Scientific Papers. Series D. Animal Science. Vol. LXII, No. 2, 2019
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

# PROTON TRANSVERSE RELAXATION TIMES OF FREE AND BOUND WATER IN RAT LIVER AND RED BLOOD CELLS FED ON CHOLESTEROL REACH DIET. THE EFFECT OF TREATMENT WITH EUTROPHIC MEDICATION

**Flory REVNIC[1], Cristian Romeo REVNIC[1,2], Silviu VOINEA[1]**

[1]"Ana Aslan" National Institute of Gerontology and Geriatrics, Bucharest, Romania
[2]Ambroise Pare` Hospital, Pierre et Marie Curie University, Paris VI, France

Corresponding author email: f_ revnic@yahoo.com

***Abstract***

*Our study done on experimental hypertensive old rats model using reach cholesterol diet associated or not with rejuvenating drugs (Aslavital/procaine was designed to investigate biophysical parameters such as the proton transverse relaxing times of intracellular free and bound water in liver tissue and of membrane permeability to water by 1H NMR method..Biological material: 32 male white Wistar rats aged 24 months old were divided into 4 groups of 8 rats each. Group A (control) group (B) received only reach cholesterol diet for 6 weeks without associated eutrophic substances. Group (C) which received high cholesterol diet associated with Aslavital treatment for 6 weeks 4mg/kg body weight intraperitoneal injections(ip). Group (D) has received associated treatment with Procain 4mg/kg body weight in order to test the antiatherosclerotic properties of this rejuvenation drug. In liver tissue, bound water , characerised by structural parameter* $T_{21}$ *presents a slight decrease în treated groups, comparatively with Control group, while free water characterised by structural parameter* $T_{22}$*, decreases following cholesterol administration and increases under Aslavital and Procaine treatment, in the case of Procaine this effect is more powerfull above the control values. In the case of intraerythrocyte water there are very subtile changes , but which remind of those which take place in the liver. As a mirrror reflexion of the behaviour previosly described , the proton trannsverse relaxing time of plasma water increases slightly in cholesterol treated rats and decreases under the actions of administrated drugs ,with a more pronounced effect under Procaine treatment. Conclusion: Liver hypertophy is associated with a decrease in proton transverse relaxing times as well as in the proportion of free water($m_2$%), associated with the decrease in proton transverse relaxing times as a consequence of the increase in protein content of liver tissue.The global decrease of proton transverse relaxation time ($T_2$) in liver is mainly due to changes in the free and bound water ratio and of a decrease in proton transverse relaxing times of free water(T22) and bound water(T21). The effects of Procaine are much more increased. In general, Aslavital is behaving like a buffered Procaine with a more adequate action. There were cases in which both drugs had the same effects:for example a decrease in proton transverse relaxing time* $T_{22}$*, characteristic of bound water structural parameter, or did not manifest any influence upon proton transverse relaxation time* $T_{21}$ *charateristic for free water structural parameter from liver.*

***Key words****: cholesterol, rejuvenating drugs, liver, blood cells.*

## INTRODUCTION

The recent data upon atherosclerosis origin have initiated a strong debate regarding the preponderant role of hypercholesterolemia in the onset of this disease in counterpart with the idea that atherosclerosis could have its origin in an inadequate immune response due to presence of vascular alterations. Despite these data an impressive amount of experimental research have shown that atherogenesis is initiated under the reciprocal influence between cholesterol, cytokine cellular secretion (IL-6 especially), appolipoprotein E and the arterial wall (Balta, 2009). Recent data have shown that cells posses two types of sensors for cholesterol: Ck receptors sensitive for extracellular cholesterol which initiate the signalling pathway responsible for gene regulations implicated in the cell cycle, cell death and homeostasis of cell cholesterol level and cytokine including IL-6 and LxR alfa receptors sensitive to intracellular oxysterols and control genes implicated in cell death, cellular cholesterol homeostasis and cytokine IL-8 (Balta, 2009).

The understanding of membrane permeability mechanisms to water and of changes in intracellular water structure will might improve the actual view about various diseases in which

water transport is directly involved or the medication influences the cellular water state (Balta, 2009). Such aspects are well revealed by the most modern nuclear magnetic resonance (NMR) techniques (Gatina et al., 1998; Petcu et al., 1995).

Water crosses cell membranes by two routes: by diffusion through the lipid bilayer and through water channels (aquaporins) (Benga, 2012).

They were termed initially as major intrinsec proteins (MIPs) but now are also known as water channels, glycerol facilitators and aquaglyceroporins, yet recent studies suggest that they facilitate the movement of other low molecular –weight metabolites as well (Zhang et al., 2007). AQP-1is found in erythrocyte membranes, in epitelia and its expression was recently confirmed in the arterial wall and in capillary endothelia in the smooth muscle vascular cells and in the atherosclerotic plaques (Shanahan et al., 2000).

Taking into account this distribution it might be supposed that vascular cells and erythrocyte membrane permeability too water is well correlated; they being modulated by the same AQP-1, controlled by the same circulating factors. The role of arginine vasopressin and atrial natriuretic peptide in aquaporine regulation of water channel activity (Schrier et al., 2001).

These aspects facilitate evaluation of cardiovascular status by NMR relaxometric measurements of blood erythrocytes. This is a most suitable technique for studies of erythrocyte membrane permeability in physiological and pathological states such as arterial hypertension experimentally induced by feeding rats on reach cholesterol diet. Erythrocyte membrane has the capacity to renew during its life span (120 days) and imagistic is a useful tool to evidence modifications in water permeability and the results may contribute to a better understanding of aging process as well as pathological mechanisms of arterial hypertension (Stoian et al., 2012; Marin et al., 2018).

## OBJECTIVE

Our study done on experimental hypertensive old rats model using reach cholesterol diet associated or not with rejuvenating drugs (Aslavital/Procaine was designed to investigate biophysical parameters such as the proton transverse relaxing times of intracellular free and bound water in liver tissue and of proton transverse relaxation times of intraerythrocyte and plasma water by 1H Nuclear Magnetic Resonance (1H NMR) method.

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## MATERIALS AND METHODS

### Biological material

Our study has been done on: 32male White Wistar rats aged 24 months old divided into 4 groups of 8 rats each: group A control, group B fed for 6 weeks on reach cholesterol diet only, group (C) and (D) received along with reach cholesterol diet also rejuvenation drugs treatment as follows: group (C) treated with Aslavital injections (4 mg/kg body weight intraperitoneal (IP); Aslavital contains in its composition; Procaine chlorhydrate, glutamic acid (as activator factor) and benzoic acid (as an antiaterogenic factor). This drug has a regenerative eutrophic, antiatherogenic (lipotrop) action and regulates fat metabolism and cholesterol levels which is used for prophylactic and curative treatment of cerebral and cardiovascular aging (Aslan, 1962; MacFarlane, 1975) restoration of the deformability of 'irreversibly' Sickled Cells by Procaine Hydrochloride.Group (D) –which received along with reach cholesterol diet for 6 weeks tretament with Procaine injections Procaine solution 4 mg/kg body weight (IP). Procaine is an ester composed of PABA (para-amino benzoic acid) and DEAE (diethyl amino ethanol). Both of these are water soluble B-vitamins. PABA stimulates the production of folic acid and vitamins K and B1. It has its greatest beneficial effects in the hair, glands and intestines (Aslan, 1962; MacFarlane, 1975). DEAE is a precursor to choline and acetylcholine. These factors are well known for their importance in nerve function. 9 Esters typically are joined by weak covalent bands. In the case of Procaine, the looseness of the bonds allows the PABA and DEAE to enter the body easily. Once inside, they separate and pursue their singular missions. PABA has an electrical charge, which makes it difficult to absorb. When joined together in the procaine molecule, however, PABA and DEAE become ionized.

Since they no longer have a charge, the body readily attracts and absorbs them (Aslan, 1962; MacFarlane, 1975). It has been obtained restoration of the deformability of 'irreversibly' Sickled Cells by Procaine Hydrochloride treatment (Baker et al., 1975).
Procaine treatment has been done in order to establish if this drug formula is efficient in preventing atherosclerotic effect of high reach cholesterol diet.
After 6 weeks treatment, rats have been antestethised with Na penthobarbital and peripherial blood was harvested on heparin and an adequate volume of $MnCl_2$ in such a way to obtain in extracellular compartment a concentration of 20 mM $MnCl_2$.
Then the animals were sacrificed by cervical dislocation, 24 hours after the last dose, then thoracic cage was opened and fragments of liver of $1cm^3$ were collected for assessment of T2 by1H NMR.
Samples were stored on ice until NMR measurements had been done (within maximum 1h) in order to prevent biochemical damage of tissue. Before the assay, the samples were brought to room temperature ($24^oC$).

**Determinations of 1H Nuclear Magnetic resonance (1H NMR)**

$^1H$ NMR method has been used for evaluating Proton transverse relaxing times of free and bound water in liver, as well and Proton transverse relaxation time changes in free water against the total water ratio from liver under the effect of Cholesterol treatment associated or not with Aslavital or Procaine modifying intra erythrocyte water and also of extra erythrocyte water, as well as for evaluation of times for exchange of water and calculus for water permeability.
The method`s principle: Consists of characterising of a system composed of two compartments - A and B –of the two relaxing times - T2a and T2b – of the same type of nuclei originating from the same compartment (Revnic et al., 2007).
$^1H$ NMR determinations have been done at room temperature on a $^1H$ NMR AREMI`78 Spectrometer (0.6T; proton resonance in impulses at a frequency of 25 MHz. The estimation of T2 was done by CARR-PURCELL-MEIBOOM-GILL pulse sequence, with 32 spin chhoes ranging from 8 to 256 ms after the 90 degree pulse,each point being the average of 16 measurements with an interval of 1 ms between impulses (Revnic et al., 2007).

**Proton transverse relaxation times and data processing**

The data were fitted to a bi-exponential as well as to a monoexponential curve. $X^2$ values were analyzed using the Student *t* test, and we arrived to the conclusion that a model with two relaxation times is adequate and therefore, we attributed the two relaxation times obtained from our data to bound water which are engaged in supporting motion of protein chain substrate and free water in which a large number of solutes are dissolved.
The two values obtained for T2 by means of a computerized program were T21for the bound water and T22 for the free water. Literature data (Gillis et al., 1991) pointed out that at high frequencies(between 10-200 MHz, bound water may be used in its usual meaning ,that is the hydration shell bound to the protein by electrostatic interactions.
The obtained relaxation times ascribed to the bound water and free water are the apparent times of the considered compartments.
It have been measured transverse proton relaxing times in intracellular compartment in the presence of water exchange between intracellular and extracellular compartment fed with $Mn^{2+}$ obtaining in such a way the apparent relaxing time $T2^1$.

**RESULTS AND DISCUSSIONS**

Figure 1 represents the value of transverse proton relaxing times (T2) of water in liver of control and treated animals. There is a decrease in this parameter in cholesterol treated animals, which is more obvious in groups which received Aslavital treatment; increase values than in controls were observed also in Procain treated . In other words, Aslavital and Procain have different effects upon water from animals` liver .Globaly, water becames more bound in case of Aslavital treatment than in case of Procaine.
Analiysing in detail proton transverse relaxing times of bound water and free water from liver of control and cholesterol and Aslavital and of

cholesterol and Procaine treated rats (Figure 2), we can observe that bound water , characerised by $T_{21}$ structural, presents a slight decrease în ltreated groups, comparatively with Control group, while free water characterised by structural parameter $T_{22}$, decreases following cholesterol administration and increases under Aslavital and Procaine treatment, in the case of Procaine this effect is more powerfull above the control values.
Modification of negative sense of transverse proton relaxing times , therefore gradualy reduction of the degree in mobility of proton from the structure of bound and free water, in the liver of treated animals are due to modifications in free water to bound water ratio – bound water in the detriment of quantity of free water, which decreases at half versus Controls (Figure 3). It must be mentioned that the treatment with above mentioned drugs, even modifies the structure of free and bound water does not act upon proportion of free water quantity.

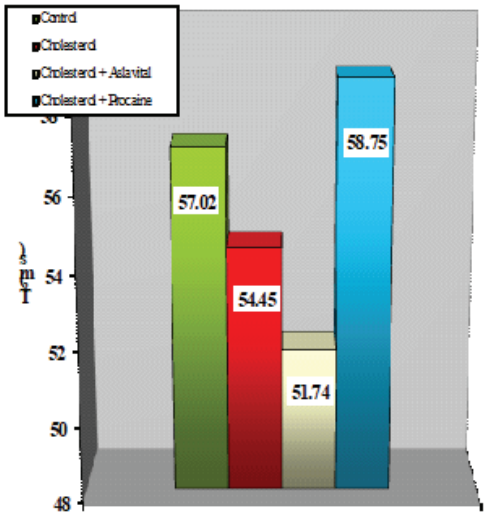


Figure 1. Proton transverse relaxation times of water ($T_2$) from control rat liver treated with cholesterol, Aslavital and Procaine in different combinations

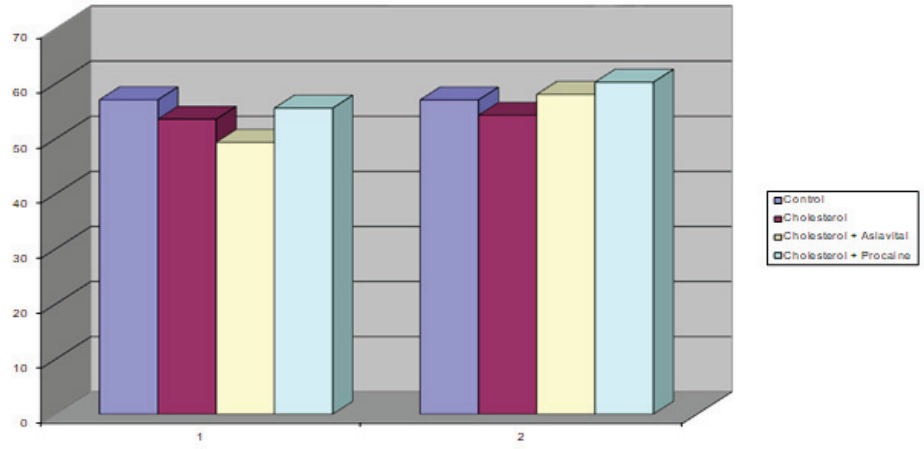


Figure 2. Proton transverse relaxation times of free (T21) and bound (T22) water from control rat liver or from rats treated with cholesterol, Aslavital and Procaine in different combinations

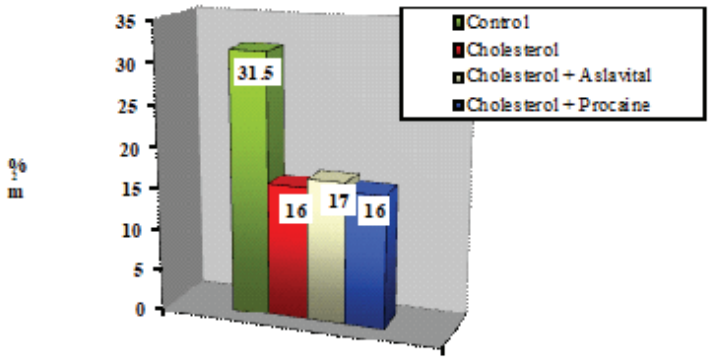


Figure 3. Free water mass ratio against total quantity of water mass from control rat liver and from trated rats with cholesterol, Aslavital and Procaine

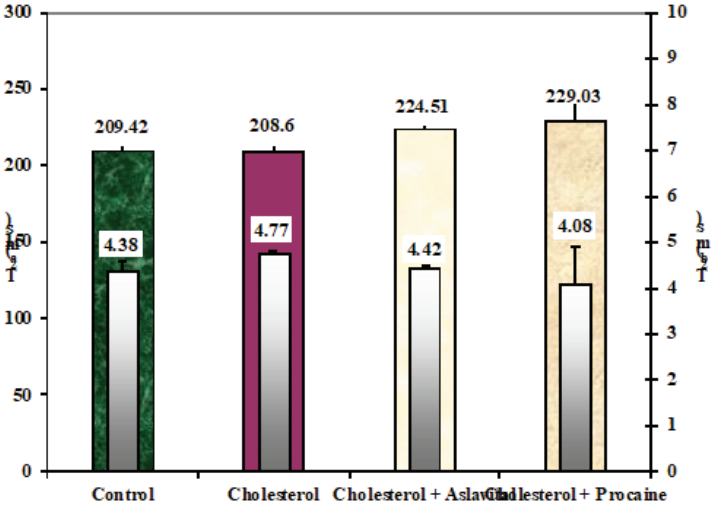


Figure 4. Proton transverse relaxing times of plasma water (T2b) and intraerythrocyte water (T2a) in control rats and in treated rats with cholesterol, Aslavital and Procaine

In the case of intraerythrocyte water there are very subtile changes , but which remind of those which take place in the liver.

Therefore, the transverse proton relaxing time of intraerythrocyte water ($\mathbf{T_{2a}}$) decreases slightly in cholesterol treated group,but thne under the effect of Aslavital and Procaine to slightly increase. (Figure 4*)*.

As a mirrror reflexion of the behaviour previosly described, în plasma the proton trannsverse relaxing time ($\mathbf{T_{2b}}$) of plasma water increases slightly in cholesterol treated rats and decreases under the actions of administrated drugs, with a more pronounced effect under Procaine treatment.

## CONCLUSIONS

There is a global decrease of proton transverse relaxation times in rat liver which is mainly due to changes in the free($\mathbf{T_2}$) and bound water ($\mathbf{T_1}$) ratio.

Liver hypertophy is associated with the decrease in these proton transverse relaxing times as well as in the proportion of free water ($m_2$%), associated with the decrease in proton transverse relaxing times as a consequence of the increase in protein content of liver tissue.

There are cases in which the two drugs Aslavital and Procaine have the same effects ie: either lead to a decrease in liver of proton