

Therapeutic perspectives of nucleases in cancer

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Nucleases are enzymes normally present in all living forms. They participate in the arrangement of nucleic acids as well as in the processes of transcription and translation through their mechanisms of recognition of specific regions of DNA and RNA, and they also work actively with the molecules that regulate cellular proliferation. Nucleases play a very significant role in the lysis of products from ill and old cells, as well as in the destruction of neoplastic cells.

It has been proved that in patients with cancer, the concentration of nucleases is modified as well as their activity in general, and they show a sharp decrease or they are not present at all in tumoral cells. The application of exogen nucleases is proposed as a therapeutic option. In this work we report the results obtained when treating patients affected by different types of cancer with deoxyribonuclease and cocarboxylase or thiamine pyrophosphate in stable solution, in addition to standard anti-cancer therapy.

Introduction

Cancer represents the disease responsible for the greatest number of deaths all over the world.¹⁴ Given its multifactorial origin, it continues to be difficult to control it.¹³ Several therapeutic approaches have been used such as radiotherapy, chemotherapy and surgery, and even more recently immunotherapy; all these treatments are successful in some cases.¹¹ In search of its control, a natural phenomenon was analyzed: the apoptosis or programmed cellular death, in which a group of enzymes, the nucleases, participate along with some oncogenes. These nucleases have different functions, among which are the capacity of recognition and destruction of neoplastic cells.² Their impor-

tance in this field was determined when observing that their concentration and/or activity is modified in the organism when a cancerous process is established.

It has also been proved that in cancerous cells the nucleases are diminished or absent.⁵ Since then, important data was published that allowed us to evaluate their function as probable antitumoral enzymes. Because they conform a big family of enzymes, they are classified as ribonucleases (RNases) and deoxyribonucleases (DNases) according to the substrate they work with. Their digestive activity permits them to desintegrate the ribonucleic and deoxyribonucleic acids (RNA and DNA) from dead cells, abscesses, hematomas, as well as those from vi-

rus and bacteria, reason for which they are amply recognized through their germicidal action.²⁰ They are universal, given that they are present in all organisms. In humans, they are in all tissues in constant concentrations outside and inside the cell under normal conditions, but they may be modified according to the conditions of the person.¹⁶ Their presence in the intracellular compartments has been related with the regulatory mechanisms of cellular proliferation,²¹ in the arrangement processes of DNA that include repair⁹ and degradation¹⁴ as well as in other activities that regulate DNA, probably related to transcription and translation.²⁸ The control of their activity is done through other proteins that act as inhibitor factors, such as actin.¹⁹ Owing to the changes that have been found in the concentration and/or activity of these enzymes in patients who suffer some kind of cancerous process, nucleases have been proposed as elements for the diagnosis, the follow-up of the illness and even to measure response to diverse treatments.^{10,24,26} As it is shown by several reports, there is a decrease in the concentration of nucleases activity during the establishment of cancer, which gives a therapeutic option that must be taken into consideration: the exogenous administration of these enzymes would make up for the deficiency in such patients. In this study, we present the results obtained when treating cancer patients with DNase simultaneously with co-carboxylase or thiamine pyrophosphate (TPP) at the same time while they received conventional antineoplastic treatments like chemotherapy, radiotherapy and immunotherapy.

Material and methods

A group of 40 patients who gave their consent were selected considering only one entrance criteria: they should have cancer with a variable time of illness. The patients were treated with DNase (0.03 mg/ml) and co-carboxylase or TPP (40 mg/ml) stables in solution by intramuscular administration with the following general scheme: 5 ml of DNase alternated with 3 ml of TPP daily for 15 days; every other day for two weeks, twice a week for a month and then once a week for a month. The general scheme varied according to the patients' evolution. At the same time, some of them continued with their

radiotherapy, immunotherapy or chemotherapy treatments if they were previously assigned to them. Follow-up was done observing the evolution for a period of three months at least and for a maximum of five years and data was compared with the evolution of patients who only received radiotherapy, chemotherapy and/or immunotherapy (30 patients). The tests performed in order to assess results included: blood count, blood chemistry, general urinalysis, ultrasound, tomography and X-ray of the tumoral tissues, depending upon the case.

Results

The results obtained are shown in Table 1.

The response of the patients was compared to the response of those who only had radiotherapy, chemotherapy and/or immunotherapy during the same period. Such response was considered good when the patient not only improved clinically, but when there was remission in the cancer process; it was considered as fair when the patient was clinically stable, but when there was no remission and no change, the patient did not respond to treatment. As observed in Table 1, 68% of the patients had a favorable response. The most significant results were obtained in patients with leukemia, who all responded. It must be taken into consideration that the treated cases correspond to acute cases diagnosed with acute lymphocytic leukemia or lymphoblastic leukemia.

To date, these patients have been under treatment for at least one and a half years to more than five years and they still keep their normal blood values as shown in Table 2. A similar result was shown by patients affected with lymphoma, in whom a remission was determined through radiographic, tomographic and ultrasound studies. None of the patients that were submitted to radiotherapy and/or chemotherapy had side effects, not in short or in long term during the treatment with DNase and co-carboxylase; on the contrary they revealed greater energy, they were asymptomatic, and the patients who had lost weight gained it back. It was also seen that patients that were treated with DNase and TPP when the illness was recently established and metastasis had not taken place, the response to treatment was more rapid and definitive.

TABLE 1
General results

Cancer type:	Total No. of cases:	Response to treatment:					
		Remission	*Time	Partial	Time	Negative	Time
Ovarian cancer	4	3	3 to 24	---	---	1	24
Breast cancer	6	4	13 to 36	---	---	2	1
Uterine cancer	3	2	15	---	---	1	1
Hepatic cancer	4	1	5	---	---	3	1
Colon cancer	1	---	---	---	---	1	6
Gastric cancer	1	---	---	---	---	1	4
Bone cancer	1	---	---	---	---	1	4
Melanoma	3	3	6 to 18	---	---	---	---
Leukemia	5	5	13 to 66	---	---	---	---
Lymphoma	6	6	4 to 60	---	---	---	---
Miosarcoma	2	1	6	---	---	1	2
Tyroma	1	---	---	---	---	1	5
Epidermoid cancer	1	---	---	1	36	---	---
Astrocytoma	1	1	9	---	---	---	---
Cancer in paranasal sinus	1	1	36	---	---	---	---

*Time is given in months.

Discussion

The favorable response showed by patients listed in Table 1 may be due to: the treatment of chemo, radio and/or immunotherapy, owing to it induce the destruction of neoplastic cells; the treatment based on the application of DNase, that induces the apoptosis of neoplastic and tumoral cells, such as it occurs in normal conditions and to the applications of cocarboxylase or TPP in all cases, which controls the metabolic alterations caused by the cancer process and improves the functions of the immune system.

Based on the differences showed by patients who were only treated with chemotherapy, radiotherapy and/or immunotherapy and the patients who also received the enzyme and the coenzyme, it may be proposed that the presence of these two molecules is determinant in the positive response to treatment, improving the specific destruction of cancerous cells and, at the same time, the release of chemical messengers which activate the cytotoxic cells⁷ allowing the elimination of the cancerous cells by a natural mechanism without homeostasis alteration.

The importance of the role played by DNase in cancer comes from studies performed in patients affected by acute leukemias and with Hodking and non Hodking lymphomas, where it has being proved that the activity of seric DNase is decreased or it is absent.⁸ There has also been reported as absent in leukemic cells of human and mice.³⁰ The cause of these enzymatic variations is not known; in some cases, it is related to genetic disorders, which would explain the absence of one or some nucleases. This agrees with one of the theories on the origin of cancer: the genetic theory.

The favorable response of patients affected by leukemia and lymphoma while treated with DNase is based on the knowledge that these disorders are originated because the lymphoid cells suffer a chromosomic translocation in specific regions of DNA that have secuencies of oligopurines/oligopyrimidines, which are also the ones that show a greater sensibility to the nucleases action,¹⁵ then, if these enzymes are exogenously supplied the destruction of the altered cells is induced by a natural process that is not taking place because, as it was earlier men-

TABLE 2
Clinical and laboratory data in some treated patients

P: A.R.A.			M.A.S.C.	
A: 61			16	
D: Multiple myeloma.			Acute lymphoblastic leukemia	
LD:	Before:	After:	Before:	After:
Hb:	6.5 g/dl	12.3 g/dl	11.2 g/dl	13.6 g/dl
Hc:	22.0%	42.1%	32.7%	41.8%
Er:	2780 000/ul	5200 000/ul	3320 000/ul	5600 000/ul
Le:	3100/mm ³	6200/mm ³	3500/mm ³	7420/mm ³
Pl:	389 000/ul	388 000/ul	113 000/ul	280 000/ul
CT:	Oral chemo-therapy	Suspended	Intravenous chemotherapy	Suspended
S:	Asthenia, adynamia, general infections, general pain	Asymptomatic	Asthenia, adynamia, lumbago, cramps, muddy sight	Asymptomatic.
P: J.M.T.			M.A.A.R.	
A: 57			38	
D: Lymphocytic leukemia, diffuse type.			Breast cancer. Metastasis to left hip.	
LD:	Before:	After:	Before:	After:
Hb:	11.5 g/dl	13.9 g/dl	14.2 g/dl	14.4 g/dl
Hc:	36.9%	43.5%	42.0%	41.8%
Er:	3100 000/ul	5800 000/ul	5200 000/ul	5350 000/ul
Le:	3600/mm ³	6200/mm ³	5800/mm ³	5600/mm ³
Pl:	200 000/ul	380 000/ul	362 000/ul	374 000/ul
CT:	Oral chemo-therapy.	Suspended	Intravenous and oral chemotherapy.	Continued.
S:	General pain, neck ganglia growth, arthralgies, weight lost	Asymptomatic. Recovered weight	Asthenia, adynamia, nausea, hyporexia, weight lost	Asymptomatic Recovered weight
P: U.A.R.			P.G.S.	
A: 61			12	
D: Chronic granulocytic leukemia			Chronic granulocytic leukemia	
DL:	Before	After:	Before:	After:
Hb:	9.8 g/dl	14.2 g/dl	12.0 g/dl	13.7 g/dl
Hc:	33.2%	41.9%	36.9%	42.2%
Er:	3600 000/ul	4950 000/ul	4200 000/ul	4400 000/ul
Le:	1500/mm ³	6800/mm ³	3200/mm ³	5400/mm ³
Pl:	166 000/ul	310 000/ul	345 000/ul	330 000/ul
CT:	Oral chemo-therapy	Suspended	Oral chemo-therapy	Continued
S:	Asthenia, adynamia, fever	Asymptomatic, Recovered weight	Weight lost	Asymptomatic Recovered weight

P: patient initials; A: age; D: diagnosis; LD: laboratory data; Before, After: treatment based on DNase and TPP; Hb: hemoglobin; Hc: hematocrit; Er: erythrocytes; Le: leucocytes; Pl: platelets; CT: conventional treatment; S: symptomatology.

tioned, the nucleases are diminished, they are not activated or they are absent in these cells. Other researchers have observed that a decrease in the concentration of nucleases is a consequence of an increase in their activity; even when a treatment is effective, the enzymes decrease within the 6th and the 9th days, what may be the answer to the tumoral necrosis therapeutically induced. In the opposite case, when there is a lack of response to treatment, there is no decrease in the concentration or activation of the enzymes.

It is also reported that the patients that show low levels of the enzymes do not respond to any treatment.²⁵ Therefore, it is proposed that these enzymes may be inhibited by specific factors such as actin and other inhibitors that regulate their lytic activity such as divalent cations, temperature and pH.⁶ This last statement is reinforced by data that we have obtained in the blood samples of patients in which we observed that pH of plasma is modified to alkaline values, when the illness is acute and tends to get back into normal values when the patient recovers with treatment.

Another important factor for the nucleases to complete their lytic function is the greater permeability of the plasma membrane of neoplastic cells,²² being this one of the determinant factors of the enzyme's penetration. In the interior of the transformed cells, the nucleases perform their lytic action when recognizing specific regions of DNA or RNA (restrictives) that are out of control.³ This has been proposed based on the regulatory role that they have in the transcription and genic translation while recognizing the active regions of chromatin. In any case, a decrease in the concentration or in the activity of nucleases is another factor that lets free the cancerous processes; then, their therapeutic administration must be considered.

The application of TPP in these patients is based in the fact that thiamine and its phosphated esters such as the cocarboxylase are not in an adequate amount.²⁷ It is known that the coenzyme is an important factor for the degranulation of basophiles, whose products help the chemotaxis of leukocytes that are in charge of the cellular immune response;¹⁸ then, the immune response in general is potentialized. Once

they are active, the lymphocytes induce to cytotoxic cells to perform their lytic action against the transformed cells. It has also being proved in several studies that the cancerous cells show their metabolism affected, given that they present an increase in glycolysis, and this brings one of the most frequent alterations in different types of cancer, that is lactic acidosis,⁴ such problem gets solved in minutes when TPP is present.¹⁷ There are also other alterations like the increase of intracellular sodium, which decreases the resting membrane potential value.²³ In several experimental models we have demonstrated that TPP regulates the intermediary metabolism¹ and it also plays a key role in the active transport at the cell membrane allowing the activation of ATPases, normalizing ionic concentrations and getting back the resting membrane potential.²⁰ In this sense, the positive effect created by the presence of TPP in patients affected by cancerous processes can be therefore explained.

Currently, we are performing some trials with several lines of cancerous cells *in vitro*, with the aim to determine the percentage of mortality when DNase is added directly at different concentrations. Preliminary results show that the enzyme induces directly the lysis of these kind of cells in proportion to the enzyme concentration.

Conclusions

The patients treated with deoxyribonuclease and cocarboxylase or thiamine pyrophosphate stables in solution as well as with the conventional anticancer treatments, show resistance to the negative effects of chemotherapy, radiotherapy and immunotherapy in comparison to the patients that only received the conventional treatment.

In 68% of the cases there is a control of the cancer process according to clinical and laboratory results. Metastasis is avoided in short and long terms when treatment is applied in early stages. It has being observed such results not only in patients that took part in this specific study, but in those that have had this therapy for the last ten years. We propose that patients affected by cancer should be treated with deoxyribonuclease and cocarboxylase or thiamine pyrophosphate stables in solution.

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RESUMEN

Las nucleasas son enzimas que se encuentran presentes normalmente en todos los organismos. Participan en el rearrreglo de los ácidos nucleicos, en la modulación de los procesos de transcripción y traducción a través de sus mecanismos de reconocimiento de regiones específicas del ADN y ARN, e interactúan con las moléculas reguladoras de la proliferación celular. Las nucleasas tienen un papel muy importante en la lisis de los productos de las células lesionadas, viejas y en la eliminación de células neoplásicas. Se ha comprobado que en los pacientes con cáncer se encuentra modificada su concentración y/o su actividad en general y disminuidas o ausentes en las células neoplásicas en particular, por lo que se propone la aplicación de nucleasas exógenas como una opción en el tratamiento del cáncer. En este trabajo se presentan los resultados obtenidos al tratar a pacientes afectados con diversos tipos de cáncer con desoxirribonucleasa y cocarboxilasa o pirofosfato de tiamina estables en solución, además de los tratamientos anticancerosos convencionales.