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Full Length Research Paper

Synergistic interaction of extracts of garlic (*Allium sativum*) and propolis against methicillin-resistant *Staphylococcus aureus*

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is a public health problem, being a cause of severe diseases in hospitals and communities in general. To confront this contingency at present, the effectiveness, in combination with diverse natural products is being studied in order to inhibit this microorganism. The objective of the present work was to evaluate the combined inhibitory effects of ethanolic extracts of garlic (*Allium sativum*) and propolis (Propolis –ppl-) against MRSA strains. We tested two types of extracts: at 20 and 30% for each. Microbial resistance assays were evaluated by the macrodilution method and the combinations were assessed by isobolographic studies. The study strains were divided into two groups based on their resistance to garlic as sensitive (36.8 ± 7.4 mg/mL) and resistant (67.2 ± 8.9 mg/mL). The results show that for strains catalogued as sensitive, the combinations in both concentrations suggest a synergistic effect; on the other hand, for strains catalogued as resistant, the combinations no longer presented a synergic effect.

Key words: Synergism, isobolographic analysis, methicillin-resistant *Staphylococcus aureus* (MRSA), garlic, propolis.

INTRODUCTION

Staphylococcus aureus is a medically important microorganism. For several years, it has been recognized as the main pathogenic agent of its genus for infections of community as well as hospital origin. *S. aureus* forms parts of the Micrococcaceae family, genus *Staphylococcus*, which comprises more than 30 different species, many of

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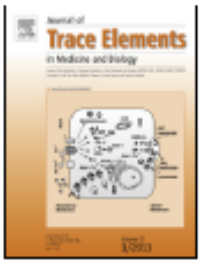

Title: Apoptosis in pancreatic β-cells is induced by arsenic and atorvastatin in Wistar rats with diabetes mellitus type 2

Authors: Tania Guadalupe Delgado-León, José Manuel Sálas-Pacheco, Fernando Vazquez-Alaniz, Ángel Antonio Vértiz-Hernández, Olga Dania López-Guzmán, Eduardo Lozano-Guzmán, Aurora Martínez-Romero, Norma Úrtiz-Estrada, Maribel Cervantes-Flores

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Research Article

Association between Urine Fluoride and Dental Fluorosis as a Toxicity Factor in a Rural Community in the State of San Luis Potosi

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Objective. The aim of this study is to investigate urine fluoride concentration as a toxicity factor in a rural community in the state of San Luis Potosi, Mexico. *Materials and Methods.* A sample of 111 children exposed to high concentrations of fluoride in drinking water (4.13 mg/L) was evaluated. Fluoride exposure was determined by measuring urine fluoride concentration using the potentiometric method with an ion selective electrode. The diagnosis of dental fluorosis was performed by clinical examination, and the severity of damage was determined using Dean's index and the Thylstrup-Fejerskov (TF) index. *Results.* The range of exposure in the study population, evaluated through the fluoride content in urine, was 1.1 to 5.9 mg/L, with a mean of 3.14 ± 1.09 mg/L. Dental fluorosis was present in all subjects, of which 95% had severe cases. Higher urine fluoride levels and greater degrees of severity occurred in older children. *Conclusions.* The results show that dental fluorosis was determined by the presence of fluoride exposure finding a high positive correlation between the severity of fluorosis and urine fluoride concentration and the years of exposure suggested a cumulative effect.

1. Introduction

There are contaminants in the environment in constant interaction with us that can affect our health through exposure to them. Drinking water can transmit numerous diseases caused by different pollutants; two of the most common chemicals in water that are capable of causing health problems are fluoride and arsenic. Fluoride (F^-) is a toxic agent that causes adverse health effects, such as dental and skeletal fluorosis, reproductive and neurological effects, and endocrine disorders [1, 2].

In 1936, it was shown that the increase of fluoride content in water causes dental fluorosis, which is an alteration of tooth enamel that can be observed as spots ranging from whitish to dark brown color and that, in severe cases, leads to the loss of tooth enamel [3]. Research suggests that fluoride affects enamel formation by making it porous. Skeletal fluorosis is a condition associated with the accumulation of fluoride in bone, resulting in brittle bones that are susceptible to tensile forces [4].

Furthermore, studies conducted in recent years suggest that fluoride is a neurotoxic agent, as research conducted in populations exposed to F^- (with water concentrations higher than 3 mg/L) supports the hypothesis that F^- decreases the intelligence quotient (IQ) of children [5, 6].

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Full Length Article

In utero exposure to fluoride and cognitive development delay in infants

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ABSTRACT

The objective of this study was to evaluate the association between *in utero* exposure to fluoride (F) and Mental and Psychomotor Development (MDI and PDI) evaluated through the Bayley Scale of Infant Development II (BSDI-II) in infants. The sample included 65 mother-infant pairs. Environmental exposure to F was quantified in tap and bottled water samples and F in maternal urine was the biological exposure indicator; samples were collected during the 1st, 2nd and 3rd trimester of pregnancy. The mean values of F in tap water for the 1st, 2nd and 3rd trimester were 2.6 ± 1.1 mg/l, 3.1 ± 1.1 mg/l and 3.7 ± 1.0 mg/l respectively; above to 80% of the samples exceeded the reference value of 1.5 mg/l (NOM-127-SSA1-1994). Regarding F in maternal urine, mean values were 1.9 ± 1.0 mg/l, 2.0 ± 1.1 mg/l and 2.7 ± 1.1 mg/l for the 1st, 2nd and 3rd trimester respectively. The infants with MDI and PDI scores less than 85 points were 38.5% and 20.9% respectively. After adjusting for potential confounding factors (gestational age, age of child, marginalization index and type of water for consumption), the MDI showed an inverse association with F levels in maternal urine for the first ($\beta = -19.05$, $p = 0.04$) and second trimester ($\beta = -19.34$, $p = 0.01$). Our data suggests that cognitive alterations in children born from exposed mothers to F could start in early prenatal stages of life.

1. Introduction

Fluorides are naturally-occurring components in rocks and soil and are also found in air, water, plants, and animals. The general population is exposed to fluoride (F) through the consumption of drinking water, foodstuff, and dental products. Populations living in areas with naturally high F levels in water and soil may be exposed to high levels of F in water, especially if drinking water is provided from wells (ATSDR, 2003; Vineet Dhar1, 2009). In the central and north areas of Mexico there are groundwater with elevated levels of F (Ortega-Guerrero, 2009). In this areas, almost 90% of the population has the practice of use tap water for food preparation and direct consumption as drinking water (Jarquín-Yañez et al., 2015). The bioavailability of F through ingestion 80–100% (ATSDR, 2003).

Epidemiological research conducted in school age children living in endemic hydrofluorosis areas have evaluated the influence of exposure of F on cognitive development assessed as intelligence quotient scores (IQ). Different intelligence tests have been used (RAVEN-Chinese version, Wechsler Intelligence Scales, Stanford-Binet Intelligence Scale) and have reported lower IQ points associated with F exposure at concentrations of 2.20–3.94 mg/l compared with residents from control areas (concentrations of F in water < 0.41 mg/l). The lack of biomarkers of exposure and control of potential confounders is an issue that has to be considered in these studies (Karimzade et al., 2014; Trivedi et al., 2012). Other well conducted research papers also reported that F decreases IQ scores (Ding et al., 2011; Rocha-Amador et al., 2007).

Cognitive development alterations associated with F exposure could start in early prenatal stages of life and come up later at school age; and likely continue into adulthood. Few studies have explored this hypothesis and the evidence is inconclusive. For

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MICRONUCLEUS IN EXFOLIATED BUCCAL CELLS OF CHILDREN FROM DURANGO, MEXICO, EXPOSED TO ARSENIC THROUGH DRINKING WATER

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Key words: genotoxicity, natural pollution, vulnerable population, environmental risk

ABSTRACT

Exposure to inorganic arsenic (As) via drinking water in Latin America and Mexico is a growing public health concern that requires rapid methods to assess risk and adverse effects. The measurement of micronucleus frequency (MNf) in oral mucosa is a non-invasive and low-cost method for monitoring the genotoxic damage in exposed populations. Mexican children from Durango city, a zone with high water arsenic concentrations, were selected and assigned to three-risk categories (low, medium, high) based on the use of tap or bottled water. Levels of As in water, urine and MNf, were measured. Results indicate a significant correlation between the groups at risk and the frequency of MNf in children (0.9 ± 1.9 MN, 1.1 ± 1.9, and 2.6 ± 2.2 per 1000 buccal cells, respectively); ($r = 0.416$; $p = 0.001$). Which demonstrated that the MNf in oral mucosa is an efficient and low-cost technique for assessing and monitoring DNA damage by exposure to As. This study also provides evidence that the modification of risk factors could reduce health risk.

Palabras clave: genotoxicidad, contaminación natural, población vulnerable, riesgos ambientales

RESUMEN

La exposición a arsénico (As) inorgánico a través del agua de bebida es un problema de salud pública en América Latina y en México que requiere métodos rápidos para evaluar riesgo y efectos adversos. La medición de la frecuencia de micronúcleos (MNf) en mucosa oral es un método no invasivo y de bajo costo para monitorear daño genotóxico en población expuesta a contaminantes ambientales. Para esta investigación,

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RESEARCH PAPER

Genetic variation in oxidative stress and DNA repair genes in a Mexican population

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Abstract

Background: Oxidative stress has been associated with several complex diseases. Effects generated as a result of oxidative stress may be modulated by various genes. Variation in these genes, particularly when located within coding or regulating regions, may be the primary cause of this modulation. The aim of this work was to determine the allelic and genotypic frequencies of *CAT C-262T*, *SOD3 Ala58Thr*, *APEX1 Asp148Glu*, *XPD Lys751Gln* and *XRCC3 Thr241Met* genetic markers in a northern Mexican population.

Subjects and methods: This study analysed 250 unrelated individuals by RT-PCR.

Results: A high allele mutant frequency was found in *SOD3 Ala58Thr* and *APEX1 Asp148Glu* genetic markers (0.395 and 0.38, respectively). A correspondence analysis showed that northern Mexicans are close to European populations. A linkage disequilibrium test between *XPD Lys751Gln* and *CAT C-262T* and *XPD Lys751Gln* and *SOD3 Ala58Thr* genetic markers was significant ($p = 0.000$).

Conclusion: The genetic markers described in this work will be a valuable resource for future functional studies in the northern Mexican population to explore comprehensively their role in the aetiology of human diseases. Furthermore, it will be necessary to replicate these studies in other regions of Mexico due to differences between Mexican sub-populations.

Keywords

DNA repair, genetic marker, oxidative stress

History

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Introduction

In normal conditions, a balance between oxidative damage and protective mechanisms is usually kept, but in some situations excessive production of free radicals or deficiencies in antioxidant defenses can generate oxidative stress (Collins, 2009). To avoid damage, antioxidant defenses have evolved to remove most of these oxidant agents. The principal reactive oxygen species (ROS) are superoxide anion ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$) and hydrogen peroxide (H_2O_2) (Miller et al., 2008).

An excess of ROS can produce harmful effects, such as peroxidation of the membrane lipids, aggression to tissue proteins and membranes or damage to DNA and enzymes. The foremost enzymes that directly eliminate ROS are superoxide dismutase (SOD) and catalase (CAT). SOD catalyses the dismutation of superoxide anion ($O_2^{\bullet -} + O_2^{\bullet -} + 2H^+ \rightarrow O_2 + H_2O_2$). Until now three isoforms of SOD have been identified: SOD1 (contains Cu and Zn as cofactors), SOD (has Mn in its reactive core) and SOD3 (has Cu and Zn in its catalytic center) (Fukui & Zhu, 2010). CAT is a heme enzyme that has a major role in controlling H_2O_2 concentrations in human cells by its decomposition to water (H_2O) and oxygen (O_2) (Valko et al., 2007).

The main effort of the mechanisms of damage repair induced by ROS is focused on the DNA molecule. Therefore, a variety of DNA repair processes such as base excision repair (BER), nucleotide excision repair (NER) and mismatch and double-strand break repairs have evolved to perform critical repair functions. *APEX*1 codifies one of the main enzymes in the BER pathway, which accounts for nearly all the basic site cleavage activity observed in cellular extracts (Chen et al., 1991). The *APEX*1 protein cleaves the phosphodiester backbone immediately at the 5′ of a basic site, via hydrolytic mechanism, in order to generate a single-strand DNA break leaving a 3′-hydroxyl and 5′-deoxyribose phosphate terminus (Demple & Harrison, 1994). The DNA repair Xeroderma Pigmentosum complementation group D (*XPD*) is essential in the NER pathway. This enzyme has a dual role: (i) uncoiling the double helix at the site of DNA lesions and (ii) transcription (Lehmann, 2001). The *XRCC*3 protein functions in the homologous DNA double strand break repair pathway

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Oxidative stress equilibrium during obstetric event in normal pregnancy

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Polymorphisms in the *GSTT1* and *GSTM1* genes are associated with increased risk of preeclampsia in the Mexican mestizo population

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ABSTRACT. Preeclampsia is a pregnancy-specific disorder in humans and a major cause of maternal and neonatal morbidity and mortality. Increasing evidence suggests that oxidative stress plays an important role in the pathogenesis of preeclampsia. The aim of this study was to investigate the relationship between null alleles of the glutathione S-transferases (GST) M1 and T1 genes and the risk of preeclampsia. This case-control study involved 112 preeclamptic and 233 normoevolutive pregnant women. The null polymorphisms were

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Lower Uric Acid Linked with Cognitive Dysfunction in the Elderly

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Oscar Arias-Carrión

Abstract: Uric acid has been associated as a risk factor for cardiovascular disease. Recently, however, there is growing evidence that uric acid plays a role as antioxidant in the brain. In cognitive dysfunction, vascular and oxidative stress mechanisms play a role, but the link remains unknown. Therefore, we investigated the link between serum uric acid-levels and cognitive function in 62 elderly subjects. The statistical analysis was adjusted to age, sex and cardiovascular risk factors. Here, we found that lower serum uric acid levels are linked to cognitive dysfunction. In a Mexican population, higher levels of uric acid are associated with a decreased risk of dementia.

Keywords: Cardiovascular risk factors, cognitive dysfunction, dementia, elderly, serum uric acid.

INTRODUCTION

The prevalence and incidence of dementia increase markedly with aging [1]. In Mexico, the prevalence of dementia has been estimated at between 6.1% [2] and 7.9% in those over the age of 60 [3], reaching a reported prevalence of 16.1% in the north of the country [4]. Current treatments to improve cognition or delay progression in dementia are only modestly effective [5]. The identification of modifiable risk factors for cognitive decline could reduce the incidence of dementia in the future.

Recent evidence indicates that oxidative stress is a relevant pathogenic factor in dementia [6-8]. In patients with Alzheimer's disease (AD) oxidative stress is elevated and accompanied by mild cognitive impairment (MCI), a clinical syndrome of deficient recent memory with minimal limitation of activities of daily living [6-9]. Uric acid is an endogenously produced water-soluble antioxidant that accounts for over half of the free radical scavenging activity in humans [10].

Over the past years, several studies were conducted to establish a correlation between serum uric acid levels and the risk for developing a neurological disease as as multiple sclerosis (MS), Parkinson disease (PD), and AD [9, 11]. So far, the role of uric acid in MS remains the area most investigated. In several different studies, a correlation between low serum uric acid levels and disease was demonstrated [11]. On the other hand, high levels of uric acid were associated with a reduced risk of incident for PD in two independent studies, the Health Professionals Follow-Up [12] and Rotterdam Studies [13]. In the same way, elevated uric acid levels have recently been found to be associated with slower disease progression in PD and other neurodegenerative diseases as Huntington disease and amyotrophic lateral sclerosis [14].

It is less clear whether uric acid is important for the development or progression of dementia [9, 15-17]. Recently, several studies have reported that higher levels of uric acid are linked with a diminished risk of dementia and cognitive function [18, 19]. Besides, cross-sectional studies of serum uric acid have identified lower concentrations in AD and MCI subjects compared to controls [9, 15], although another report found no difference [14]. Further, other authors have even suggested that elevations of uric acid could increase the risk of cognitive dysfunction in older subjects [20, 21]. These divergent findings have generated controversy on this hot topic.

Very few studies have examined the association between plasma uric acid levels and the presence of MCI, and none have reported the frequency of hypouricemia in demented patients. Here, we hypothesized that: 1) uric acid serum levels are reduced in subjects with cognitive impairment; 2) uric acid is correlates with rates of cognitive decline in elderly Mexican subjects.

EXPERIMENTAL PROCEDURES

Study Population

This transversal study was conducted from January to December 2013 in accordance with the Declaration of Helsinki - Ethical Principles for Medical Research Involving Human

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Original Article

Association of *COMT* G675A and *MTHFR* C677T polymorphisms with hypertensive disorders of pregnancy in Mexican mestizo population

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Polymorphisms

ABSTRACT

Objective: To investigate the relationship between *COMT* G675A and *MTHFR* C677T polymorphisms and hypertension disorders of pregnancy (HDP) in a Mexican mestizo population.

Design and methods: This case-control study involved 194 HDP and 194 normoevolutive pregnant women. The polymorphisms were genotyped by real time PCR.

Results: Our results showed that the *COMT* AA genotype increases the risk to HDP (OR: 2.67; 95% CI 1.33–5.35), preeclampsia (OR: 2.69; 95% CI 1.00–7.22) and gestational hypertension (OR: 3.87; 95% CI 1.25–12.0). Furthermore, the double mutant genotype (*COMT*AA/*MTHFR*TT) potency the risk to HDP more than two times (OR: 5.21; 95% CI 1.12–24.3, $p = 0.019$).

Conclusion: Our work provides evidence that *COMT* 675AA genotype is a risk factor for HDP and that this risk is increased by the presence of *MTHFR* 677TT genotype in a Mexican mestizo population.

Introduction

Hypertensive disorders of pregnancy (HDP) are common complications that affect 5–10% of all pregnancies [1]. The contribution of HDP to maternal mortality is well documented [1–3]. The classification scheme of HDP proposed by the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy includes preeclampsia/eclampsia, preeclampsia superimposed on chronic hypertension, gestational hypertension, and chronic hypertension [4]. Secular increases in chronic hypertension, gestational hypertension and preeclampsia (PE) [5] have occurred as a result of changes in maternal characteristics (such as maternal age and pre-pregnancy weight), whereas declines in eclampsia have followed widespread antenatal care and use of prophylactic treatments (such as magnesium sulfate) [6,7]. HDP are associated with higher rates of maternal, fetal and infant mortality and severe morbidity, especially in cases of severe PE, eclampsia with or without association of hemolysis, elevated liver enzymes and low platelet syndrome (HELLP) [8,9].

Etiology of HDP is complex and like other common complex disorders both genetic and environmental factors influence the risk of developing the disorders. Genetic factors are suggested to be responsible for >35% of the liability

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Relationship between Blood Lead Levels and Hematological Indices in Pregnant Women

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Article

Polymorphisms in DNA Repair Genes (*APEX1*, *XPD*, *XRCC1* and *XRCC3*) and Risk of Preeclampsia in a Mexican Mestizo Population

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Abstract: Variations in genes involved in DNA repair systems have been proposed as risk factors for the development of preeclampsia (PE). We conducted a case-control study to investigate the association of Human apurinic/apyrimidinic (AP) endonuclease (*APEX1*) Asp148Glu (rs1130409), Xeroderma Pigmentosum group D (*XPD*) Lys751Gln (rs13181), X-ray repair cross-complementing group 1 (*XRCC*) Arg399Gln (rs25487) and X-ray repair cross-complementing group 3 (*XRCC3*) Thr241Met (rs861539) polymorphisms with PE in a Mexican population. Samples of 202 cases and 350 controls were genotyped using RTPCR. Association analyses based on a χ^2 test and binary logistic regression were performed to determine the odds ratio (OR) and a 95% confidence interval (95% CI) for each polymorphism. The allelic frequencies of *APEX1* Asp148Glu polymorphism showed statistical significant differences between preeclamptic and normal women ($p = 0.036$). Although neither of the polymorphisms proved to be a risk factor for the disease, the *APEX1* Asp148Glu polymorphism showed a tendency of association (OR: 1.74,

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RESEARCH ARTICLE Open Access

Arsenic exposure and risk of preeclampsia in a Mexican mestizo population

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Abstract

Background: Exposure to arsenic in drinking water has been associated with various complications of pregnancy including fetal loss, low birth weight, anemia, gestational diabetes and spontaneous abortion. However, to date, there are no studies evaluating its possible association with preeclampsia.

Methods: This case–control study involved 104 preeclamptic and 202 healthy pregnant women. The concentrations of arsenic in drinking water and urine were measured using a Microwave Plasma-Atomic Emission Spectrometer.

Results: We found relatively low levels of arsenic in household tap water (range of 2.48–76.02 μg/L) and in the urine of the participants (7.1 μg/L vs 6.78 μg/L in cases and controls, respectively).

Conclusions: The analysis between groups showed for the first time that at these lower levels of exposure there is no association with preeclampsia.

Keywords: Preeclampsia, Arsenic, Drinking water

Background

Preeclampsia (PE) is a disorder peculiar to pregnancy and a major cause of maternal death and adverse fetal outcome [1]. In developing countries where access to health care is limited, PE is a leading cause of maternal mortality, with estimates of more than 60,000 maternal deaths per year [2] Although the exact pathophysiologic mechanisms of PE remain elusive, studies to date have implicated multiple processes, including the following: abnormal trophoblastic invasion, vasospasm, platelet activation, imbalance in the vasomotor-regulating factors and placental ischemia [3]. PE is characterized by increased oxidative stress due to the imbalance between lipid peroxidation and antioxidant defense mechanisms, leading to endothelial dysfunction and free radical mediated cell injury [4].

Arsenic-contaminated drinking water represents a major public health problem internationally [5–8]. The World Health Organization (WHO) and U.S. Environmental Protection Agency (EPA) standard for arsenic level in drinking water is 10 μg/L [9, 10]. Arsenic (As) is an established carcinogen and is also associated with a wide range of other chronic illnesses, such as diabetes, hypertension, and vascular diseases [11].

Oxidative stress has been identified as an important mechanism of As toxicity and carcinogenicity. In particular, As induces oxidative DNA damage and lipid peroxidation [12–16]. Oxidative stress and disrupted antioxidant systems have been shown to be involved in a wide range of pregnancy complications such as impaired fetal growth, PE, and miscarriage [17, 18].

Besides the generation of oxidative stress as a possible mechanism by which As may be associated with PE, Shin Le et al. reported that exposure to environmentally relevant concentrations of As (2.5 μM of AsNaO2) inhibit the migration of EVT cells (a human extravillous trophoblast cell line) in vitro, therefore, a similar mechanism may be occurring in vivo [19].

Several studies have been conducted to determine the association between chronic As exposure and adverse pregnancy outcome. Excess spontaneous abortion,

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ORIGINAL ARTICLE

IL-2 Expression and T lymphocyte Phenotyping in Young Children Suffering from Upper Respiratory Tract Infection with *Streptococcus Pyogenes*

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ABSTRACT

T cells are components of adaptive immunity and are involved in the resolution of respiratory infections, which are a major cause of morbidity and mortality in young children worldwide. Activation and differentiation of T cells is given mostly by the cytokine IL-2. This study aimed to determine the phenotype of T cells and IL-2 expression in children suffering from upper respiratory tract infection with *Streptococcus pyogenes* (*S. pyogenes*). For this purpose, IL-2 expression at its gene and protein levels and quantitation of CD4+ and CD8+ T lymphocytes were assessed in children aged 0-5 years old suffering from upper respiratory tract infection with *S. pyogenes* and healthy children of the same age. Children with *S. pyogenes* infection had a higher expression of IL-2 gene and a lower level of this cytokine expression at protein level than healthy children. The numbers of $CD4^+$ T lymphocytes were similar among the groups. In contrast, difference in the numbers of $CD8^+$ T lymphocytes among the groups was found. We conclude that infections by *S. pyogenes* in young children lead to an increased expression of IL-2 mRNA. (*Int J Biomed Sci* 2016; 12 (2): 53-57)

Keywords: IL-2 expression; phenotyping; T lymphocytes; *Streptococcus pyogenes*; infection

INTRODUCTION

T cells belong to the adaptive immune system and perform a wide range of functions in immune regulation, inflammation and protective immune response (1). The maturation of these cells is subjected to positive and negative selection to produce $CD4^+$ and $CD8^+$ lymphocytes. At maturation, lymphocytes leave the thymus, in that moment are considered naive cells until they are activated by signals, then they start to proliferate and differentiate into effector cells (helper and cytotoxic). The activation of na-

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Modulation of the immune response by infection with *Cryptosporidium* spp. in children with allergic diseases

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SUMMARY

It has been demonstrated that the allergic response can be ameliorated by the administration of pathogen derivatives that activate Toll-like receptors and induce a Th1-type immune response (IR). Cryptosporidium *is a parasite that promotes an IR via Toll-like receptors and elicits the production of Th1-type cytokines, which limit cryptosporidiosis. The aim of this study was to investigate allergy-related immune markers in children naturally infected with* Cryptosporidium. *In a cross-sectional study, 49 children with or without clinical diagnosis of allergies, oocysts of* Cryptosporidium *spp. in the faeces were screened microscopically. We microscopically screened for leucocytes, examined T and B cells for allergy-related activation markers using flow cytometry and evaluated serum for total IgE using chemiluminescence. Children with allergies and* Cryptosporidium *in the faeces had significantly lower levels of total IgE, B cells, $CD19^+CD23^+$ and $CD19^+CD124^+$ cells as well as a greater percentage of interferon-gamma ($IFN\text{-}\gamma^+$) and $IL\text{-}4^+$ $CD4^+$ cells than children with allergies without* Cryptosporidium. *This is the first description of the modulation of the IR in children with allergic diseases in the setting of natural* Cryptosporidium *infection. Our findings suggest the involvement of $CD4^+$ cells producing IL-4 and IFN-γ in the IR to* Cryptosporidium *in naturally infected children.*

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Keywords *allergy, children,* Cryptosporidium, *interferon-gamma, IgE, interleukin-4, Th1, Th2*

INTRODUCTION

Allergic disease is the result of an immunological reaction regulated by T helper (Th) cells with a pattern of Th2 activation. Th2 cells release interleukin (IL)-4 and IL-13 (1), which are required for the production of IgE by B cells (2). Additionally, IL-4 inhibits the generation of Th1 cells by decreasing the production of cytokines such as IL-2, interferon-gamma (IFN-γ) and IL-12. In sensitized individuals, exposure to an allergen results in a cross-linking of allergen to IgE-bound FcεRI on the surface of mast cells. As a consequence of allergen-IgE cross-linking, mast cells release inflammatory mediators responsible for the allergic symptomatology (3). In the last phase, the Th2 inflammatory response is characterized by the recruitment of eosinophils, neutrophils, basophils and activated $CD4^+$ T cells, which are associated with the severity of a reaction and a reduction in the quality of life in allergic individuals (4–7).

The role of exposure to nonpathogens, pathogens or their derivatives in the control of the allergic response has been previously studied. Studies on the efficacy of live bacteria and bacterial extracts for the treatment of allergic diseases suggest the participation of IL-10-producing B cells and Treg cells (8, 9). In some experimental models and in children with allergies, suppression of the allergic response has been observed through the administration of whole cell inactivated mycobacterial vaccines, including administration of *Mycobacterium vaccae*, the live attenuated strain of *Mycobacterium bovis* (bacillus Calmette-Guérin, BCG) (10–12) or lipopolysaccharide, via the activation of Toll-like receptors capable of inducing

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Diagnosis of Caprine Brucellosis by Serology and Multiple PCR

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Abstract: *Our aim was to perform the diagnosis of caprine brucellosis by serology and multiple Polymerase Chain Reactions (PCR). The field work was conducted on the Ejidos la Victoria MunicipioTlahualilo Public Lands of San José de Bellavista y Bermejillo, Mapimi Municipality, state of Durango, México. Meetings were held with the producers to explain to them the objectives and benefits to be obtained with the carrying out of this investigation. Samples were taken from 114 native breed animals crossed with Saanen and Alpine breed animals from the jugular vein, obtaining 114 blood samples in tubes without anticoagulant for the obtention of serum in order to process the Rose Bengal Plate Test (RBPT) and 114 whole blood samples for processing multiple PCR. We carried out DNA extraction of control strains of Brucella abortus RB51 and Brucella melitensis RM1 employing the phenol:chloroform:isoamyl alcohol method with the oligonucleotide sequence of Brucella genus, Brucella abortus, Brucella melitensis, Brucella suis, and IS711. General seroprevalence was 26.31% and seroprevalence by Tlahualilo Municipality was 41.86%, while this in Mapimi was 18.18%. In the multiple PCR sample analysis, we found that 30 samples corresponded to B. melitensis, obtaining 100% sensitivity and specificity. The PCR technique described in this study presented 100% sensitivity and specificity with the RBPT, allowing for the simultaneous identification, between and genus and species, the implementation of the multiple-PCR variant capable of identifying different species of the Brucella genus, the latter leading to a better diagnosis of the disease.*

Keywords: *Brucella melitensis, goats, sensitivity, specificity.*

I. Introduction

Brucellosis is cataloged as a bacterial zooonosis found worldwide whose genus is *Brucella* and that consists of multiple species [1]. Its incidence ranges from between 1.3 and 70.0 cases per 100,000 inhabitants, differences due to the characteristics of each nation. Mexico is one of the countries with the greatest incidence of human brucellosis in Latin America, causing economic loses generated in domestic cattle-raising and its impact on public health [2]. The highest brucellosis incidence rates were found in bovines, followed by caprines and ovines. The genus *Brucella* includes three important species for human pathology: *Brucella melitensis*, which preferentially affects goats, but that can affect bovines and pigs. In Mexico, the highest incidence of bovine brucellosis is observed in stabled livestock and in high-animal-density areas, such as the central, southeastern, and coastal zones. Caprine brucellosis possesses a wider distribution with greatest frequency registered in entities with high goat concentrations [3].

At present, the Mexican Ministry of Health carries out educative health activities, informing the population of the public health problem represented by the consumption of non-pasteurized lactic products and contact with the meat of animals suffering from brucellosis. Preventive measures for professional risk include the following: consume pasteurized milk, subproducts, and derivatives of these, rejecting those of doubtful origin; limit close cohabitation with animals; wash hands with soap and water before eating and after contact with animal or subproducts and waste; cleanliness, disinfection, and separation by means of fences in places for the raising/breeding of livestock (caprine, bovine, and porcine); identification and elimination of sick animals and vaccination prior to 3 months of age; timely stimulation of medical care and treatment termination; motivation of the medical and paramedical area to conduct patient follow-up at 30, 60, 90, and 120 days, and maintain surveillance of blood donors being brucellosis-negative [4].

Maximal focus cases have been reported of up to 18.3% of brucellosis in humans, caused by the ingestion of caprine- and ovine-origin lactic products in which *B. abortus* and *B. melitensis* were involved.

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Full Length Research Paper

Standardization of the method to obtain therapeutic-quality platelet-rich plasma

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Platelet-rich plasma (PRP) is a reliable source for obtaining cells to regenerate tissues, with ease of availability inorder to implement and standardize the ideal methodology in centrifugation strength and time for obtaining therapeutic-quality PRP, allowing its application to provide better and rapid recovery of muscular injuries, tendinitis, bone and ligament lesions. To evaluate PRP therapy, 150 patients with muscular lesions, tendinitis, shoulder, knee, ankle, hand and elbow injuries were treated. On application of PRP, we obtained 100% clinically significant symptomatic improvement in all 150 patients treated, who had musculoskeletal and ligament injuries, with a marked reduction of pain and inflammation. We concluded that the ideal concentration for obtaining PRP is at 1000 rpm with a time of 5 min; in addition, under these conditions the plasma lacks leukocytes and erythrocytes. The results were reproducible because the experiment was repeated at two institutions under the same conditions and similar results were obtained. The regeneration obtained in the affected patients is due to the fact that growth factors were released from the activated platelets; these initiate and modulate cicatrization in the tissues, which is a recent innovation to promote cicatrization, accelerating the power of tissue regeneration, with a platelet concentrate suspended in plasma.

Key words: Growth factors, platelet activation, application, tissue regeneration, therapeutic quality.

INTRODUCTION

Platelet-rich plasma (PRP) is a reliable source for obtaining cells to regenerate tissues, with ease of availability. In short term clinical practice, it is utilized to concentrate growth factor-rich plasma (GFRP) by up to 388% above values found in normal plasma, for later application in tissues, in a search to enhance the osteo-induction biological cascade. The pharmaceutical way in which PRP is utilized clinically is obtained by means of its gelling on adding thrombin and $CaCl_2$ to it. PRP gel is a compound of fibrinogen and activated platelets (by the addition of

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Scientific Research and Essays

Full Length Research Paper

Regulation of cytokine gene expression during *Brucella abortus* infection

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Toll-like receptors (TLR) play a key role in antimicrobial host defense. Bacterial cell wall components and lipopolysaccharide (LPS) are recognized by macrophages via TLR, resulting in activation of professional antigen-presenting cells, initiation of acquired immune responses and further elimination of the invasive bacteria. TLR2 and TLR4 have been shown to recognize bacterial components. TLR2 is required for signaling by numerous ligands from gram-negative and gram-positive bacteria such as lipoteichoic acids, peptidoglycan and lipoproteins. In contrast, TLR4 fails to confer responsiveness to gram-positive bacteria and their components, but it is the main LPS signaling receptor. LPS is a major constituent of the outer membrane of gram-negative bacteria, such as *Brucella*, and is known to activate neutrophils, monocytes, macrophages, and other cell types to up-regulate expression of adhesion molecules and produce a number of pro- and anti-inflammatory cytokines. This study demonstrates that the attenuated strain *Brucella abortus* RB51 can stimulate cells through TLR4 and MyD88, resulting in NF-κB activation. The virulent strain *B. abortus* 2308 can also stimulate the cells by a MyD88-dependent pathway without involving either TLR4 or TLR2. It also induced NF-κB activation and nuclear translocation, suggesting that *B. abortus* RB51 induces activation of the proinflammatory response by a TLR4-dependent pathway with the subsequent NF-κB activation and nuclear translocation; nevertheless, the 2308 strain induced NF-κB nuclear translocation that was activated by an alternative pathway, different from that induced by TLR.

Key words: *Brucella abortus*, RB51, TLR, NF-κB, transduction signals, cytokines.

INTRODUCTION

Brucellosis is a major zoonotic disease that causes a serious health and economic problem worldwide. In spite of the growing number of countries declared Brucella-free, the disease remains one of the main zoonotic infections throughout many parts of the world with major economical and public health implications. About 500,000 new cases occur annually worldwide with predominance in the Middle East, Mediterranean countries, South

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SIP1/NHERF2 enhances estrogen receptor alpha transactivation in breast cancer cells

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ABSTRACT

The estrogen receptor alpha (ERα) is a ligand-activated transcription factor that possesses two activating domains designated AF-1 and AF-2 that mediate its transcriptional activity. The role of AF-2 is to recruit coregulator protein complexes capable of modifying chromatin condensation status. In contrast, the mechanism responsible for the ligand-independent AF-1 activity and for its synergistic functional interaction with AF-2 is unclear. In this study, we have identified the protein Na+/H+ Exchanger RegulatoryFactor 2 (NHERF2) as an ERα-associated coactivator that interacts predominantly with the AF-1 domain of the nuclear receptor. Overexpression of NHERF2 in breast cancer MCF7 cells produced an increase in ERα transactivation. Interestingly, the presence of SRC-1 in NHERF2 stably overexpressing MCF7 cells produced a synergistic increase in ERα activity. We show further that NHERF2 interacts with ERα and SRC-1 in the promoter region of ERα target genes. The binding of NHERF2 to ERα in MCF7 cells increased cell proliferation and the ability of MCF7 cells to form tumors in a mouse model. We analyzed the expression of NHERF2 in breast cancer tumors finding a 2- to 17-fold increase in its mRNA levels in 50% of the tumor samples compared to normal breast tissue. These results indicate that NHERF2 is a coactivator of ERα that may participate in the development of estrogen-dependent breast cancer tumors.

INTRODUCTION

The hormone estrogen (17β-estradiol, E2) has a key role in cell proliferation and differentiation. The effects of E2 have been widely analyzed in human mammary gland where it is responsible for normal epithelial growth and for the development of 70–80% of human breast cancer tumors (1). The biological effects of E2 on mammary epithelium are mediated by the estrogen receptor α (ERα), a ligand-activated transcription factor. Structurally, ERα is organized in functionally independent domains that include an N-terminal domain, a DNA-binding domain, formed by two cysteine-rich zinc-finger motifs, and a C-terminal ligand-binding domain (LBD) (2). ERα transactivation is mediated by two transcriptional activating domains, designated AF-1 and AF-2. AF-1 is located at the N-terminal region of ERα and is characterized by a ligand-independent transcriptional activity (3,4). AF-2 is located within the LBD domain of ERα and its transcriptional activity shows a strong ligand-dependency.

Structural and functional studies have shown that ligand binding induces a major conformational change in the LBD domain of ERα. The structural rearrangement creates a new docking interphase that allows AF-2 to interact

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Holocarboxylase synthetase acts as a biotin-independent transcriptional repressor interacting with HDAC1, HDAC2 and HDAC7

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ABSTRACT

In human cells, HCS catalyzes the biotinylation of biotin-dependent carboxylases and mediates the transcriptional control of genes involved in biotin metabolism through the activation of a cGMP-dependent signal transduction pathway. HCS also targets to the cell nucleus in association with lamin-B suggesting additional gene regulatory functions. Studies from our laboratory in *Drosophila melanogaster* showed that nuclear HCS is associated with heterochromatin bands enriched with the transcriptionally repressive mark histone 3 trimethylated at lysine 9. Further, HCS was shown to be recruited to the core promoter of the transcriptionally inactive hsp70 gene suggesting that it may participate in the repression of gene expression, although the mechanism involved remained elusive. In this work, we expressed HCS as a fusion protein with the DNA-binding domain of GAL4 to evaluate its effect on the transcription of a luciferase reporter gene. We show that HCS possesses transcriptional repressor activity in HepG2 cells. The transcriptional function of HCS was shown by in vitro pull down and in vivo co-immunoprecipitation assays to depend on its interaction with the histone deacetylases HDAC1, HDAC2 and HDAC7. We show further that HCS interaction with HDACs and its function in transcriptional repression is not affected by mutations impairing its biotin-ligase activity. We propose that nuclear HCS mediates events of transcriptional repression through a biotin-independent mechanism that involves its interaction with chromatin-modifying protein complexes that include histone deacetylases.

1. Introduction

In human cells, the enzyme holocarboxylase synthetase (HCS) catalyzes the covalent attachment of the vitamin biotin to five biotin-dependent carboxylases [1–3]. Carboxylase biotinylation occurs as a two-step, ATP-dependent, reaction that generates 5′-biotinyl-AMP as an intermediary product [2]. The relevance of HCS in maintaining metabolic homeostasis was first recognized through the study of the genetic disorder multiple carboxylase deficiency (MCD). MCD patients express a HCS protein with reduced affinity for biotin, with the Km of the mutant enzyme elevated up to 70 times over the normal enzyme [4,5]. The resulting disruption in gluconeogenesis, amino acid catabolism and fatty acid metabolism leads affected individuals to develop life treating ketoacidosis and organic acidemia that requires life-long pharmacological doses of biotin to be resolved [1,2].

Our laboratory demonstrated that HCS also participates in the transcriptional regulation of genes involved in biotin transport and metabolism [6].

Mechanistically, 5′-biotinyl-AMP activates a signal transduction cascade involving soluble guanylate cyclase (sGC) and cGMP-dependent protein kinase (PKG) [6–8]. The HCS–cGMP–PKG pathway has a seemingly paradoxical role because under conditions of biotin deficiency it reduces the expression of genes required for biotin utilization in the liver, kidney and muscle but not in the brain [7]. We proposed that this mechanism of regulation is aimed at sparing the essential function of biotin in the brain at the expense of the liver and other organs during biotin deprivation [7]. In cells from MCD patients, we found a reduced expression of genes involved in biotin utilization suggesting that some of the biochemical manifestations of the disease may be associated with down-regulation of biotin utilization in the liver because of the impaired activity of HCS.

In addition to its roles in carboxylase activation and transcriptional expression, HCS has also been found localized in the nucleus of human cells in association with the nuclear lamina [9]. Several studies have proposed that nuclear HCS could affect gene expression through histone biotinylation and modification of chromatin structure [10–13]. However, the participation of biotin in the post-translational modification of

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EGF Regulates Claudin-2 and -4 Expression Through Src and STAT3 in MDCK Cells

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Epidermal Growth Factor (EGF) is a key regulator of epithelial paracellular permeability, a property that depends on tight junctions (TJ) and can be evaluated through the measurement of the transepithelial electrical resistance (TER). EGF increases the TER of MDCK monolayers by inducing ERK1/2-dependent downregulation of claudin-2 (CLDN-2) and upregulation of claudin-4 (CLDN-4). Because either increments or decrements in TER often involve Src activation and epithelial cell differentiation occasionally depends on STAT3, here we investigated whether EGF might control CLDN-2 downregulation and CLDN-4 upregulation through those proteins. We found that EGF induces Src activation necessary for the reduction of CLDN-2 at the TJ, the degradation of this CLDN, the reduction of the cellular levels of its mRNA and the resulting increase of TER. EGF-induced changes on CLDN-2 protein and mRNA also depend on STAT3 activity. This growth factor increases the levels of STAT3 phosphorylated at Y705 in the nucleus, a process that depends on Src activation. Interestingly, Src and STAT3 activation do not exclusively mediate the EGF-induced downregulation of CLDN-2, but they are also implicated in the EGF-induced CLDN-4 transcription, translation, and exocytic fusion into TJ. Our results indicate that EGF controls the levels of CLDN-2 and -4 proteins and mRNAs through Src and STAT3 activity.

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Epithelia cover all superior animal surfaces and selectively interchange matter and energy between the internal milieu and the environment (Cereijido and Anderson, 2001). This interchange partly consists of the transport of substances across epithelial cells either through the transcellular pathway or between these cells, using the paracellular pathway governed by tight junctions (TJ) (Cereijido et al., 1991). The expression of a particular set of TJ proteins, especially those belonging to the claudin (CLDN) family, confers upon the paracellular pathway a specific permeability and selectivity that can be evaluated via transepithelial electrical resistance (TER) (Cereijido et al., 1978). In the case of the renal system, the epithelia that line the tubular surface gradually increase their TER from approximately 10 Ω cm^2 at the proximal tubule (Boulpaep and Seely, 1971; Lutz et al., 1973) to several thousand Ω cm^2 at the collecting duct (Helman et al., 1971; Rau and Frömter, 1974) and up to hundreds of thousands of Ω cm^2 at the bladder (Lewis et al., 1977; Lavelle et al., 2000). The resistance gradient results from a number of epithelial cell adaptations, including reduced cell size and junctional membrane tortuosity, a progressive increase in the number of TJ strands and the expression of a specific set of CLDNs in each nephron segment. While the fluids that bathe apical membranes, such as urine, semen, and milk, are radically distinct from each other, the interstitial milieu that contacts the basolateral membranes maintains a remarkably constant composition due to the actions of powerful homeostatic mechanisms. This difference suggests that substances in the apical media might regulate specific epithelial properties. We sought to determine whether substances in the extracellular milieu might also induce TER changes and identified several factors in canine urine capable of affecting the TER of Madin Darby canine kidney cells (MDCK) (Gallardo et al., 2002). We identified one of these as the EGF (Flores-Benitez et al., 2007), a substance previously known to increase the TER of epithelial LLC-PK1 cells (Mullin et al., 2000). Urinary EGF reduces the

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ORIGINAL PAPER

Hyper-response to Novelty Increases c-Fos Expression in the Hippocampus and Prefrontal Cortex in a Rat Model of Schizophrenia

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Abstract
Schizophrenia is a debilitating disorder that may have a neurodevelopmental origin. For this reason, animal models based on neonatal insults or manipulations have been extensively used to demonstrate schizophrenia-related behaviors. Among those, the neonatal ventral hippocampus lesion (nVHL) is largely used as a model of schizophrenia-related behavior as it mimics behavioral and neurochemical abnormalities often seen in schizophrenic patients including hyperlocomotion in a novel environment. To investigate the neuroanatomical basis of coding novelty in the nVHL rat, we assessed the behavioral locomotor activity paradigm in a novel environment and measured expression of c-Fos, a marker of neural activation, in brain regions involved in the process of coding novelty or locomotion. Upon reaching adulthood, nVHL rats showed hyperlocomotion in the novel environment paradigm. Moreover, in nVHL rats the expression of c-Fos was greater in the prefrontal cortex (PFC) and CA1 region of the dorsal hippocampus compared to sham rats. Whereas similar expression of c-Fos was observed in the basolateral amygdala, nucleus accumbens and dentate gyrus region of hippocampus of nVHL and sham rats. These results suggest that the nVHL disrupts the neural activity in the PFC and CA1 region of hippocampus in the process of coding novelty in the rat.

Keywords Animal model · CA1 region of hippocampus · c-Fos · Novel environment · Prefrontal cortex · Schizophrenia

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Introduction

Schizophrenia is a devastating neurodevelopmental disorder affecting 1% of the population worldwide [1]. Importantly, schizophrenia symptoms start in early adulthood during the time when synapses are pruned [2]. This mental disorder induces a constellations of symptoms often referred as positive, negative, as well as cognitive deficits [1]. The neural mechanisms underlying schizophrenia are however not fully known, and available pharmacological treatments are often ineffective to restore physiological functionality in the schizophrenic patients [2]. Therefore, the use of animal models of schizophrenia-related behavior are thus needed to elucidate the pathophysiology of this disorder.

Animal models represent powerful tools to explore the pathology of schizophrenia as well as to screen novel treatments [2, 3]. Among the numerous animal models of schizophrenia-related behavior, the neonatal ventral hippocampus lesion (nVHL) is widely used as it mimics numerous

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RESEARCH ARTICLE

WILEY Molecular Carcinogenesis

ATP2A3 gene as an important player for resveratrol anticancer activity in breast cancer cells

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The Ca^{2+}-ATPases from the Sarco/endoplasmic reticulum (SERCA) are fundamental for maintaining intracellular [Ca^{2+}] homeostasis by pumping Ca^{2+} into the endoplasmic reticulum (ER) of eukaryotic cells. SERCA enzymes are encoded by three different genes (*ATP2A1-3*), whose expression occurs in a tissue and development stage-specific manner. It has been reported alterations in the expression of SERCA2 and SERCA3 pumps in different types of cancer: oral, lung, colon, stomach, central nervous system, thyroid, breast, and prostate. Resveratrol (RSV), a phytoalexin produced by a wide variety of plants in response to stress situations can modulate cellular processes involved in all stages of carcinogenesis. In this work, we used breast cancer cell lines (MCF-7 and MDA-MB-231) to evaluate mRNA levels of *ATP2A2* and *ATP2A3* genes in response to RSV treatment. Our results demonstrate that RSV treatment induced the expression of *ATP2A3* gene in both cell lines in a time and concentration-dependent manner, while the expression of *ATP2A2* gene remained unaffected. The RSV-induced expression of SERCA3 in these breast cancer cell lines produced decreased cell viability, triggered apoptosis and changes in cytosolic Ca^{2+} levels, as well as changes in the capacity for Ca^{2+} release by the ER. These data suggest an important participation of SERCA3 genes in RSV-mediated anti-tumor effect in breast cancer cell lines. Nevertheless, further research is needed to elucidate the molecular mechanisms underlying this effect.

KEYWORDS
apoptosis, breast cancer cells, calcium, resveratrol, SERCA

1 | INTRODUCTION

The calcium ion concentration ([Ca^{2+}]) is an intracellular signal that operates with versatility in the regulation of several processes such as differentiation, growth, and cell death.[1] Alterations in the expression or function of components of the [Ca^{2+}] homeostasis machinery have been implicated in several diseases, compromising the normal cellular function.[2] An important component of this machinery are the Sarco/Endoplasmic Reticulum Ca^{2+} ATPase (SERCA) pumps. Ca^{2+} is actively accumulated in the endoplasmic reticulum (ER) by SERCA enzymes. SERCA-dependent Ca^{2+} transport is the only mechanism for Ca^{2+} uptake in ER so that regulation of SERCA expression and activity constitutes an essential path to maintain Ca^{2+} homeostasis in the different cell types and states of differentiation.[3] Deregulated expression of specific Ca^{2+} channels and pumps are characteristic features of some cancers;[3] understanding the role of [Ca^{2+}] in controlling cell proliferation, and death might provide opportunities for therapeutic intervention.[4]

The SERCA enzymes are encoded by three different genes (*ATP2A1-3*), whose expression occurs in a tissue and development stage-specific manner, and with different biochemical features such as Ca^{2+} affinity or transport speed.[5] SERCA1 isoforms (a and b) are expressed exclusively in fast twitch skeletal muscle[6]; SERCA2 has three isoforms (a–c) that are expressed in slow skeletal muscle, heart, smooth muscle, and hematopoietic cells [7]. SERCA2b is the only isoform of SERCA expressed in all tissues.[7] SERCA3 has six isoforms (a–f), they are expressed in endothelial and epithelial

Abbreviations: ER, endoplasmic reticulum; ERec, estrogen receptor; RSV, resveratrol; SERCA, Sarco/Endoplasmic Reticulum Ca^{2+}-ATPase Pump.

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Tristetraprolin Represses Estrogen Receptor α Transactivation in Breast Cancer Cells*

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Background: Estrogen receptor α (ERα) mediates the effects of 17β-estradiol in mammary gland, and it is associated with the development of breast cancer tumors.
Results: Tristetraprolin (TTP) represses ERα transactivation through its interaction with histone deacetylases.
Conclusion: TTP acts as a novel ERα corepressor.
Significance: TTP reduces estradiol-induced cell proliferation and tumor growth, suggesting it may be important in breast cancer development.

Estrogen receptor α (ERα) mediates the effects of 17β-estradiol (E2) in normal mammary gland, and it is a key participant in breast cancer tumor development. ERα transactivation activity is mediated by the synergistic interaction of two domains designated AF1 and AF2. The function of AF2 is to recruit coactivator and corepressor proteins that allow ERα to oscillate between the roles of transcriptional activator and repressor. In contrast, the mechanism responsible for AF-1 transcriptional activity is not completely understood. In this study, we identified tristetraproline (TTP) as a novel ERα-associated protein. TTP expression in MCF7 cells repressed ERα transactivation and reduced MCF7 cell proliferation and the ability of the cells to form tumors in a mouse model. We show that TTP transcriptional activity is mediated through its recruitment to the promoter region of ERα target genes and its interaction with histone deacetylases, in particular with HDAC1. TTP expression attenuates the coactivating activity of SRC-1, suggesting that exchange between TTP and other coactivators may play an important role in fine-tuning ERα transactivation. These results indicate that TTP acts as a *bona fide* ERα corepressor and suggest that this protein may be a contributing factor in the development of E2-dependent tumors in breast cancer.

The estrogen receptor α (ERα)[4] is a ligand-activated transcription factor that mediates the effects of the hormone estrogen (17β-estradiol; E2) on cell proliferation and differentiation in normal mammary gland. However, ERα is also associated with tumor development and progression in 70–80% of breast cancer patients (1). ERα is a 595-amino acid protein characterized by the presence of functionally independent domains that include a DNA-binding domain (DBD), a ligand-binding domain, and two transactivation domains designated AF1 and AF2, respectively (2). AF1 is located at the N-terminal region of ERα, and its transcriptional activity is constitutive. In contrast, AF2 is located at the C-terminal region of the nuclear receptor and exhibits ligand-dependent transcriptional activity (3, 4). Both domains mediate ERα transactivation activity through the synergistic interaction of their unique functional properties.

The mechanism responsible for ERα transactivation is best understood in the context of AF2. This transactivation domain acts as a docking interphase for the recruitment of coactivator or corepressor protein complexes. Mechanistically, the binding of E2 induces a major structural rearrangement in ERα, enabling the ligand-binding domain/AF2 domain to interact with multiple coactivator proteins (5, 6). Coactivators, such as SRC-1, SRC-3, and CBP/p300, possess histone acetyltransferase activity, which enhances transcriptional activation through the relaxation of chromatin structure (7, 8). In the absence of E2, AF2 interacts with repressor protein complexes that include NCoR and SMRT and histone deacetylases (HDACs) (9, 10) that promote chromatin condensation (11, 12). The exchange of coactivators and corepressors constitutes the basis of a sophisticated regulatory mechanism that fine-tunes ERα transactivation activity and allows this transcription factor to oscillate between the roles of activator and repressor of gene expression (13).

In contrast, the mechanism responsible for the E2-independent transcriptional activity of AF1 is not clearly understood. Molecular and functional studies have shown that phosphorylation of several serine residues in AF1, in particular serine 118, plays a key role in the cross-talk between the steroid and growth factor-dependent signal transduction pathways (14, 15). This process is thought to play an important function in the coordinated regulation of multiple genes by mitogenic stimuli in hor-

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[4] The abbreviations used are: ERα, estrogen receptor α; E2, estradiol; DBD, DNA-binding domain; HDAC, histone deacetylase; TTP, tristetraprolin; UAS, upstream activating sequence; TSA, trichostatin A; ERE, estrogen response element(s).

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BMC Physiology

RESEARCH ARTICLE Open Access

Androgen receptor is expressed in mouse cardiomyocytes at prenatal and early postnatal developmental stages

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Abstract

Background: Previous studies show that androgens are involved in hypertrophy and excitability of cardiomyocytes and that their effects are mediated through their receptor. The aim of this study was to evaluate the presence of androgen receptor (AR) in mouse heart during prenatal and early postnatal stages.

Results: The expression of AR and related genes, alpha myosin heavy chain -Myh6-, beta myosin heavy chain -Myh7- and atrial natriuretic factor –Nppa- was simultaneously evaluated by semiquantitative RT-PCR. AR was also detected by immunohistochemistry. Androgen receptor mRNA was detected in hearts from 10.5 days post coitum to 16 postnatal days. A higher expression of AR mRNA in atria compared to ventricles was observed in neonatal mouse. A positive correlation between mRNA levels of AR and Nppa was observed in mouse heart at early postnatal development. Androgen receptor expression is similar in males and females during cardiac development. Finally, androgen receptor protein was observed by immunohistochemistry in myocardial cells of atria and ventricles from 12.5 days onwards and restricted after 16.5 days post-coitum to nuclei of cardiomyocytes.

Conclusion: Present results provide evidence that androgen receptor is expressed from prenatal stages in mouse heart, supporting the proposition that androgens could be involved in mammalian heart development.

Keywords: Androgen receptor, Mouse embryo, Cardiac myocytes, Atrial natriuretic factor, Heart development

Background

The involvement of androgens in gender-related cardiovascular diseases [1] explains the interest for the study of the role of sexual steroids on cardiac myocytes. There are sex-related differences in mRNA expression of alpha- and beta-myosin heavy chains (MHC) and other functional proteins in rat myocardium [2]. The MHC composition changes in ventricular myocytes of castrated rats and it is restored by testosterone treatment [3]. Moreover, androgens influence the expression of genes regulating intracellular calcium and contractile performance of ventricular myocytes in postnatal rats [4]. A sex-related difference in the cardiac response to atrial natriuretic peptide has been described in spontaneously hypertensive rats. On the other hand, atrial natriuretic peptide is differentially expressed between atria and ventricles in the human heart and has been related to cardiac hypertrophy and remodeling [5–7].

The presence of androgen receptor in embryonic heart would be important to indicate a role of androgens in prenatal cardiac development. The aim of this work was to determine the expression of androgen receptor simultaneously with the expression of alpha and beta myosin heavy chain genes (Myh6 and Myh7) and atrial natriuretic peptide gene (Nppa) at prenatal and early postnatal stages of mouse heart development. The presence of mRNA and the protein of the androgen receptor was observed in the nuclei of cardiac myocytes from embryonic stages and a positive correlation between AR and Nppa mRNA's was registered at 2 and 9 postnatal days.

Methods

Animals

CD1 mice were caged with food and water ad libitum under a 12-h light/12-h dark cycle in a room with a

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Expression of Human Chloride Channels ClC1 or ClC2 Revert the Petite Phenotype of a *Saccharomyces cerevisiae* GEF1 Mutant

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Abstract

The mechanism of activation of the yeast ClC chloride channel/transporter *GEF1* is unknown, and in this study we tested the ability of human ClC1 and ClC2, two channels with different activation kinetics, to revert the *petite* phenotype of a strain whose *GEF1* gene was deleted. We found that when the human channels are expressed in a low-copy plasmid, the reversion of the phenotype does not occur; in contrast, when the channels are over expressed by means of a strong transcriptional promoter in a multiple-copy plasmid, the cells reach the normal size, and show a normal membrane surface and oxygen consumption. To determine the size variationsof individual cells, we employed flow-cytometry as a quantitative tool to evaluate the *petite* phenotype.

These results suggest that the human ClC channels, when abundantly present in the cells, can support the metabolism disrupted in the knock-out strain. We also observed that the fluorescence emitted by GFP-tagged channels was found mostly towards the periphery of the *wt* yeast, whereas in the *GEF1* knock-out it was detected in intracellular clusters. GFP-tagged channels expressed in *X. laevis* oocytes produced robust currents and did not show any evident difference with respect to the normal ClCs, whereas Gep1p did not show voltage-dependent activation.

Keywords: chloride channel, functional complementation, voltage-clamp, *Xenopus laevis* oocytes

1. Introduction

Chloride channels/transporters (ClCs) are members of a large family present in a wide variety of organisms from bacteria to higher eukaryotes. ClCs carry out multiple physiological roles, from plasma membrane and cell volume modulation to the control of vesicular pH (Fahlke, 2001; Jentsch, Stein, Winreich & Zdebik, 2002; Sardini et al., 2003; Soleimani & Xu, 2006; Jentsch, 2008). A clear example of this functional diversification is illustrated by comparing the properties of mammalian ClC1 and ClC2. They are both located in the plasma membrane; however, whereas ClC1 is activated by plasma membrane depolarization and thus is responsible for the repolarization current in muscle fibers, ClC2 is activated by hyperpolarization, as well as by other mechanisms such as changes in pH and cell volume (Conte, De Luca, Mannini, & Vrbovà, 1989; Steinmeyer, Ortland, & Jentsch, 1991; Klocke, Steinmeyer, Jentsch, & Jockusch, 1994; Jordt & Jentsch, 1997).

The mechanism of activation of the *Saccharomyces cerevisiae* Gef1p, the sole ClC found in this species of yeast, is still not clearly understood. Gef1p plays a critical role in yeast iron metabolism and is found mainly in the *trans*-Golgi (Greene, Brown, DiDomenico, Kaplan & Eide, 1993; Schwappach, Stobrawa, Hechenberger, Steinmeyer & Jentsch, 1998). Mutations of the GEF1 gene lead to an iron requirement for growth on non-fermentable carbon sources due to a failure to load copper onto the iron uptake system; thus, knocking down the expression of GEF1 leads to *petite* (*pet*) colonies when grown in these conditions (Gaxiola et al., 1998). Gef1p forms a Cl^- transporter/channel in the plasma membrane of the yeast that does not show

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ION CHANNELS, RECEPTORS AND TRANSPORTERS

Anion permeation in calcium-activated chloride channels formed by TMEM16A from *Xenopus tropicalis*

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Abstract Calcium-activated chloride channels (CaCC) formed by anoctamin1/TMEM16A subunits are ubiquitously expressed, and these channels are known to prevent polyspermy in amphibian oocytes. Here, we describe a TMEM16A clone isolated from *Xenopus tropicalis* oocytes (xtTMEM16A) and how the anion permeation properties are modified in single-site mutants of the ion pore. The anion permeability sequence was $SCN^->I^->Br^->Cl^->$gluconate (relative permeabilities 5.6:3.0:2.1:1:0.2, respectively). Dose–response curves indicated that the voltage-dependent half-maximal concentration for Ca^{2+} activation (K_d of the Hill equation at +100 mV) was 120 nM in normal external Cl^-, whereas it was displaced leftward to 75 nM Ca^{2+}, when I^- replaced Cl^-. The I^-:Cl^- mole fraction (MF) of the external solution was varied in order to gain insight into the permeation mechanism of the pore. No anomaly in MF behavior was observed for conductance, but it was observed for current reversal potential, which deviated from the prediction of the Goldman–Hodgkin–Katz equation. Mutations of positively charged amino acids in the pore, R646 and R761, to glutamate resulted in reduction of the relative permeability to I^-. Data from the wild type and mutants could be well fitted by a three-barrier, two-site permeation model. This suggests a multi-ion pore with at least two binding sites for anions, with R646 mole fraction closer to the extracellular membrane surface—being important for the stability of both sites—and R761—located deeper within the membrane—mainly affecting the innermost binding site. Considerations of xtTMEM16A putative pore region topology are discussed in the light of two alternative topological models of the protein.

Keywords Anion channel · Anoctamin1 · Anomalous mole fraction effect · Permeation

Introduction

Calcium-activated chloride channels (CaCCs) were initially described in *Xenopus laevis* oocytes, in which a transient outward current is activated upon stepping the plasma membrane to about +25 mV [3, 15]. CaCCs are ubiquitously expressed and play important roles in several processes such as transepithelial Cl^- secretion, gastrointestinal motility, and neural signaling, among others [1, 2, 12, 14]. TMEM16A has been postulated as the molecular correlate of CaCCs [5, 19, 26], since this channel presents the same biophysical and pharmacological properties as native CaCCs, e.g., anion permeability ($NO_3^->I^->Br^->Cl^->F^-$) and inhibition by niflumic acid, NPPB, and DIDS [19, 26]. TMEM16A interacts with calmodulin through a conserved intracellular site located in the N-terminal region; this site—a regulatory calmodulin-Ca^{2+} motif or RCBM—serves as the Ca^{2+} sensor regulating the activation of the channel [24].

No high-resolution structure of this channel has yet been obtained, but biochemical evidence suggests that the channel assembles as a dimer [8, 21, 23], and each subunit is thought to have eight transmembrane domains (TMs) and intracellular NH2 and COOH termini. A re-entrant loop between the fifth

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Study of permeation and blocker binding in TMEM16A calcium-activated chloride channels

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Keywords: calcium-activated chloride channels, ion permeation, pore, pore blockers, TMEM16A, *Xenopus tropicalis* A-9-C, anthracene-9-carboxylic acid, DIDS, 4,4'-Diisothiocyano-2,2'-stilbenedisulfonic acid

We studied the effects of mutations of positively charged amino acid residues in the pore of *X. tropicalis* TMEM16A calcium-activated chloride channels: K613E, K628E, K630E; R646E and R761E. The activation and deactivation kinetics were not affected, and only K613E showed a lower current density. K628E and R761E affect anion selectivity without affecting Na^+ permeation, whereas K613E, R646E and the double mutant K613E + R646E affect anion selectivity and permeability to Na^+. Furthermore, altered blockade by the chloride channel blockers anthracene-9-carboxylic acid (A-9-C), 4, 4'-Diisothiocyano-2,2'-stilbenedisulfonic acid (DIDS) and T16inh-A01 was observed. These results suggest the existence of 2 binding sites for anions within the pore at electrical distances of 0.3 and 0.5. These sites are also relevant for anion permeation and blockade.

Introduction

Calcium-activated chloride channels (CaCC) are relevant for a variety of processes, such as fluid secretion, prevention of polyspermy in amphibian oocytes, and regulation of synaptic transmission, vascular smooth muscle tone, and gastrointestinal motility.[1] At least some members of the TMEM16 (TMEM16A-K) protein family-particularly TMEM16A and TMEM16B-are known to form CaCC.[2] Functional channels have been generated from purified TMEM16 proteins by means of liposome reconstitution, indicating that no additional subunits are required.[3,4] Although the crystal structure of a TMEM16 protein has been reported, the structure of the pore of these channels is still enigmatic.[5]

In this context, we continued our study of residues which could be important for the determination of the properties of the pore of *Xenopus tropicalis* TMEM16A (xtTMEM16A). We have previously reported[6] that positively charged residues R646 and R761 are important for ion permeation. Further examination suggested that residues in the TM5 transmembrane domain should also contribute significantly to the properties of the pore. In fact, the crystal structure indicates that TM5 forms part of the protein surface inside the 'subunit cavity', which includes residues previously related to channel function.[5]

Thus, we worked with mutants R646E, R761E, the double mutant (R646E+R761E) and the newly generated mutants in TM5 (namely, K613E, K628E and K630E), and we characterized the permeation properties of these mutants. In addition, we studied whether the mutations affected blocker interactions within the pore, based on the assumption that the blockers may act as organic anions, which might interact-much like inorganic anions do-with positively charged residues in the permeation pathway of the pore.[7]

The results of our present study further confirm and extend the view of a pore with 2 anion-binding sites, at electrical distances of 0.3 and 0.5, where blockers bind, thus interfering with anion permeation. The different phenotypes associated with the various mutations point to a complex anion permeation pathway.

Results

Most single-site mutations do not affect current amplitude or kinetics

We investigated whether the tested mutations affected current amplitude or kinetics. As shown in Figure 1, with the exception of K613E, no differences in comparison to the WT were observed among the mutants. In the case of K613E, a modest, yet significant reduction in current amplitude was observed, which was not accompanied by a change in current kinetics.

Mutations resulting in compromised anion selectivity in favor of increased sodium permeability

In TMEM16A proteins from other species, mutations in residues homologous to R646 (R621 in mouse; Yang et al.[8]) and to

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Glutathione release through connexin hemichannels: Implications for chemical modification of pores permeable to large molecules

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Cysteine-scanning mutagenesis combined with thiol reagent modification is a powerful method with which to define the pore-lining elements of channels and the changes in structure that accompany channel gating. Using the *Xenopus laevis* oocyte expression system and two-electrode voltage clamp, we performed cysteine-scanning mutagenesis of several pore-lining residues of connexin 26 (Cx26) hemichannels, followed by chemical modification using a methanethiosulfonate (MTS) reagent, to help identify the position of the gate. Unexpectedly, we observed that the effect of MTS modification on the currents was reversed within minutes of washout. Such a reversal should not occur unless reducing agents, which can break the disulfide thiol–MTS linkage, have access to the site of modification. Given the permeability to large metabolites of connexin channels, we tested whether cytosolic glutathione (GSH), the primary cell reducing agent, was reaching the modified sites through the connexin pore. Inhibition of gamma-glutamylcysteine synthetase by buthionine sulfoximine decreased the cytosolic GSH concentration in *Xenopus* oocytes and reduced reversibility of MTS modification, as did acute treatment with tert-butyl hydroperoxide, which oxidizes GSH. Cysteine modification based on thioether linkages (e.g., maleimides) cannot be reversed by reducing agents and did not reverse with washout. Using reconstituted hemichannels in a liposome-based transport-specific fractionation assay, we confirmed that homomeric Cx26 and Cx32 and heteromeric Cx26/Cx32 are permeable to GSH and other endogenous reductants. These results show that, for wide pores, accessibility of cytosolic reductants can lead to reversal of MTS-based thiol modifications. This potential for reversibility of thiol modification applies to on-cell accessibility studies of connexin channels and other channels that are permeable to large molecules, such as pannexin, CALHM, and VRAC.

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INTRODUCTION

Cysteine substitution followed by thiol modification has long been used to explore the structure and topology of ion channels and transporters. Recently, wider structural insights have been achieved by combining chemical modification with data from crystal structures. New thiol derivatives have provided novel resources to determine near-neighbor interactions, including salt bridges and hydrophobic bonds, state-dependent accessibility via cross-linking that traps specific conformational changes, protein dynamics via fluorescent derivatives, etc. (Karlin and Akabas, 1998; Newell and Czajkowski, 2007; Zhu and Casey, 2007; Gandhi and Olcese, 2009). These data provide experimental constraints that can be used in molecular modeling of ion channels and transporters, including those for which little is known structurally because of the lack of high resolution structures. The chemical modification strategy can serve to test, validate, and refine the models provided by crystal structures in specific conformational states.

The most commonly used thiol reagents are those derived from MTS. The MTS reaction with thiols is reversible upon treatment with reducing agents such as DTT and TCEP, a property that serves as an experimental control to show specificity of the modification. Ideally, to assess MTS accessibility to the channel pore, one would like to use the inside-out or outside-out patch configuration to avoid modification of other cellular targets that could affect interpretation of the results. However, for many ion channels, the excised patch configuration is problematic because of rundown and instability over the time course of the experiments. Chemical modification studies can be profitably performed in whole cells (e.g., *Xenopus laevis* oocytes) for channels and transporters where excised patches are not feasible, including P2X, STIM, and Hv1 channels (Li et al., 2008; Gonzalez et al., 2010; Allsopp et al., 2011; Kawate et al., 2011; Amcheslavsky

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Abbreviations used in this paper: BSO, L-buthionine-S,R-sulfoximine; Cx26, connexin 26; GSH, glutathione; maleimide ES, *N*-2-sulfoethyl maleimide; MTSES, 2-sulfonatoethyl MTS; rGSH, reduced GSH; $TBHO_2$, tert-Butyl hydroperoxide; TSF, transport-specific fractionation.

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