THERMODYNAMIC CONSIDERATIONS ON THE EFFECT OF HYPERTHERMIA AND ELECTROTHERAPY IN THE TUMOR HeLa CELLS

CONSIDERACIONES TERMODINÁMICAS SOBRE EL EFECTO DE LA HIPERTERMIA Y LA ELECTROTERAPIA EN CÉLULAS TUMORALES HeLa

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Cervical cancer is one of the leading causes of death for women in the world [1]. Current therapeutic strategies are targeted at surgical intervention, followed by radio and chemotherapies [2, 3]. The negative side effects of these conventional therapies, which in many cases deteriorate the patient's quality of life and even lead to premature death, are well known.

In this sense, other kinds of adjuvant therapies should be considered as well. This includes electrotherapy [4] and hyperthermia [5]. They have local character, are easy to implement and minimally invasive. These therapies significantly reduce the volume of the tumor mass and even the total elimination of the tumor without reported regression [6,7]. Therapies cause a variation in intra- and extracellular (electrotherapy) [8], as well as a local increase in temperature (hyperthermia) that could affect the cellular metabolism of tumor cells, in particular the glycolysis process.

Despite the numerous attempts to explain and clarify the possible mechanisms of action of these therapies [9,10], there is still no consensus on their mechanism of action. This is the main limitation for their implementation as a therapeutic modality in the treatment of solid tumors [11] in the practice of Clinical Oncology.

It is well-known that most of tumor cells show a high glycolytic rate compared with normal cells. This phenomenon is known in the literature as Warburg's effect [12]. The significant increase of glycolysis rate observed in tumors has been recently verified, yet few oncologists or cancer researchers understand the full scope of Warburg's work [12,13] despite of its great importance. Altered energy metabolism is proving to be as widespread in cancer cells as many of the other cancer-associated traits that have been accepted as hallmarks of cancer [14].

The goal of this work is to determine the possible influence of electrotherapy and hyperthermia on the mechanism of glycolysis in the tumor HeLa cells, by calculating the entropy production rate.

For this purpose, we consider the metabolic model proposed by Marín et al. [15] from experimental studies performed on the HeLa tumor line (cervical cancer cells) of three phenotypes: hypoglycemic, normoglycemic and hyperglycemic for extracellular glucose (Gluc) concentrations. For the modeling of the metabolic network the software COPASI v. 4.6.32 was used, available on the website http://www.copasi.org and the values of the parameters and concentrations reported by Marín et al. [15].

In previous works, we have shown how the production of entropy per unit of time can be used to select the fundamental steps in a mechanism of a complex network of biochemical reactions [16,17] and, in turn, how it represents a distinctive behavior associated with cancer robustness [18] and associated with prognostic capacity.

The entropy production rate $\frac{\delta S_i}{\mathrm{d}t} \equiv S_i$, at constant temperature T and pressure P and neglecting diffusive and viscous effects, of each of the reactions of the glycolysis process was evaluated without loss of generality [16] as

$$S_i = -\frac{1}{T}\Delta G_k \xi_k \tag{1}$$

where ξ_k represents the generalized flow, and $\frac{1}{T}\Delta G_k$ the generalized force, i.e. the Gibbs free energy variation, of the reaction of the glycolysis process. The reaction velocity is obtained for each reaction by Copasi simulations.

The Gibbs free energy of the k-*th* reaction is written [19] as

$$\Delta G_k = \Delta G_k^{\oplus}(pH, T, I) + RT \sum_i \nu_i \ln c_i, \tag{2}$$

where ν_i c_i represent the stoichiometric coefficients and concentrations respectively of the involved biomolecules in each reaction and is the standard Gibbs free energy adjusted taking into account its dependence on temperature T, pH and ionic force I [20,21], in the physiological conditions used experimentally [15,21]: $I = 0.18$ M, $T = 310.15$ K and $pH = 7$. In electrotherapy the local temperature of the tumor mass remains constant and the ionic force is not affected [6]. The values of extracellular pH used were those reported in the literature: for cathodic $pH \approx 8-12$ and anodic $pH \approx 2-6$ electrotherapy [6]. The relation between extracellular and intracellular pH was taken into account. On the other hand, in hyperthermia, the temperature moves in the interval: $T \approx$ 309K−335K and pH and ionic force are not appreciably affected [7].

To calculate the rectified standard Gibbs free energy $\Delta G_k^{\oplus}$ the equation (3) was used

$$\Delta G_k^{\oplus}(pH, T, I) = \sum_n \Delta G_n^{\oplus}(pH, T, I)$$

$$\Delta G_n^{\oplus}(pH, T, I) = \frac{T}{298.15}\Delta G_n^{\theta} + \left(1 - \frac{T}{298.15}\right)\Delta H^{\theta} + \tag{3}$$

$$+ N_H RT \ln 10 pH - \frac{RT\alpha(z^2 - N_H)\sqrt{I}}{1 + 1.6\sqrt{I}},$$

where $\alpha = 1.20078$ (kg/mol)$^{1/2}$ is the Debye-Hückel constant, z is the species charge, $R = 8.31$ J/(mol K) is the universal gases constant and N_H is the average number of hydrogen atoms bond to the species.

As shown in Fig. 1, the reaction catalyzed by the enzyme *ATPase* is significantly affected by the application of anodic electrotherapy (acidification of the extracellular medium: an anode electrode at the center of the tumor and a cathode subcutaneous, at the periphery [6]). A marked decrease in the entropy production rate is observed, which makes the process less robust [18,22].

It is known that *ATPase* is overexpressed in many types of cancers, which is associated as a consequence of the accelerated glycolysis of these cells [23]. This reaction is involved in the maintenance of a slightly alkaline intracellular pH (pH_i) which is advantageous for tumor cells and in turn acidifies the extracellular medium, which is known as reverse gradient, through pumping from the intracellular medium to vacuoles and to the extracellular medium, causing the death of healthy cells, favoring metastasis, radioresistance and immune leakage [24,25].

In this way a decrease could be interpreted as the loss of *ATPase* activity which would lead to a decrease in the tumor mass resulting from the cellular decrease, probably due to the important role it plays in the regulation of the cytoplasmic [16,19].

It is known that prolonged treatments with *ATPase* inhibitors induce cellular apoptosis [26–28], while short treatments with these same substances induce cell stress and autophagy [29]. Recent studies have reported that inhibition of *ATPase* induces the formation of substances that potentiate apoptosis as the pro-apoptotic protein PNIP3 among others [26,30].

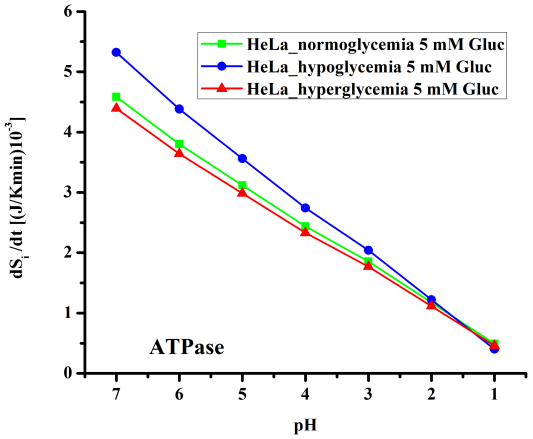

Figure 1. Dependence of the S_1 with the pH for the reaction catalyzed by *ATPase* during the application of anodic electrotherapy.

Hyperthermia therapy consists in raising the local temperature of the tumor to approximate values of 60^o [31]. It is known that at these temperatures the proteins are denatured [31]. Generally the efficacy of the same has been attributed precisely to this effect [32]. However, in the peripheral area of the tumor where most proliferating cells are found [33] the temperature only reaches the 45^o [5]. These values apparently do not produce noticeable changes in the cells [31].

In Fig. 2, the dependence of the S_i with the T for the reaction catalyzed by the enzyme *LDH* (Lactate Dehydrogenase) is shown. This reaction guarantees the maintenance of the rate of glycolysis by regenerating the NADH (Nicotinamide adenine dinucleotide reduced), which contributes to redox balance. In turn, it causes acidification of the extracellular medium [34,35].

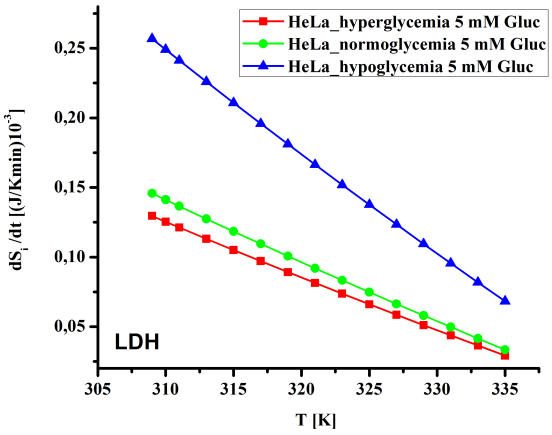

Figure 2. Dependence of the S_1 with the pH for the reaction catalyzed by *LDH* during the application of hyperthermia.

As observed with the increase in temperature decreases the S_i. This phenomenon can be interpreted as a decrease in the robustness of such a reaction [16, 19]. It is known [36] that many therapies have targeted the inhibition of *LDH* and therefore the inactivation of *MCT*1 transporters (responsible for the transport of lactate to the outer cell) [37]. An analogous result using low frequency electromagnetic waves was recently found [38].

This reaction is also affected by cathodic therapy (basification of the extracellular medium: a cathode electrode at the center of the tumor and a subcutaneous anodic at the periphery [6]). As can be seen in Fig. 3, the increase in extracellular *pH* causes a marked decrease of that of this reaction. This is a clear indicator that lactate production is significantly affected; in this way the process becomes less robust [18, 21].

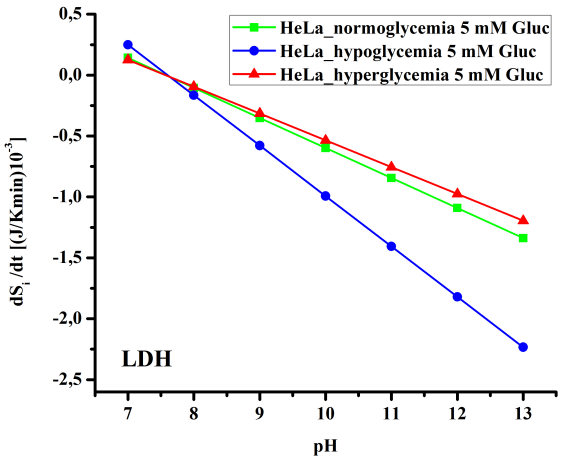

Figure 3. Dependence of the S_1 with the *pH* for the reaction catalyzed by *LDH* during the application of cathodic electrotherapy.

It appears that the positive effects of these therapeutic techniques can be attributed to their ability to selectively affect fundamental reactions within the glycolytic process of cancer. This causes a decrease in the robustness of the metabolism of cancer cells. In this way, with the joint implementation of such therapies an increase in the effectiveness of the treatments would be achieved.

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