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Androgen Deprivation and Cytoprotective Parameters in Rat Lung

Silvina M Álvarez, Verónica S Biaggio, María E Ciminari, Silvana N Piguillem, Anahí B Pacheco Guiñazú, Sophia M Escobar Correas, María V Perez Chaca, Nidia N Gomez

Departamento de Bioquímica y Ciencias Biológicas
Facultad de Química, Bioquímica y Farmacia
Universidad Nacional de San Luis
IMIBIO-CONICET, San Luis, Argentina.

Daiana G Álvarez-Olmedo, Mariel A Fanelli

Laboratorio de Oncología
IMBECU (CCT) - CONICET- Mendoza, Argentina.

Dra. Nidia N. Gómez*

Laboratorio de Anatomía y Fisiología,
Bloque I, Universidad Nacional de San Luis
Ejercito de los Andes 950,5700, San Luis, Argentina.
**ngomez@unsl.edu.ar, gomez.nidia@gmail.com*

Abstract: *The aim was to study the effect of androgen deprivation, focusing on oxidative stress, and cytoprotective markers, in order to better understand the possible causes of pulmonary diseases in elder people.*

Adult male Wistar rats were divided into three groups: control (Co), a group subjected to castration by simple orchidectomy (Ca) and a group subjected to orchidectomy that received testosterone (100 µg/kg body weight) daily from day 25 to day 30 after castration (Ca + T). Bronchoalveolar lavages (BAL) and lungs were obtained.

We observed a decrease in body weight in the castrated groups. Protein content in BAL was increased in both castrated groups while nitrites did not show differences among the groups. TBARS, Catalase (CAT) and Glutathione Peroxidase (GPx) activities were significantly increased in the castrated group, returning to the control values after the administration of testosterone. AR expression was increased in the castrated groups. Nrf-2 factor was increased in both castrated groups, while NADPH oxidase (NOX) and GPx expression increased in the Ca group but showed a decrease to control values in the Ca+T group. Superoxide Dismutase (SOD) did not show differences among the treatments. The immunohistochemistry showed a decreased expression of Hsp27 and increased expression of Hsp70

The absence of androgens induces oxidative stress in lung, together with changes in the expression of cytoprotective markers. This would lead to weak lung stroma, susceptible to undergo several pulmonary diseases.

Keywords: *Cytoprotective mechanisms, castration, lung*

1. INTRODUCTION

Respiratory diseases are the major causes of morbidity, mortality, and an economic burden worldwide [1,2]. Several studies have shown an increased prevalence of chronic pulmonary diseases, such as chronic obstructive pulmonary disease (COPD) in men older than 65 years old [3]. On the other hand, it is known that in elder men, testosterone declines [4].

It has been shown that testosterone is the most important androgen in men. 95% of the androgenic activity is due to testosterone and only 5% is represented by adrenal androgens [androstenodione and dehydroepiandrosterone (DHEA)] and the peripheral transformation of testosterone [5]. Only

the free fraction of testosterone, approximately 1-3% of total testosterone is capable of penetrating the target cells and bindig to the cytoplasmic receptor [5].

Male steroid hormones's mechanism is mediated by its specific receptor named androgen receptor (AR). This receptor is a member of the class I subgroup of the nuclear receptor superfamily [6]. In the absence of the ligand, AR is bound to protein complexes that stabilize its tertiary structure and prevent its activation. In response to androgens, cytoplasmic AR rapidly translocates to the nucleus and interacts with sequence-specific androgen response elements (ARE) in the transcriptional regulatory regions of target genes [7, 8]. The formation of dimers of these nuclear receptors conditions the efficiency of the union between the receptor and its ARE.

Several studies have shown that AR is expressed in different tissues such as liver, kidney, muscle and lung [9, 10, 11, 12]. Regarding lungs, previous studies have shown that AR is expressed by mesenchimal cells such as fibroblasts [13] and also by epithelial cells [14]. Recent studies showed that sexual hormones can modulate lung development and physiology, and they can also interfere with some pathologies [15]. It was also shown that sexual hormones play an important role in airway related diseases and the inmune response, leading to pumonary injuries [16]. Understanding the phisiologycal responses of the stroma to hormonal variations is of relevance for its application in different pathological states such as resistance to drugs and cancer treatments, among others.

On the other hand, it is known that the redox balance is important in the airways because it is the first contact with environment contaminants, particles, cigarette smoke and pathogens. Therefore, an imbalance between oxidants/antioxidants induces stress, which has been implicated in the development of airway diseases. Several antioxidant enzymes are critical for maintaining cellular homeostasis and preventing cellular damage. There are very few studies that correlate oxidative stress with androgen deprivation and pulmonary diseases.

Molecular chaperones are involved in folding, activation, trafficking, and transcriptional activity of most steroid receptors, including the androgen receptor. In absence of the ligand, AR is predominantly cytoplasmic, maintained in an inactive but highly responsive state by a large dynamic heterocomplex composed mainly of heat-shock proteins (Hsp). Ligand binding leads to a conformational change in the AR and dissociation from the large Hsp complex. Dissociation of the AR-chaperone complex after ligand binding is viewed as a general regulatory mechanism of AR signaling [17]. It is known that Hsp27 complexes with estrogen receptor (ER) and glucocorticoid receptors (GR) and is ATP independent [18]. Hsp27 is a cytoprotective chaperone expressed in response to many stress signals to regulate key effectors of the apoptotic machinery, including the apoptosome, the caspase activation complex [19, 20], and proteasome-mediated degradation of apoptosis-regulatory proteins [21, 22]. Although Hsp27 is induced by estrogens and glucocorticoids [23, 24], its relationship with AR and androgens is undefined.

On the other hand, increasing evidence suggests that there is a complementary regulation between Hsp70 and inflammatory mediators, such as cyclooxygenase (COX-2) (EC 1.14.99.1) and NF-kB, among others [25]. These anti-inflammatory actions of Hsp70 are mediated by the binding of Hsp70 to NF-kB and its subsequent inhibition. During oxidative stress, Hsp70 has been found to reduce the expression of COX-2 and the production of nitric oxide (NO) [26, 27]. However, the role of Hsp70 and Hsp27 during androgen deprivation is still unknown.

Therefore, the aim of this work was to study the effect of androgen deprivation, focusing on oxidative stress parameters and cytoprotective markers, in order to better understand the possible causes of pulmonary diseases in those who undergo a decrease or eventual deprivation of testosterone.

2. MATERIALS AND METHODS

2.1. Animals and Feeding Procedure

Adult male Wistar rats weighing 200 ± 20 g were housed individually in a controlled environment with a 12 h light - 12 h dark cycle and temperature maintained at 21 ± 2°C. Rat chow (Cooperación, 16-014007 Rata Ratón Extrusado, Asociación de Cooperativas Argentinas, ACA) and tap water were available *ad libitum*. Rats were divided randomly into three groups. Group I was used as control (Co), group II was subjected to castration by simple orchidectomy (Ca) and

group III was subjected to orchidectomy and received testosterone (100 µg/kg body weight) daily from day 25 to day 30 after castration, by intramuscular injection (*im*) (Ca+T). During the experiment, body weights were registered weekly.

All experiments were conducted in accordance to the National Institutes of Health Guide for the Care and Use of laboratory Animals (National Institutes of Health Publication no. 80-23) and the National University of San Luis Committee´s Guidelines for the Care and Use of Experimental Animals (ordinance CD 006/2).

2.2. Isolation of Bronchoalveolar Lavages (BAL)

All animals were killed 30 days after surgery. Before that, rats were anesthetized intraperitoneally (*ip*) with sodium pentobarbital (50 mg/kg). The trachea was cannulated and the lungs were filled with 2.5 ml ice-cold 0.9% saline solution. Bronchoalveolar lavages were then collected. This procedure was repeated five times with ice-cold 0.9% saline. BAL samples were centrifugated at 4ºC for 10 min at 3000 rpm to sediment cells. Blood samples were obtained by heart punction and collected in tubes. They were incubated at 37ºC and centrifuged at 3000 rpm for 10 min in order to obtain serum. Lungs were extracted and frozen in liquid nitrogen, and they were stored at -80°C until used.

2.3. Thiobarbituric Acid–Reactive Substance (TBAR'S) Determination

Lung homogenates and serum samples were used for TBA assay [28], and the levels of lipid peroxidation products, mainly malondialdehyde (MDA), were determined spectrophotometrically as TBAR'S.

Lung was homogenized at pH 7.4 and proteins were precipitated with 20% trichloroacetic acid (TCA, Sigma-Aldrich Co.). The supernatant containing malondialdehyde (MDA), the end product of the lipid peroxidation, was incubated with a 0.7% thiobarbituric acid solution (TBA, Sigma-Aldrich Co.) to measure the TBAR'S content. An acid hydrolysis product of 1,1,3,3-tetramethoxy propane (TMP) was used as standard.

2.4. Measurement of NO_2^-

Nitric oxide (NO) formation was measured indirectly by assaying nitrite, a stable product of NO oxidation [29]. Nitrites were determined alone, using the Griess reagent. Optical density (OD) was measured at 540 nm. The level of NO was expressed in µmols of NO_2^- per ml after being equilibrated with a solution of $NaNO_2$ (10 mM).

2.5. Protein Determination

Protein concentration was determined by the method of Lowry et al. [30] using bovine serum albumin as a standard.

2.6. Measurement of Catalase (CAT) (EC 1.11.1.6) and Glutathione Peroxidase (GPx) (EC 1.11.1.9) Activities

In order to process the tissue for measuring the antioxidant enzymes activities, lungs were homogenized in 30 mM PBS buffer, with 120 mM KCl, pH 7.4, containing 1× protease inhibitors (Pepstatin A and PMSF) and 50× Triton, followed by centrifugation at 3000 rpm, for 30 min at 4ºC. The pellets were discarded and the supernatants were collected and used for the measurement of CAT and GPx activities [31]. The enzyme determinations were performed immediately after the rats were killed.

Catalase (CAT) activity was determined by measuring the decrease in absorption at 240 nm in a reaction medium containing 50 mM phosphate buffer (pH 7.3) and 3 mM H_2O_2. The pseudo-first-order reaction constant (k':k [CAT]) of the decrease in H_2O_2 absorption was determined and the catalase content in units/mg protein was calculated using: $k = 4.6 \times 10^7 M^{-1} s^{-1}$ [32, 33]. One unit of catalase activity is defined as the amount of enzyme required to decompose 1mM of H_2O_2/min.

GPx activity was measured with 100 mM Potassium phosphate solution, 1 mM EDTA (pH7.7), 10 mM Sodium Azide, 10mM Tert-butyl hydroperoxide (BHT), 10µM NADPH, 10U/ml Glutathione Reductase (GR) and 100 µM reduced glutathione (GSH).

GPx activity was determined following the NADPH oxidation rate at 340 nm [34]. Results were expressed as international units (1 IU oxidizes 1 µmol NADPH/min at pH 7.7 at 30ºC per milligram of protein) (IU/mg protein).

2.7. RNA Isolation and RT-PCR Analysis

A lung lobe was used to study the expression of different genes. Total RNA was isolated by using TRIzol (Life Technologies). All RNA isolations were performed as instructed by the manufacturers. Gel electrophoresis and Gel Red staining confirmed the purity and integrity of the samples. Quantification of RNA was based on spectrophotometric analysis at 260/280 nm. 10 µg of total RNA were reverse-transcribed with 200 units of MMLV Reverse Transcriptase (Promega Inc.) using random hexamers as primers in a 20 µl reaction mixture, following the manufacturer's instructions. PCR was performed in 20µl of reaction solution containing 0.2 mM dNTPs, 1.5 mM MgCl2, 1.25 U of Taq polymerase, 50 pmol of each rat specific oligonucleotide primers and RT products (1/20 of RT reaction). The expected PCR product of Nrf-2 is 160 bp, Superoxide Dismutase (SOD) (EC 1.15.1.1) is 191 bp, NADPH oxidase (NOX-2) (EC 1.6.3.1) is 150 bp, GPx is 245 bp, AR is 423 bp and S28 is 200 bp. The samples were heated to 94°C for 2 min, followed by 35 temperature cycles. Each cycle consisted of three periods: (1) denaturation, 94°C for 1 min; (2) annealing, 59°C for GPx, SOD, Nrf-2, NOX and S28 [35] and 65°C for AR [36] during 1 min; (3) extension, 72°C for 1 min. After 35 reaction cycles, the extension reaction continued for 5 more minutes. The PCR products were electrophoresed on 2% agarose gel with 0.01% Gel Red. The image was visualized and photographed under UV transillumination. The intensity of each band was measured using the NIH Image software Scion Image and reported as the values of band intensity units. The relative abundance of each target band was then normalized according to the expression of S28.

2.8. Immunohistochemistry

Details of the antibodies used are provided in Table 1. Streptavidin-biotin immunoperoxidase method was used, as previously described [37, 38]. The endogenous peroxidase activity was inhibited with 1% (v/v) H_2O_2, and non-specific binding was blocked with 10% (v/v) normal goat serum. All sections were incubated with primary antibodies for 18 hours at 4°C and then for 30 min at room temperature. The visualization of antigens was achieved by using the Vectastain ABC kit (Vector laboratories, Burlingame CA). Finally, the slides were washed in distilled water, counterstained with Mayer's hematoxylin, dehydrated and mounted.

Table1. *Antibodies used*

Antibodies	Clone	Supplier	Dilution
Primary Antibodies			
Hsp 25/27	Hsp27	Gaestel	1:1000
HSPA1A	Hsp72/70i	Stressgen	1:1000
PCNA	Clone PC10	Dako	1:700
Bcl-2	Clone 124	Dako	1:80

2.9. Image Analysis

Image analysis was performed using an Image Pro-Plus 3.0.1 system (Media Cybernetics, Silver Spring, MA, USA). For the immunohistochemistry technique, images were digitized by a Leica microscope (Leica ICC50 HD microscope, Leica Mycrosistems, Germany), using an objective magnification of X40. This was achieved by recording an empty field reference image for the correction of unequal illumination (shading correction) and by calibrating the measurement system with a reference slide to determine background threshold values. The reference slides contained a series of tissue sections stained in the absence of a primary antibody. The methodological details of image analysis have been described earlier [39, 43]. The average density (% of immunopositive area) was calculated as a percentage of total area evaluated through color segmentation analysis, which extracts objects by locating all objects of a specific color (brown stain). These values were verified and normalized with the controls carried across various runs using the same region (verified by image comparison) for calibration. Sections were analyzed with the observer blinded to treatment. The images were then transformed to a bi-level scale TIFF (Tagged Image File Format) format.

2.10. Statistical Analysis

All data are expressed as means ± SEM. For comparisons that involved three experimental groups, one way ANOVA (GraphPad Prism 5.0, software) was used. Having passed statistical significance by ANOVA, individual comparisons were made with the Dunnett´s multiple-comparison test. Differences between means were considered significant at the $p< 0.05$ level.

3. RESULTS

3.1. Body Weight and Biochemical Parameters Measured During Androgen Deprivation

As shown in **Table 2**, we observed a significant decrease in body weight values in the castrated group when compared to the control group ($p<0.01$). Testosterone supplementation during 5 days showed a trend to reach the control level.

We analyzed the protein content in BAL and found that the values were significantly elevated in both castrated groups when compared to the control group ($p<0.05$). Nitrites concentrations in serum and BAL were unchanged in all the treated groups.

Table2. *BAL and serum biochemical parameters after 30 days of treatment*

	Co	Ca	Ca+T
Body Weight (gr)	277.8 ± 9.2	248.3 ± 10.3*	256.1 ± 14.6
Nitrites in BAL (µM/mg prot)	15.72 ± 0.31	16.76 ± 1.00	18.51 ± 1.18
Nitrites in Serum (µM/mg prot)	15.65 ± 0.36	14.81 ± 2.51	18.9 ± 3.07
Proteins (BAL) (mg/ml)	2.46 ± 1.49	5.03 ± 1.15*	6.65 ± 0.71*

3.2. Lipid Peroxidation Products and Antioxidant Enzimes

Figure 1 shows that the levels of MDA / mg of protein increased significantly in the Ca group in lung, while in the animals treated with testosterone TBAR'S levels achieved the control group values.

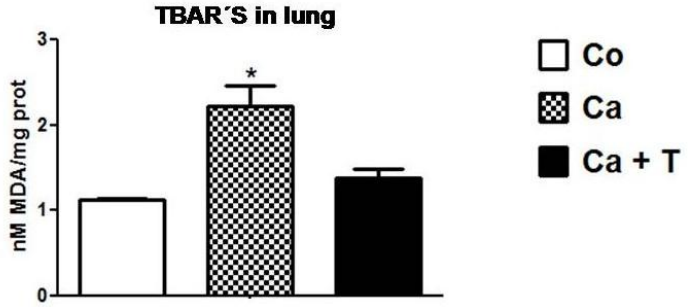

Fig1.*TBAR'S in lung. Data are presented as mean ± SEM, for 10 rats in each group. *p< 0.05. Abbreviations used: Co: control group. Ca: castrated group. Ca + T: castrated group and testosterone supplementation.*

Figure 2A shows Catalase activity in lung. It showed a significant increase in the castrated group ($p<0.05$) while the addition of testosterone induced a trend to return to the control values. Glutathione Peroxidase activity (Figure 1B) also displayed an increase in its activity in the Ca group ($p<0.001$) when compared to the control group, and the administration of Testosterone returned the activity values to those seen in the control group.

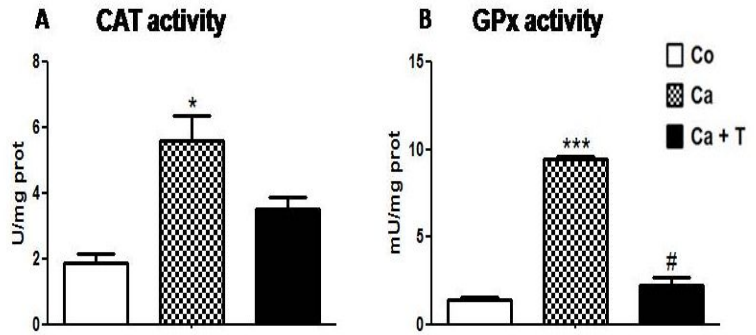

Fig2. *Enzymes activity in lung. A) CAT activity. Data are presented as mean ± SEM, for 10 rats in each group. B) GPx activity. Data are presented as mean ± SEM, for 10 rats in each group. *p < 0.05, ***p < 0.001 significantly different when compared to control. # p<0.01 significantly differente when compared to Ca. Abbreviations used: Co: control group. Ca: castrated group. Ca + T: castrated group and testosterone supplementation.*

3.3. Expression of AR In Rat Lung After Androgen Deprivation

We analyzed the expression of the androgen receptor in lung and we detected a significant increase in the Ca group, while the Ca+T group showed an AR expression similar to the control group (**Figure 3**). This correlates with an upregulation of the AR induced by the absence of circulating testosterone.

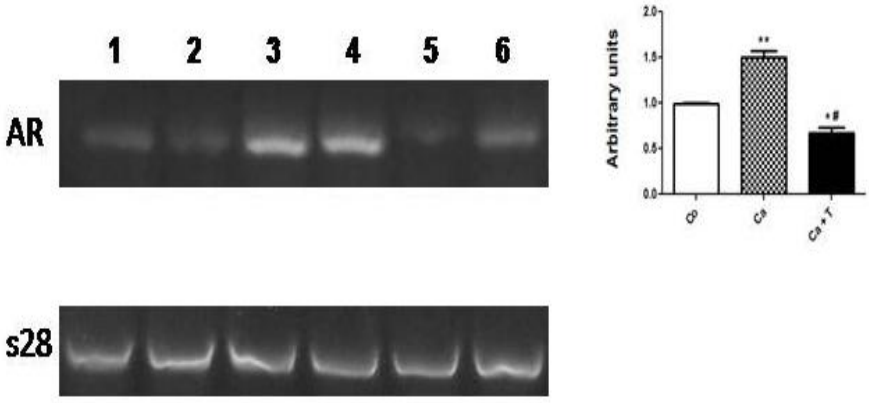

Fig3. *Expression of AR in Control, Castrated and Castrated + Testosterone rats. Gel Red–stained agarose gel of AR PCR products and quantification of the intensity of the fragment bands in relation to the intensity of the internal control bands (S28) (n = 6 cases per group). ** p < 0.01 and *p < 0.05 significantly different when compared to control; + p < 0.05 significantly different when compared to castrated group. Lanes 1, 2: control samples. Lanes 3, 4: Ca samples. Lanes 5, 6: Ca + T samples.*

3.4. Expression of nrf-2 factor, NOX-2 and antioxidant enzymes in rat lung after androgen deprivation

We analyzed the expression of the nrf-2 factor and we found that it was increased in both Ca and Ca+T groups ($p<0.01$) (Figure 4). We also studied the expression of NOX-2 and we observed a significant increase in the Ca group ($p<0.05$), while the Ca+T group showed control like values. GPx also exhibited a significant increase in Ca group ($p<0.05$) while Ca+T returned to the control values. SOD expression did not change among the studied groups (Figure 4).

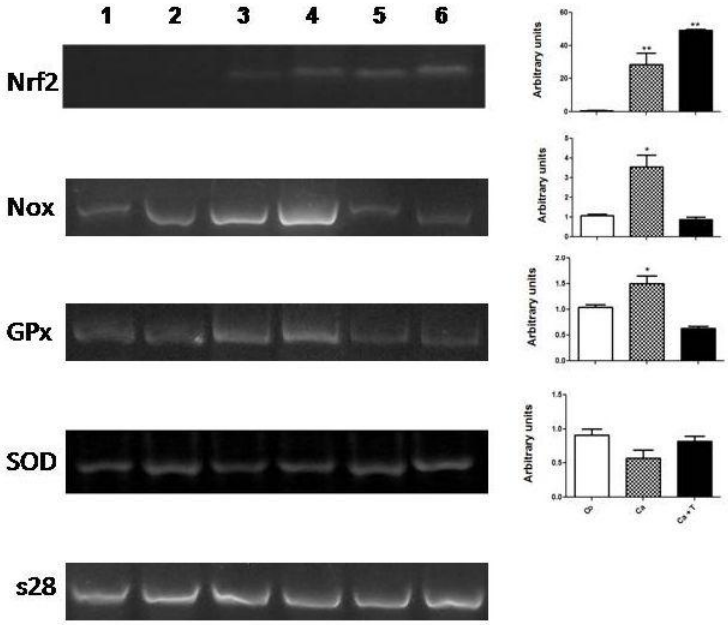

Fig4. *Expression of nrf-2, NOX-2, GPx and SOD in Control, Castrated and Castrated + Testosterone rats. Gel Red–stained agarose gel of nrf-2, NOX-2, GPx and SOD PCR products and quantification of the intensity of the fragment bands in relation to the intensity of the internal control bands (S28) (n = 6 cases per group). *p < 0.05, **p < 0.01 significantly different when compared to control. Lanes 1, 2: control samples. Lanes 3, 4: Ca samples. Lanes 5, 6: Ca + T samples.*

3.5. Bcl-2 and PCNA Expression in Lung During Androgen Deprivation

During androgen deprivation, Bcl-2 expression was increased almost two-fold ($^{**}p<0.01$) when compared to the control group. The group supplemented with testosterone elevated Bcl-2 expression nearly one-fold ($^{+}p<0.05$). However, we observed that PCNA expression did not change among the groups. The data also revealed the presence of weak cytoplasmic staining in the cells and alveolar spaces in pulmonary parenchyma; this staining was considered as part of the background, and it was not included in the analysis (**Figure 5: A-F).**

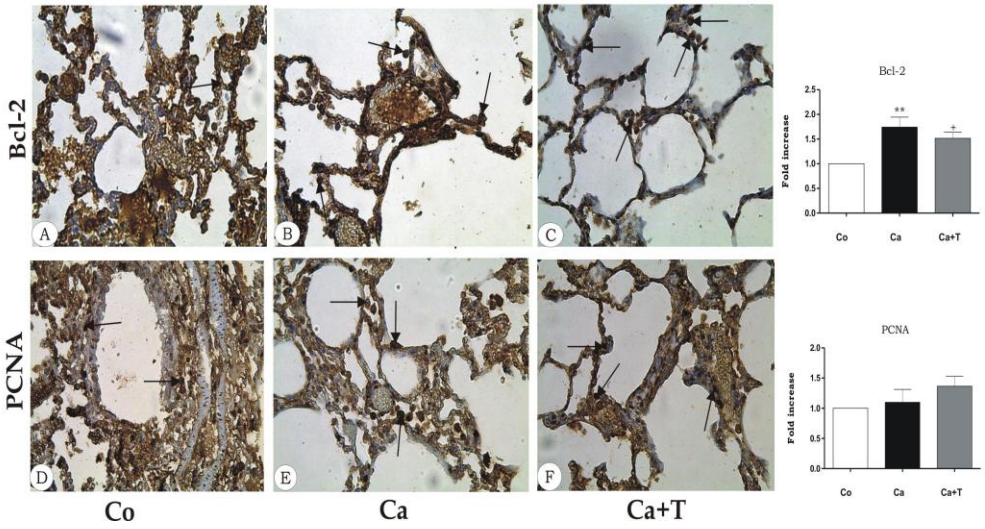

Fig5. *Sections of lung parenchyma immunostained with Bcl-2 and PCNA. Representative images of lung parenchyma show the positive area (brown). Both antibodies appeared mainly in nuclear zone of cells. The positive reaction was visualized as brown deposits; the nuclei were counterstained with hematoxylin. The bars represent the quantification of the protein expression. Results are given as means ± SD, n = 8 for each case. (***) p<0.001; (**) p<0.01 Ca vs. Co group. Co: control group; Ca: castrated group; Ca+T: castrated + Testosterone supplementation; Bcl-2 (B-cell lymphoma-2): anti-apoptotic protein. PCNA: proliferating cell nuclear antigen. Magnification 40X.*

3.6. Hsp27 and Hsp70i Expression in Lung During Androgen Deprivation

We found that Hsp27 immunostaining decreased one-fold in Ca group ($^{***}p<0.001$), compared to Control (**Figure 6: A, B and C**). On the contrary, the expression of Hsp70i increased nearly two-fold in Ca group ($^{***}p<0.001$). During the androgen replacement period, Hsp70i elevated one-fold than the control group ($^{++}p<0.01$). Hsp27 showed a trend towards an increase in the same group (**Figure 6: D, E and F**).

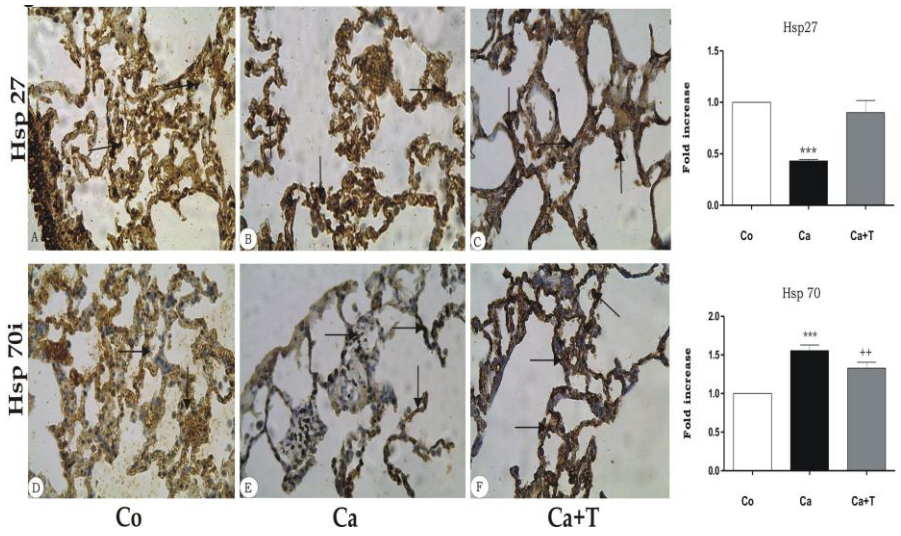

Fig6. *Sections of lung parenchyma immunostained with Hsp27and Hsp70. Photomicrography shows positive immunolabelling (arrows). The positive reaction was visualized as brown deposits; the nuclei were counterstained with hematoxylin. The bars represent the quantification of the protein expression. Results are given as means ± SD, n = 8 for each case. (***) p<0.001; (**) p<0.01 Ca vs. Co group.*

Co: control group; Ca: castrated group; Ca+T: castrated + Testosterone supplementation; Hsp27: heat shock protein 27 (small molecular weight); Hsp70i: heat shock protein 70 inducible (high molecular weight). Magnification 40X.

4. DISCUSSION

The goal of this study was to examine the effect of androgen chronic deprivation in lung and wether its variations are related to changes in different oxidative stress parameters as well as to modifications in cytoprotection markers.

In the present study we found that one month of castration significantly increases the androgen receptor expression in lung. This situation is clearly associated to androgen deprivation (Figure 3). In our experimental design, the low gain of body weight observed in Ca when compared to Co animals confirms previous results [44, 45]. These changes are similar to those previously observed in our laboratory using surgical and pharmacological castration during 21 days [44]. When we evaluated the protein concentration in BAL after castration, the increased content we found can be attributed to increased capillar-alveolar membrane permeability. The fact that in the Ca+T group the difference in the permeability is also significant indicates that the administration of testosterone did not reverse the effect of castration at this level.

The increased level of reactive oxygen species (ROS) is reflected by the accumulation of oxidative damaged cellular macromolecules and chromosomal breakage. Lipid hydroperoxides and protein-associated carbonyl groups are related to the development of chronic lung disease [46]. We showed that the peroxidation products in castrated rats after one month of treatment increased, as indicated by lung TBAR´S; but when the rats were injected with testosterone, the level of TBAR´S decreased (Figure 1). We suggest that these changes are related to the development of oxidative stress in lung due to the absence of testosterone. In addition, there is a strong positive correlation between lipid peroxidation and lung injury. It is known that part of ROS production is normally generated by the tightly regulated enzyme NAD(P)H oxidase. Inappropriate gene transcription or activation of NOX during various pathologies suggests its specific involvement in the diseases [47]. In addition, this is one of the first communications that relates the changes of NOX-2 expression to androgen deprivation in lung. Our results demonstrated that NOX-2 transcription is increased in the castrated group; so the superoxide production could explain, at least in part, the pathological conditions found in our experimental model. At the end of the testosterone supplementation period, the level of NOX-2 decreases when compared to the control group, suggesting that the superoxide concentration may be decreasing and the condition in the castrated animals improves (Figure 4).

Oxidative stress is a threat to well-being. The antioxidant enzymes SOD and CAT serve as a primary line of defense, destroying the free radicals produced by oxidative stress. SOD reduces the radical superoxide (O_2-) to form hydrogen peroxide (H_2O_2) and oxygen (O_2); then, CAT and GPx work simultaneously with glutathione to reduce H_2O_2 [48]. The increased MDA level suggests that was the cause of oxidative stress, while CAT and GPx increased their activity in order to protect the cells by eliminating the toxic oxidative products.

The antioxidant defense system protects the biological integrity of cells and tissues against the harmful effects of superoxide free radicals [48]. This implies that the depletion of the antioxidant defense system results in loss of cell and tissue integrity. In our laboratory we observed that castration provokes significant changes in lung. These changes may be related, at least partially, to alterations in the chemical composition of cell membranes and lung surfactants [44, 49]. The change observed could be a consequence of the alteration in the composition of the phospholipids that make up the alveolar surface (data not shown). Besides, the most widespread tool to examine the cellular immune response in lungs is to study the broncoalveolar lavages (BAL). When we compared BALs of castrated rats versus control rats, we observed a clear increase of lymphocytes and neutrophiles (data not shown).

In order to appreciate the precise contributions of NO to a pathological process in lung induced by castration, we determined the levels of stable metabolites of NO, measured as nitrite. The data obtained provide evidence that NO-related species are not accumulated in serum and BAL during the course of castration in rats.

Several studies have demonstrated a clear link between defects in the lung antioxidant defense system, regulated by nuclear factor erythroid 2-related factor 2 (nrf-2) and excessive oxidative stress, increased apoptosis, inflammation and exacerbated emphysema [50]. Inversely, ROS mediate the induction of redox-sensitive signal cascades, leading to an increased expression of antioxidant enzymes [51, 52].

Antioxidant response element (ARE)-mediated expression and coordinated induction of antioxidant enzymes is a critical mechanism for protection against chemically induced oxidative/electrophilic stress. NF-E2-related nuclear factors (nrf-1 and nrf-2) bind to ARE and regulate ARE-mediated gene expression. We found an increased expression of nrf-2 factor in Ca as well as in Ca+T group, suggesting that the oxidative stress caused by the androgen deprivation (previously inferred by the increased levels of TBAR'S in lung) induced the activation of nrf-2 in order to maintain redox homeostasis. This is consistent with what Mann et al demonstrated, that activation of nrf-2 transcription may be triggered by cellular oxidative stress [53] and is a key enhancer of a number of antioxidant and cytoprotective genes [50].

Given the fact that we found oxidative stress in our model, we measured the expression of Bcl-2. Bcl-2 protein protects cells from oxidative stress [54] and suppresses the activation of caspases [55]. We found this protein elevated in the castrated rats and with regard to the administration of testosterone for 5 days, it would not be enough to counteract the anti-apoptotic process in lung.

Finally, in order to demonstrate the cytoprotective function of Hsp in lung stroma, we studied the expression of Hsp27 and Hsp70i. Heat shock proteins (HSP) are involved in the processes of folding, activation, trafficking and transcription of most steroid receptors including the androgen receptor. Accumulating evidence links rising heat shock protein 27 (Hsp27) levels to the development of castration-resistant prostate cancer [56].

Hsp27 showed a significant decrease of the immunostained area in the androgen deprived group when compared to control group, and there were not significant differences between control and Ca+T group, suggesting that with respect to this heat shock protein in the lung, its function is recovered with androgen addition. The cytoprotective effects of Hsp27 result from its ubiquitin binding and degradation of IκB [57], direct interference of caspase activation, modulation of oxidative stress, and regulation of the cytoskeleton [58]. Androgen, AR, and Hsp27 activation cooperatively interact to regulate the genomic activity.

In contrast, Hsp70i showed a significant increase of the immunostained area in androgen deprived group when compared to the control group. But this is not surprising since Hsp70 has an inducible expression in response to extracellular and intracellular stresses and it constitutively exists in cells [59]. In a previous work we studied the effects of zinc deficiency in rat lung parenchyma and we found the same condition in zinc deficient lungs [60].

In summary, the decrease in the expression of Hsp27 denotes an absence or decline of cytoprotective properties, which would correlate with the increased level of TBAR´S found in lung. On the other hand, an elevated expression of Hsp70 has been found to reduce the production of nitric oxide (NO) [27], therefore the increased immunostaining of Hsp70 would explain the absence of variations in the NO concentration in BAL of castrated rats (measured as nitrites).

Taken together, all the results of this work suggest that the absence of androgens induce oxidative stress and lipid peroxidation in lung, synchronically with changes in the expression of cytoprotective markers. This would lead to weak lung stroma, susceptible to undergo several pulmonary diseases.

These results should be considered in order to better understand the regulation of inflammatory pathways and propose therapies for patients that suffer of lung diseases due to androgen deprivation.

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AUTHORS' BIOGRAPHY

Dr Silvina Monica Alvarez holds a Master in Molecular Biology and a Ph.D. in Biochemistry, both from the National University of San Luis, Argentina as well as two post-doctoral positions at Virginia Commonwealth University, Richmond, VA, USA. She is Assistant Professor of Physiology at National University of San Luis (Argentina) and is also a scientific member of the National Council of Science and Technology of Argentina (CONICET). She co-directs two research projects regarding the toxic effect of heavy metals and is the director of one of the lines of a research project that studies the effect of androgen deficiency. She has directed several master theses and is co-directing doctoral theses at National University of San Luis, Argentina.

Dr Veronica Silvina Biaggio has a degree in Biochemisry and holds a PhD in Biochemistry from the National University of San Luis, and a postdoctoral position at the National University of Cuyo, Mendoza, Argentina. She is Laboratory professor of Physiology at National University of San Luis (Argentina) and is also a scientific member of the National Council of Science and Technology of Argentina (CONICET). She is a member of two research projects, one of which is related to the effects of heavy metal poisoning during pregnancy and the other one focuses on androgen deficiency in the lung. She directed two master theses.

Dr. María Eugenia Ciminari holds a Master in Biological Sciences and a Ph.D. in Biological Sciences, both from the National University of San Luis, Argentina. She is currently Assistant Professor of Anatomy, Histology and Physiology at National University of San Luis (Argentina). She is a member of a research project that studies the effect of androgen deficiency in the cardiovascular system. She is directing several master theses.

Daiana Alvarez Olmedo holds a Master degree in Molecular Biology from National University of San Luis and is pursuing her PhD in Biological Sciences oriented towards cellular and molecular biology at the Oncology Laboratory, IMBECU-CCT, Mendoza, Argentina.

Silvana Noemi Piguillem holds a Master in Biological Sciences from the National University of San Luis, Argentina. She is an Instructor of Anatomy and Physiology at National University of San Luis. She is a member of a reaserch project that studies the effect of androgen deficiency in the cardiovascular system.

Anahí B. Pacheco Guiñazú holds a Master in Molecular Biology and is pursuing a Ph.D. in Biology at the Laboratory of Lipids and Acrosomal Exocytosis, IHEM-CONICET, Mendoza, Argentina.

Sophia Escobar Correas holds a Master in Molecular Biology and is pursuing a Ph.D. in Biology at the Laboratory of Physiology, IHEM-CONICET, Mendoza, Argentina.

Dr Maria Veronica Perez Chaca holds a Master in Biological Sciences and a Ph.D. in Biological Sciences, both from the National University of San Luis, Argentina. She also was awarded an internship at University of Granada, Spain. She is Assistant Professor of Anatomy at the National University of San Luis. She has directed several Master theses.

Dr. Mariel Fanelli has a degree in Biological Sciences and a Ph. D. in Biology, both from de National University of Buenos Aires and she is also an associate researcher of the National Council of Sciences and Technology of Argentina (CONICET). She co-directs research projects regarding the heat shock proteins and cell adhesion proteins in oncology and the impact of heavy metals on these proteins. She has directed two Master theses and actually is directing and co-directing doctoral Theses at the National University of Cuyo, Mendoza, Argentina.

Dr. Nidia N. Gomez has a degree in Biochemistry and holds a PhD in Biochemistry from the National University of San Luis, Argentina. She is Head of the Physiology and Morphology Laboratory in the department of Biochemistry and Biological Sciences at the National University of San Luis, Argentina, and is also a scientific member of IMIBIO, National Council of Science and Technology of Argentina (CONICET). Dr Gomez is globally recognized for researching the biochemistry of lung under different situations such as nutritional deficiency (Zn deficiency) or infections. Her main research contributions are in the area of lipid metabolism and oxidative stress in nutritional deficiency and toxic effect of heavy metals. She directs a research project that studies the effect of androgen deficiency. She has directed several Master theses and is directing doctoral theses at National University of San Luis, Argentina.