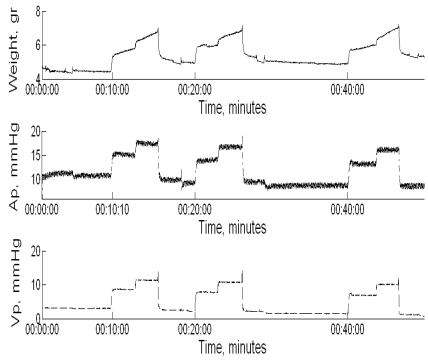

Fig. 1 Experimental rise of the venous pressure at different times, 10, 20 and 40 minutes is shown. Venous pressure (Vp, mmHg), Arterial pressure (Ap, mmHg) and Weight (gr) graphs are pointed out as stimulus-response paradigm.

B. *Population and Instrumentation*

Sixteen pulmonary blocks of Wistar rats were used. The rats were managed according to NOM 062-ZOO-1999 [12]. The experimental protocol was accepted by the Ethical Committee for Animal Experimentation at the National Institute for Medical Science and Nutrition "Salvador Zubiran" in Mexico City, under registers numbers CEX- 36-10/ 11-1.

The pulmonary block was wrapped in a plastic mesh to fixit it and hooked to the weight sensor which stands for one µg of resolution [12]. Also this mesh was handy to fix the spectrometer probe at the right lung lobe through the perfusion catheters. In addition, the pulmonary block was perfused by saline solution at 37 ºC using the arterial pressure (Ap) produced by the peristaltic pump (Masterflex 7523-30, Kent Scientific Inc) at constant flux (7-8 mL/min). The block was ventilated by means of a tracheal cannula (#16), introducing ambient air (21 % of O2) at respiratory frequency of 60 breaths/min with PEEP between 2-3 cm H2O (RSP 1002, Kent Scientific Inc). The Ap, the venous pressure (Vp) and weight changes were recorded all time by pressure and force transducers (Kent Scientific p-23). The set-up model was connected to an acquisition system (DT300, Data Translation, Inc) running at sampling frequency of 1 Hz per channel.

A specific display user interface was designed in Matlab 2008b (Matworks, Inc) for easy visualization and data post-processing. Increments of the Vp were controlled viewing the elevation in the column of the saline solution output (see Figure. 2, item 8).

The impedance spectrometer was a research instrument designed specifically to work in the range of 215 Hz to 1

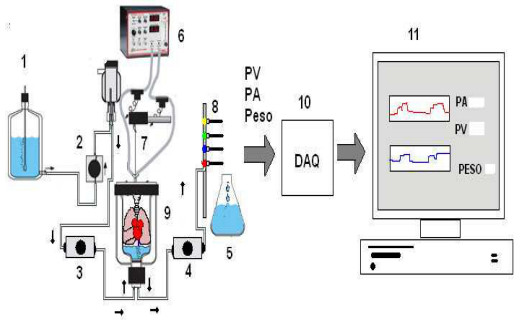

Fig. 2 Schematic diagram of the rat lung isolated perfusion model is shown. (1) Input saline solution reservoir. (2) Peristaltic pump. (3) Invasive arterial pressure sensor. (4) Invasive venous pressure Sensor. (5) Output saline solution reservoir. (6) Ventilator. (7) Weight sensor. (8) Output saline solution column. (9) Isolator glass set-up, (10) Data acquisition system. (11) Display user interface

MHz with an excitation current of 1 mA in a tetrapolar electrode configuration. This instrument is able to measure continuously the complex impedance and satisfies International regulation standards (BS EN 60601 1:1990 and ANSI/AAMI ES1:1993) [13]. The device has a built-in cannula type probe with four electrodes at the tip (Ag/AgCl with a length of one meter) in the form of rings separated coaxially from each other the same distance (3.5 mm) [13]-[15]. The probe was placed in transversal way over the external surface of the left lung at the middle distance between apex and base.

C. Data Analysis

The work of Beltran N, Sacristan E. *et al* [16] established that one of the most important parameters to evaluate the tissue ischemia was related the maximum reactance at low frequencies. Hence, this study was performed under the premise that the maximum reactance can be found in the range of 68 to 464 KHz as it is shown in figure 3.

The statistical analysis was performed to find the following parameters:

a) Time perfusion differences at basal Vp: weight and reactance changes at 0, 16, 30 and 50 minutes were analyzed by repetitive measurements. The One-way-Anova analysis was used accepting $p< 0.01$ when statistical differences appear during those times.

b) Vp Level differences: To probe the effect of the venous pressure level with respect to time, weight and reactance changes were analyzed by repetitive measurements. Anova with two factors was used (time and Vp) with $p< 0.01$.

c) Finally, weight and reactance correlation was search using lineal correlation index (Pearson correlation coefficient), and a Bland-Altman analysis.

III. Results

The Figure 3 shows reactance spectrum at different times when the Vp basal value is maintained, the data are shown in logarithmic scale.

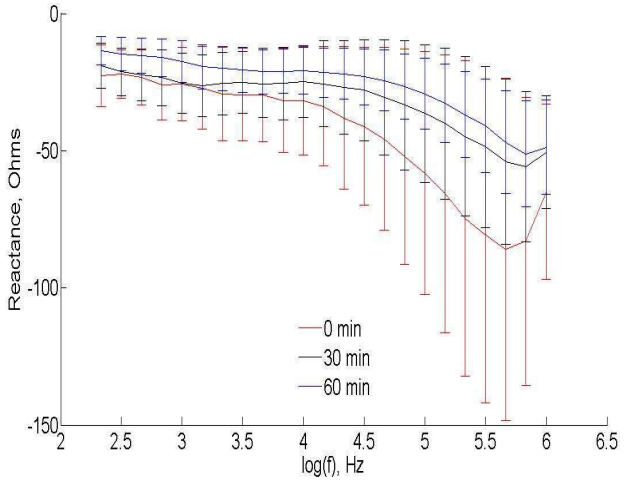

Fig. 3 Reactance Spectrums at different times is shown. Mean ± SD are graphed through out the range from 2 to 6.5 Hz.

There are differences between curves, however in range below 2 KHz an overlap between 30 an 50 minutes curves exist, that it makes difficult the analysis at those frequencies. The figure 4 shows how the weight and reactance are associated with Vp value, visually. It is important to observe for each Vp increment or decrement correspond apparently an increment or decrement in the weight and reactance, this implies a relation between reactance changes and WPC.

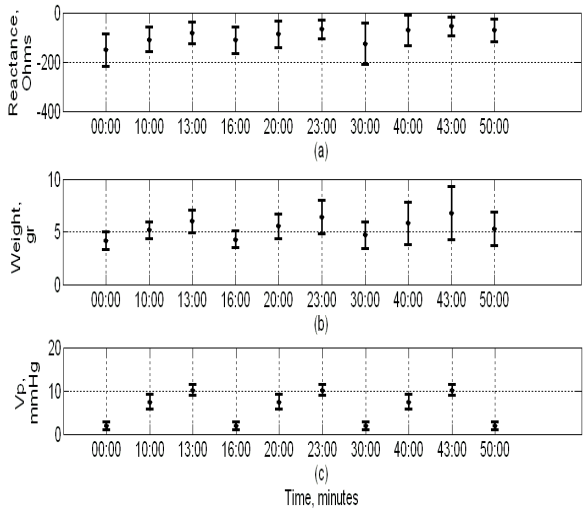

Fig. 4 Mean ± SD are show for (a) reactance, (b) weight and (c) Vp, during the experiment, n=16.

TABLE I
DATA ANALYSIS RESULTS

	n	*Time Perfusion At basal Vp*	*Time of Vp increment*	*p of Vp Level Anal*
Weight	16	0.023*	0.0091*	0.24
Reactance	16	0.000025*	0.0058*	0.04*

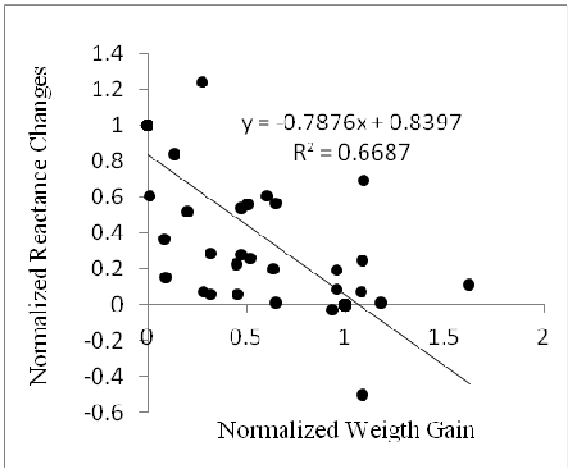

Fig. 5 The linear correlation between reactance changes vs. weight changes is shown.

Reactance and weight gain were normalized by reactance and weight value on initial time division. Table 1 shows of Anova results for each data analysis. Good correlation was obtained to the linear regression (Figure 5). Nevertheless, nonlinear behavior is observed.

IV. Conclusions

The results show that weight gain is clearly related with edema formation, when maneuvers of venous pressure were raised (see Fig. 4). Weight gain is a function of time perfusion and the repetition of venous pressure rise. Reactance decrement was observed as a function of WPC, also the reactance modification at basal Vp could suggest a permeability modification of cellular membrane that is confirmed by the weight gain at basal Vp. High correlation coefficient of 0.6 was obtained. These overall outcomes proves author's hypothesis. These findings make to think that reactance monitoring could be used as a technique to pulmonary edema diagnosis. However, the medical aside problem to measure the integrity and functionality of the human lung tissue is still as a challenge that encourage for more research.

References

[1] M. De Perrot, L. Mingyao, T. Waddell, S. Kesavjee S," Ischemia reperfusion induced lung injury", Am. J. Respir. Crit. Care Med, vol 167, 2003, p 490.

[2] S Tsutomu, T. Keiji, O. Nobuo, O. Obuo, K. Osamu, R. Thomas, Kurt H., Michael A. Matthay, "Ischemia-reperfusion lung injury in rabbits: mechanisms of injury and protection ", Am. J. of Phys Lung Cell Mol, Vol. 276, 1999, p 137-145.

[3] R. Neale, P. Lange and S. Daniel, "The measurement of lung water" Critical Care Vol. 3, 1999, p R19–R24.

[4] S. Zlochiver, M. Arad, M. Radai, D. Barak-Shinar, H. Krief , T. Engelman, R. Ben-Yehuda, A. Adunsky, S. Abbouda,"A portable bio-impedance system for monitoring lung resistivity", Medical Engineering & Physics, Vol. 29, 2007, p 93–100.

[5] AC. Guyton *Textbook of medical physiology*, 8th ed. Philadelphia, PA: W.B. Saunders Company, 1991, p. 419–20..

[6] C. James, I. Parke, I. Mary Townsley "Evaluation of lung injury in rats and mice" Am. J. of Phys Lung Cell Mol Physiol. Vol 286, 2004, p L231–L246.

[7] A. Cesar, V. Cleva, O. Salah, N. Raúl y E. Sacristán, "Impedance spectroscopy for monitoring ischemic injury in the intestinal mucosa", Physiol Meas. , vol 24, 2003 pp 277–289

[8] J. Webster "Electrical Impedance tomography", 1990.

[9] G. Sverre, G. Orja "Bioimpedance and Bioelectricity Basics", Second Edition, Elsevier, 2008, p. 7- 55, 93- 100,

[10] G. Ursula, B. Ingvar, D. Antonio, D Paul, "Bioelectrical impedance analysis part II: review of principles and methods" Clin Nutr, vol. 23, 2004, pp. 1226–1243

[11] E. Beltran, E. Sacristan, "Caracterización y Validación de Clínica de Espectros de Impedancia de la mucosa Gastrica", UAM-I. Thesis degree, 2006

[12] C. Bravo, P. Santillan, "Medición del Coeficiente de Filtración Capilar mediante el método de doble oclusión en pulmones aislados y preservados", Thesis dregree, UNAM 2007.

[13] R. Sacristan, "Impedance Spectroscopy Method for Monitoring Ischemic Mucosal Damage in Hollow Viscous Organs", No. Patent US 6, 965, 795, 2005.

[14] R. Sacristan, "Impedance Spectroscopy System and Catheter for Ischemic mucosal Damage Monitoring with Hollow Viscous Organs" No. Patent US 6,882,879, 2005.

[15] J. Davis "Muse and Rat Anesthesia and Analgesia", Current Protocols in Neuroscience, 2008,Appendix 4B.

[16] N. Beltran, E Sanchez- Miranda, G. Godinez, E. Sacristan "Gastric Impedance spectroscopy in elective cardiovascular surgey patients" Physiol. Meas. ,vol 27, 2006, p. 265-277