Modulation of radical pairs dynamics immersed in an ELF-EMF: The effect on hepatocarcinogenesis

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Abstract. The most suitable mechanism of action of electromagnetic fields (EMF) on biological systems is the effect on the radical pair (RP) recombination through the Zeeman effect and hyperfine interaction, which changes the rate of reactions or the product distribution. Enzyme reactions with RP intermediates can be altered by EMF, like those catalyzed by cytochrome P450 enzymes (CYP450), a heme-thiolate family protein that detoxifies xenobiotics and involved in chemical carcinogenesis. CYP450 activate chemical carcinogens producing an enormous amount of free radicals, which damage the DNA resulting in the malignant transformation of cells. During the activation, CYP450 produce spin-correlated RP intermediates that can either go to recombination or to continue the catalytic process. As CYP450 are electron carrier proteins, it is possible that RP intermediates may be affected by EMF. It was previously found that periodic treatment with extremely low frequency electromagnetic fields (ELF-EMF) inhibits more than 50% the number and area of preneoplastic lesions in rats with chemically induced hepatocarcinogenesis. In this work, we developed a quantum mechanical model based on RP mechanism in order to explain the experimental effects of ELF-EMF on the free radicals produced in the early stages of chemical carcinogenesis.

1. Introduction

Although mild, several studies have demonstrated the influence of extremely low-frequency electromagnetic fields (ELF-EMF; < 1200 Hz, C≈ 1 km) on biological processes [1] and chemical reactions [2, 3]. One of the most studied mechanism of action of ELF-EMF on chemical reactions, and, therefore, biological systems, is the radical pair mechanism (RPM). Radicals, molecules or atoms which contain unpaired electrons in its outer orbital, are short-lived intermediates in a wide diverse of reactions that are produced in pairs, by what they are called radical pairs (RP) [3]. RP are involved in many biological processes like oxidation and, due to this, in aging and diseases. Since radicals have unpaired electron spins they, therefore, can present one of two spin states, singlets (S) or triplets (T). RP are produced simultaneously in reactions by an electron transfer reaction in spin-
correlated states. These unpaired electrons are very unstable and hazardous. Although its lifetime is in the order of nanoseconds, they collide with other molecules and atoms generating more free radicals, creating a chain reaction in which millions of molecules could be altered in a few nanoseconds.

The RPM is used to study the effect of EMF on the rate of the RP recombination dynamics, \(i.e.,\) the interconversion of the S and T states through the Zeeman effect and hyperfine interaction due to the associated magnetic momentum to each electron spin [4]. RPM effects of ELF-EMF only are significant with EMF of more than 1mT [2] where they have measurable effects on the kinetics of chemical reactions through their effect on the spin correlation rate of the unpaired electrons and consequently over the radicals lifetime [5]. However, since ELF-EMF are too weak, they do not produce any temperature increase in biological systems [6].

In the absence of EMF, there is not S-T interconversion. On the other hand, ELF-EMF can alter the course of reactions through the regulation of the interconversion from S to T spin states or its regeneration from the S spin state. When an EMF is applied, two of the three triplets levels, namely \(T_{+1}\) and \(T_{-1}\), become progressively decoupled restricting S-T interconversion to the S and \(T_0\) states. Thus, RP can either to recombine to form the original molecule or to separate into free radicals and create products in other chemical reactions [3]. Since radicals usually react and recombine when they are in the S state [3], the result is a change in the rate of reactions or in the product distribution [2, 3, 7]. The dynamics of the RP is generated by a magnetic interaction provoking a Zeeman splitting of the energy levels and a hyperfine coupling between an electron and nucleon. The RP oscillates as a combination of S and T states [8]. RPM is considered an excellent, and currently the best, theoretical model for understanding the effects that magnetic fields exert on chemical reactions [3, 9].

Enzymes with RP intermediates exhibit magnetic field-dependant parameters [2], and numerous enzymes with radical mechanisms are known such as cytochrome P450 (CYP450) [10]. CYP450 enzymes are a heme-thiolate monooxygenase family of proteins expressed in the liver and other tissues [11]. The main function of CYP450 is the oxidation of substrates [12]. CYP450 use the energy from an electron provided by a donor protein to initiate its catalytic process [13]. CYP450, which normally detoxify xenobiotic compounds, are important enzymes because they are involved in chemical carcinogenesis since this enzyme activates procarcinogens [14]. Chemical carcinogenesis is a multi-step process characterized by the electrophile attack to DNA, which produces mutations, resulting in the malignant transformation of cells [15]. Although most chemical carcinogens tend to be stable (procarcinogens), once they are incorporated into the organism, suffer an activation in the liver by CYP450 [14] converting the procarcinogen into a toxic compound. During the oxygenation of the substrates by CYP450, bursts of electrophiles near the site of oxidation are generated [16] inducing oxidative stress (OS) [17, 18, 19]. The relevance of CYP450 in carcinogenesis has made this enzyme the target of chemoprevention [20].

CYP450 are electron carrier proteins that produce spin-correlated RP intermediates [2] susceptible of being modulated by ELF-EMF. Therefore, since ELF-EMF modifies the spin orientation and, therefore, the interconversion rate between S and T spin states of RP populations, it is plausible to expect that the formation of products derived from the metabolization of chemical carcinogens, \(i.e.,\) free radicals, might be modified.

2. Problem statement

In a previous work, our group experimentally analyzed the effect of ELF-EMF on chemically induced hepatocarcinogenesis employing the modified resistant hepatocyte model (MRHM) in Fisher-344 rats [21]. The main chemical carcinogen used in the MRHM, diethylnitrosamine (DEN) is a well-known potent hepatocarcinogenic agent [22] present in many products for human consumption [23]. DEN generates free radicals causing necrosis, hyperplasia, and tumors [24]. Besides, DEN produces single strand breaks in the DNA and also induces changes in enzymes.
involved in the DNA repair [24]. The second chemical used in the MRHM is 2-acetilaminofluorine (2-AAF), which also induces tumorigenesis in the rat liver [25]. Both chemical carcinogens are activated by CYP450 producing an unregulated enormous wave of electrophiles that cause OS, leading to carcinogenesis initiation. After administration of carcinogens, partial hepatectomy promotes the development of preneoplastic lesions in the liver of rats [21]. The experimental results showed that periodic treatment with 4.5 mT - 120 Hz ELF-EMF (50 minutes each day) inhibits more than 50% the number and area of preneoplastic lesions in rats with chemically induced cancer through the reduction of cell proliferation [21]. Despite these findings, the molecular mechanisms responsible for these effects are still unclear. Nonetheless, RPM is an important mechanism that could explains part of the observed experimental effects of the ELF-EMF.

In this work, it was theoretically analyzed the experimental effects of ELF-EMF on RP intermediates and OS produced by CYP450 during procarcinogen activation. The objective was to establish a theoretical methodology to model the possible mechanism of the cytoprotective effect of ELF-EMF on the liver with induced carcinogenesis. The model uses the RP theory to study charge migration in the electron transfer reactions catalyzed by CYP450, involving free radicals dynamics interacting with the ELF-EMF. It is plausible to assume that through the RPM, the RP involved in free radicals generation could be modulated, as has been reported for other reactions [5, 26], and directed to a lower energetic states in such a way that the activation of oxidative products would be diminished and the electrophile damage reduced.

3. The model
Several attempts to model the effects of ELF-EMF on reaction kinetics have been developed [27]. Here, in order to model the dynamics of RP intermediates involved in OS produced by chemically induced hepatocarcinogenesis when an external ELF-EMF (consisting of both, static and pulsating fields) is applied, the RPM was used [3] because it involves the recombination of short-lived reactive free radicals with correlated electron spins [28]. A theoretical semi-classical (or semi-quantum) model was used to describe the possible modulation of the high amount of RP produced during CYP450 carcinogen activation by the ELF-EMF. Then, the spin Hamiltonian operator for the weakly coupled RP with an external EMF was used [3]:

$$\hat{H}_{RP} = \hat{H}_{mag} + \hat{H}_{ex},$$

$$\hat{H}_{mag} = \mu_B B_a B S_{1z} + a \vec{I} \cdot \vec{S}_1 + \mu_B B_b B S_{2z},$$

$$\hat{H}_{ex} = -J(\vec{r})(2\vec{S}_1 \cdot \vec{S}_2 + 1/2),$$  \hspace{1cm} (1)

where $\hat{H}_{mag}$ is the Hamiltonian for the ELF-EMF and $\hat{H}_{ex}$ the exchange Hamiltonian operator term for the spin exchange interaction; $\mu_B B_a$ and $\mu_B B_b$ correspond to the Zeeman interaction, with $g_a$ and $g_b$ as isotropic values of the $a$ and $b$ radicals of the RP; and $a$ is the hyperfine coupling constant with spin nucleus $\vec{I}$. The interaction energy depends on the distance by the term $J(\vec{r})$.

The chemical reactivity of free radicals is controlled by spin dynamics, which consists in the transformation of nonreactive T spin states into the reactive S state and vice versa [29]. Hyperfine coupling, the Zeeman effect and the differences in Larmor precession rates can alter the spin orientation [3]. Then, the recombination probability of S states of the RP through the EMF influence by the hyperfine interaction was studied to assess the spin states development during S-T inter-conversions. Since S-T interconversion can be influenced by an external EMF, the conservation of the angular momentum will favor one of the states, either S or T, over the other in one or another reaction pathways. Thus, the production rate of S-T populations of free radicals is modified due to differences in energy among eigenstates caused, in turn, by changes in the spin eigenstructure of the Hamiltonian of the system. Such effect is called spin selectivity of the RP recombination [30]. The hypothesis is that the periodic exposure to ELF-EMF will alter the progression of RP of free radicals generated by CYP450 catalysis of carcinogens in such a way that
the electron transfer through S-T interactions is modified, with the result of a reduction of OS. The consequence is a reduction in the formation of preneoplastic lesions. Two main steps were followed to construct the model: 1) RP analytical solution to study the influence of the ELF-EMF on the S-T interconversion employing a system composed of two spins from electrons and one spin from one nucleus; 2) numerical solution by Lanczos method in order to study complex systems composed by many spins using the tridiagonal transformation matrix obtained from the Hamiltonian.

3.1. Modeling one nucleus and two electron spins
The first approximation was to solve analytically the three spin system composed by two electron spins and one nuclear spin modulated by the ELF-EMF. Interactions between RP intermediates and electron transfer were modelled employing the RPM (see equation 1). The recombination of the free radicals by pairs was studied through the magnetic influence of the hyperfine interaction including the damping of the two quantum states to analyze the spin states development during the S-T interconversion. The relaxation time of the enzymatic reaction was assumed to be greater than the lifetime of the RP. This feature provides the adequate boundary conditions that confer the system with the capability to sustain oscillatory behavior between the spin states due to the interaction between the two electrons and the nucleus. This was assured by allocating the elemental cell target in an average position where the OS was generated. The studies were performed in the steady state, and an isotropic hyperfine interaction was assumed as an essential mechanism in the time evolution of the spin states. In the recombination study, a simple kinetic model [27, 31, 32] was used in which the quantum modulation of RP by the EMF is involved. The purpose of this was to examine the behavior of the spin population in a steady state to evaluate the recombination probability (Fig 1). Since there has been included both, the static magnetic field and the pulsating, Timmel and Hore’s theory was used [33]. As the system consisted of two electron spins and one nuclear spin modulated by hyperfine interaction, eight basic states were separated into two sets: 1) one with the energy equal to M/4 and degeneracy of 6, and the other with energy equal to -3M/4 and degeneracy of 2. For an EMF directed along the z-axis, the spin Hamiltonian operators are:

\[
\hat{H}_0 = \alpha S_z \cdot I - \omega_{\text{ELF}} (S_{ax} + S_{bz} + I_z) + \omega_S (S_{ax} + S_{bz}),
\]

\[
\hat{H}_1 = \omega_I (S_{ax} + S_{bz}).
\]

The first term includes the static magnetic field \(\omega_S(S_{ax} + S_{bz})\) and the pulsating magnetic field \(\omega_{\text{ELF}}(S_{ax} + S_{bz} + I_z)\). \(S_{ax}, S_{bz}, I_z\) are the spin operators and nuclear isospin.

3.2. Lanczos numerical approximation
RPM modelling allow us to understand the way in which the RPM works and to know what is the main effect of the ELF-EMF for the transition between S and T₀ spin state. For more complex systems composed by many pairs of electrons and nucleus and other transitions in the system, it is necessary to use numerical methods in order to simplify calculations. The Lanczos method with the Stochastic Liouville equation were used as a way to evaluate eigenvalues and eigenvectors of a matrix, which represents their typical dynamical evolution. With the Lanczos method [4, 34, 35] the matrix of the system was transformed to a tridiagonal form, which simplifies the finding of eigenvalues and eigenvectors. In this way, a vector space tailored can be created to contain the dynamical quantities needed to describe the time evolution of relevant parameters. The advantage of this method is that it allows managing smaller dimension of the vector space spanned than the original dimension of the system, eliminating redundant quantities as in the case of degenerate energy eigenvalues. The evolution equation is:

\[
\frac{\partial}{\partial t} \langle a_k | \hat{\rho} | a_l \rangle = - \sum_{j,m} L(kl, mj) \langle a_m | \hat{\rho} | a_l \rangle,
\]

where \(L(kl, mj)\) is the matrix of the transition operator in the super-operator basis, \(\hat{\rho}\) the spin population operator.
4. Results and discussion

In this work, matrix methods were applied to the quantum model that describes the effects of EMF on the RPM in order to determine the evolution of the spin system proposed. The eigenstates and eigenvalues of the system were calculated and used to perform an expansion of an arbitrary state. To know the possibilities of the method, the cases in which results can be calculated analytically were determined. These results allowed to study the magnetic effects that appear as a result of the interplay between the spins of electrons and the spin of the nucleus.

We analyzed the dynamics of the RP (governed by rules of spin coupling and the conservation of angular momentum) generated from two weakly coupled spins when CYP450 enzymatic reaction is carried out. A cyclic kinetics was used to examine the EMF influence on the spin-states inter-conversions. Such kinetic processes were introduced using them as an intermediate oscillatory process to account for an energy conversion step due to the spin selectivity. In the calculations, it was considered an elemental target cell exposed to the OS generated by the DEN activation and submitted to a periodic external EMF. Figure 1 shows the densities of singlet populations $\rho_1(t)$, triplet populations $\rho_2(t)$, and both $|\rho_2(t)|^2$ as a function of ELF-EMF ($B$) and time ($t$). It can be seen that, when ELF-EMF is turned off ($B = 0$), there are no oscillations. By contrary, oscillations increase quickly up to a maximum value when ELF-EMF intensity increases, modulating the behavior of spin populations. The resultant combined effect in the recombination process was 40% from the static EMF plus 25% from the pulsating EMF. Thus, since there are less active S spins, the number of initiated cells diminish, and, therefore, the preneoplastic lesion formation, which is the main cytoprotective effect of the EMF found experimentally.

![Figure 1](image)

**Figure 1.** Time evolution of spin populations submitted to ELF-EMF for the components of the $\rho(t)$ for the initial state $\rho(0)=(0,0,0,1)$. Plots for density of spin populations for a) singlets; b) triplets, and c) for S and T spin populations combined. $B$: EMF; $t$: time (see text).

The mathematical model presented in this work is a simple approximation that contributes to the understanding of chemical carcinogenic process based on the behavior of charged particles, which are the direct responsible of cell damage. Evidently, the scope of this approach is simplistic since biological systems are highly complex and indeed exist mechanisms that were not taken into account. For this reason, it is necessary to improve and complexify the approximations developed in the present study. As far as we know, this is the first study in which RPM is used to explain and model processes related with enzymatic RP intermediaries involved in OS and carcinogenesis. The results of this work could constitute the basis for the design of strategies or clinical applications of ELF-EMF as co-adjutant in the treatment of several diseases related with oxidative damage, including cancer.

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