

## Studying Of The Biological Effects Of Stannous Chloride On The Cell Membrane

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**Abstract:** In nuclear medicine, the process of labeling of cells and molecules with Technetium-99m almost always requires the use of a reducing agent, since the eluate obtained in the generator as pertechnetate ion is not easily connect to other chemical species. Since the use of products containing stannous chloride is increasingly growing by humans, has become a stimulating attempt to better understand the biological effects of salt. The analysis suggests that the salt of tin presents an action-oxidant which could enable the production of free radicals which could alter the structural properties of the plasma membrane. [New York Science Journal 2010;3(7):70-76]. (ISSN: 1554-0200).

**Key words:** red blood cells, rats, stannous chloride, free radicals.

### Introduction

The use of stannous ion was the key to the development of many radiopharmaceuticals. None showed a reducing agent, so far, an efficiency of marking the radioactive tracer superior to that obtained with the use of stannous chloride, thereby justifying his preference, not only in nuclear medicine, but also the marking of various structures of biomedical interest (RAO et al, 1986; SAHA, 1998; BERNARDO-FILHO, 1999).

One of the features of biological importance of SnCl<sub>2</sub> is its ability to form cationic organ metallic compounds of high lipid solubility, enabling them to cross biological membranes and exert its toxic effects within the cells (DANTAS et al, 1996).

All blood cells are formed in the bone marrow, and the process is known as hemopoiesis. The cells are basically involved in the transport of O<sub>2</sub> and CO<sub>2</sub> and work exclusively within the vascular system. The leukocytes are a part of the immune system, and thus act mainly out of blood vessels, i.e., in tissues. Once the leukocytes circulating in the blood are found only in transit between its various places of activity. Platelets play a vital role in controlling the bleeding (homeostasis) through the

buffering of changes in the walls of blood vessels and contribute to the activation of the cascade of blood coagulation (COMARCK et al, 1991; JUNQUEIRA & CARNEIRO, 2004).

The plasma membrane of red blood cells and composed of a double lipid layer that incorporates several globular proteins in accordance with the pattern of the fluid mosaic structure of the membrane. A network of cytoskeleton proteins form a set of underlying plasma membrane by one or more proteins to them incorporated. The membranes of red blood cells have different proteins, which are of two types: (i) protein members, which are firmly fixed in bicameral lipid, and (ii) extrinsic as proteins, which are non-covalently linked to members of the membrane proteins. Members of the membrane protein of red blood cells, the glycoforin (name derived from Greek, meaning "carrier of sugar"), seems only play a structural role, although it is responsible for determining the blood group (ALBERTS et al, 1996; STRYER et al, 2004).

Thorium-232 ((<sup>232</sup>Th), a natural radionuclide from the actinide family, is abundantly present in monazite and other ores. It is used as one of the prime fuel materials in nuclear industry and

may pose an exposure risk to nuclear workers and members of the public. Scanning electron micrographs showed that erythrocytes transformed into echinocytes and/or spherocytes after  $(^{232}\text{Th})$  treatment. Further examination of erythrocyte by atomic force microscopy suggested significant increase in surface roughness after  $(^{232}\text{Th})$  treatment. Experiments on neuraminidase treated and/or anti-GpA antibody blocked erythrocytes suggested significant role of membrane sialic acid and glycophorin A (GpA) protein in aggregation or hemolytic effects of  $(^{232}\text{Th})$ . Further results showed that  $(^{232}\text{Th})$  caused hemolysis by colloid osmotic mechanism, as evidenced by potassium efflux, osmotic protection and osmotic fragility studies. Osmoprotection experiments indicated that hemolysis get elicited through the formation of membrane pores of approximately 2.0 nm in size. Hemolysis studies in presence of inhibitors (TEA, bumetanide, DIDS and amiloride) revealed the role of  $\text{K}(+)$  channel,  $\text{Na}(+)/\text{K}(+)/2\text{Cl}(-)$  channel,  $\text{Cl}(-)/\text{HCO}_3(-)$  anion exchanger and  $\text{Na}(+)/\text{H}(+)$  antiporter in  $(^{232}\text{Th})$  induced erythrolysis. Presence of non-diffusible cation (N-methyl D-glucosamine) or anion (gluconate) in erythrocyte suspending medium further confirm the role of  $\text{Na}(+)$  and  $\text{Cl}(-)$  influx in hemolytic effect of  $(^{232}\text{Th})$ . These findings provide significant insight in structural, biochemical and osmotic toxic effects of  $(^{232}\text{Th})$  on human erythrocytes. (KUMAR et al., 2010)

Abali et al (2010) showed that CPF (Chronic exposure to chlorpyrifos) caused significant increase in erythrocyte fragility and MDA (malonaldehyde) concentration, which were ameliorated by pretreatment with vitamin C. In conclusion, the study showed that CPF-evoked erythrocyte fragility due to increased lipoperoxidative changes was ameliorated by pretreatment with vitamin C.

The labeling of red cells labeled with  $\text{Tc-99m}$  is a procedure among the various cellular structures that are labeled with this radionuclide (SAMPSON, 1996; BERNARDO-FILHO, 1999).

In studies of the molecular mechanisms of lesions in DNA caused by stannous chloride, it was found that the lethal effect of this salt could be mediated by the generation of free radicals (RL) (DANTAS et al, 1996). It was reported that the effect of chloride must depend on the presence of functional restoration mechanism (BERNARDO-FILHO et al, 1994; BERNARDO-FILHO, 1999).

The stannous chloride is known to inhibit the immune response in rats by changing the gene expression and induce the generation of tumor in the thyroid gland. There is no agreement on broader issues in relation to its genotoxicity and was discussed whether the effects of salt could depend on

the physicochemical conditions and the manner of its administration. This salt is directly administered in humans, intravenous, when it is used as a reducing agent to prepare radiobiocomplexes labeled with technetium-99m (SAHA, 1997).

### Aims

Studying the effect of stannous chloride in the osmotic fragility of red blood cells isolated from blood samples isolated by cardiac puncture from rats treated with 6.0 mg / mL of  $\text{SnCl}_2$ .

To evaluate the effect of stannous chloride in the osmotic fragility of red blood cells isolated from blood samples isolated by cardiac puncture from rats treated with 60 mg / mL of  $\text{SnCl}_2$ .

To analyze the effect of stannous chloride in the osmotic fragility of red blood cells isolated from blood samples isolated by cardiac puncture from rats treated with 600 mg / mL of  $\text{SnCl}_2$ .

### Relevance and Justifying

Since the use of products containing stannous chloride by the population, level of nuclear medicine, food and industry as a whole is increasing, and reported behavior of neurological diseases and cancer in organic level related to the effects of the salt, it is stimulating better understand the real effects of this molecule, therefore, decided to study the biological effects of stannous chloride in the osmotic fragility of red blood cells isolated from blood samples of rats which were obtained by cardiac puncture.

### Material and Methods

#### Collection of samples

The blood was collected from rats anesthetized with ether, in a glass bell, for 2 to 3 minutes. Samples of 5 mL of blood will be withdrawn by cardiac puncture in syringes with approximately 0.2 mL of anticoagulant (heparin). Following this procedure the rats are separated by their complete recovery. During the period of 7 days the animals will not be manipulated again. This conduct is important to prevent infection and reduce the stress and suffering of animals. Thus, you can perform various experiments replacing animals without sacrifice them.

The stannous chloride used in this experiment was the Reagen S. A., Brazil.

#### Concentrations of stannous chloride

The diluent for the preparation of solutions of stannous chloride was the saline solution ( $\text{NaCl}$  0.9%). The concentrations of stannous g / mL, chosen chloride used were those of: 0.6, 6.0, 60 and 600 according to the effects reported in the literature-

based whose results were reported to experiment with different concentrations of  $\text{SnCl}_2$  (BERNARDO-FILHO, 1988) and in accordance with the biological effects described above (DANTAS et al, 1996).

Sample of blood (0.5 mL) obtained by cardiac puncture of rats were added to 0.5 mL of saline solution (0.9% NaCl) and centrifuged. This procedure was performed twice. It was collected the pellet of red cells in 0.5 mL of NaCl and incubated with 0.5 mL of NaCl incubated with 0.5 mL of different concentrations of  $\text{SnCl}_2$  (0.6, 6.0, 60, 600 mg / mL). In the control incubation tube of blood was performed with 0.5 mL of NaCl (0.9%).

#### Osmotic fragility test

Tubes containing 0.5 mL of NaCl in various concentrations (0.9, 0.75, 0.65, 0.55, 0.50, 0.45, 0.40, 0.30, 0.20, 0, 10%) were incubated with 0.5

mL of samples obtained from each tube with their np incubated as explained above.

The tubes were centrifuged, the supernatants were read in spectrophotometer ( $\lambda = 545\text{nm}$ ) and the rate of lysis was determined (% L). The tube containing 0.1% NaCl was considered 100% lysis and containing 0.9% NaCl was used as control for the reaction. The curve was constructed by plotting the L% versus the corresponding concentration of NaCl.

#### Results

Blood samples were incubated with different concentrations of stannous chloride and then aliquots were incubated with the same different concentrations of NaCl. The reading of the L% was determined by spectrophotometer and the values were plotted curves as shown in the table above.

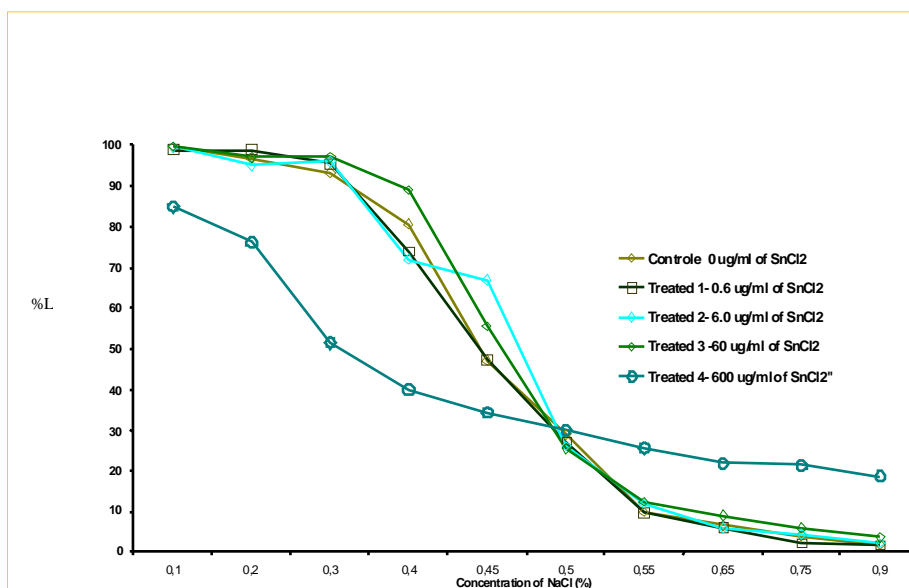


Figure 1 - Curves of osmotic fragility of red blood cells of rats.

#### Discussion

The use of products containing tin has grown over the years, soon become an extremely important in a more scientific evaluation of the biological effects of products consumed. Since it was reported that the metals have desirable and undesirable effects, it was decided whether the stannous chloride salt would be able to interfere with the cellular ultrastructure of red cells by assessing the osmotic fragility. As reported in the literature, the marking of blood constituents with Tc-99m, as sodium pertechnetate, depends on the presence of a reducing agent and stannous chloride is widely used. The

determination of optimal concentration of stannous chloride is a predominant factor in the technique of marking with Tc-99m (RAO et al, 1986; HLADIK III, SAHA & STUDY, 1987). In the case of red blood cells, they capture the extra means of stannous ion cell. When the ion concentration is low, it must be incorporated by virtually all cells. Thus, in the maximum concentration of reducing agent, the cells have the highest percentages of marking, possibly due to the "completion" of sites to link up the molecules of hemoglobin. After treatment of cells with high concentrations of stannous chloride, the system that controls the flow of this ion is saturated

and is not even able to capture this form of ion undefined, which causes the increase of staff in the extracellular environment. Be added to the Tc-99m as pertechnetate ion, this would have to cross this barrier of reducing agent, which prevented from reaching the red blood cells, thus causing a low efficiency of marking (BERNARDO-FILHO, 1988).

High throughput methodologies that measure the distribution of osmotic fragilities in red blood cell populations have enabled the investigation of dynamic changes in red cell homeostasis and membrane permeability in health and disease. The common assumption in the interpretation of dynamic changes in osmotic fragility curves is that left or right shifts reflect a decreased or increased hydration state of the cells, respectively, allowing direct inferences on membrane transport from osmotic fragility measurements. However, the assumed correlation between shifts in osmotic fragility and hydration state has never been directly explored, and may prove invalid in certain conditions. It was investigated whether this correlation holds for red cells exposed to elevated intracellular calcium. The results showed that elevated cell calcium causes a progressive increase in osmotic fragility with minimal contribution from cell hydration (<8%). Loss of membrane area by the release of 160+/-40nm diameter (mean+/-SD) vesicles is shown to be a major contributor, but may not account for the full non-hydration component. The rest must reflect a specific calcium-induced lytic vulnerability of the membrane causing rupture before the cells attain their maximal spherical volumes. (CUEFF et al., 2010).

The mechanism of transport of ions to the intracellular environment has not yet been fully established, but the evidence suggests that the chloride must cross the plasma membrane by selective calcium channels (GUTFILEN, BOASQUEVISQUE & BERNARDO-FILHO, 1992; SAMPSON, 1996) and pertechnetate by ion transport system "anion-Band 3" (CALLAHAN & RABITO, 1990, SAMPSON, 1996).

The presence of certain drugs in the blood could change% of the radioactivity of Tc-99m linked to the blood because these elements could act: (a) competing with SnCl<sub>2</sub> or with Tc-99m (b) changing the permeability of cell membrane favoring or blocking the mechanism of transport of these elements, (c) occupying sites of binding of Tc-99m or preventing SnCl<sub>2</sub> they occupy the (d) facilitating the connection Tc-99m to plasma proteins or (e) as a reducing agent or oxidizing agent changing the valence of stannous ions and / or pertechnetate (HLADIK III, SAHA & STUDY, 1987, SANTOS et al, 1995).

In this study it was observed that the stannous

chloride in relation to the results obtained in the analysis with the osmotic fragility of red blood cells treated with 600µg/mL salt that showed a decrease of hemolytic in hypotonic medium (0.1 to 0.6% NaCl), although, it was observed that the% L increased significantly in isotonic medium (0.75 to 0.90% NaCl).

The red blood cells and one of the most studied biological structures. It is much more about it in the member red blood cells than on any other membrane of eukaryotic cells. The easy availability and ease of storage of red blood cells make them ideal object to search for anyone who can make use of a microscope of good quality (STRYER, 2004).

The change of morphology of red blood cells induced by the action of various drugs and effects (Vidal et al, 1998; OLIVEIRA et al, 2000; DIRE et al, 2002 b; OLIVEIRA et al, 2002; OLIVEIRA et al, 2003 a) and the possible consequent change the transport of ions and pertechnetate and stannous into the red blood cells could cause such diverse effects such as level radiopharmacological a decrease in the marking of this structure with Tc-99m.

There are many evidences that have shown that the shape of the cell depends on the structural organization of the membrane proteins and proteins adsorbed to the surface (STRYER, 2004). Thus, one can suggest that the salt here has tested the effect of changing the morphology of red blood cells. Furthermore, in vitro studies with natural extracts such as *Nicotiana tabacum* (tobacco) (VIDAL et al, 1998), *Maytenus ilicifolia* (holy thorn) (OLIVEIRA et al, 2000), *Paullinia cupana* (guarana) (OLIVEIRA et al, 2002), *Ginkgo biloba* (MORENO et al, 2004), *Fucus vesiculosus* (OLIVEIRA et al, 2003a), *Coffea arabica* (OLIVEIRA et al, 2003b) demonstrated a link between the change in labeling of red blood cells with Tc-99m and changes in quality in the morphology of red blood cells. As reported by SILVA et al (2002) the salts of tin can induce the generation of reactive oxygen species which could alter the properties of the plasma membrane causing structural and metabolic changes. To analyze the results can be suggested that the salt of tin study, stannous chloride, in its highest concentration was able to induce changes in the architecture of the plasma membrane and its ultrastructure in making it more susceptible to osmotic lysis, probably this could be related to the generation of free radicals on the properties of reducing salt.

## Conclusion

The analysis suggests that the salt of tin presents an action-oxydante which could enable the production of free radicals which could alter the structural properties of the plasma membrane making

it more susceptible to osmotic lysis in isotonic medium. Possibly, this optimizes metabolic changes, since the transport of ions by membrane, and other elements, would be compromised for the loss of stability of the membrane, thus making it more unstable in the cell level structural.

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