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Importancia de la polietilenimina en biomedicina y sus aplicaciones en terapia génica.

Margarita López-Viota Gallardo, Raúl Megías Iglesias, Mª. Adolfina Ruiz Martínez, Jose Luis Arias Mediano.
Department of Pharmacy and Pharmaceutical Technology. University of Granada.

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Correspondence/Correspondencia:
Margarita López-Viota Gallardo
Dpto. Farmacia y Tecnología Farmacéutica.
Facultad de Farmacia. Universidad de Granada.
Campus de Cartuja s/n 18071 Granada.
e-mail: mlvg@ugr.es

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RESUMEN

La Terapéutica es la rama de las ciencias de la salud, que se ocupa de los medios empleados y su forma de aplicarlos en el tratamiento de las enfermedades con el fin de aliviar los síntomas o de producir la curación. Hoy en día existen al alcance multitud de herramientas terapéuticas para combatir los diversos problemas de salud a los que se hace frente día a día. Con esta evolución de la Medicina, es inevitable que vayan surgiendo cada vez más y mejores estrategias que conducen a un espectro terapéutico más innovador. Dentro de las nuevas estrategias, nos encontramos con la Terapia Génica que es una de las que más potencial tiene actualmente. Este tipo de terapia se basa en utilizar material genético como sustancia activa frente a diversas patologías y es aquí donde se encuentra el centro de atención de la presente revisión bibliográfica. Con la gran demanda de sistemas que vehiculizan el material génico han surgido en la última década, diversas formas de transportar dicho material. Una de estas formas consiste en el empleo de vectores no virales. Los cuales no son más que transfectores que eluden los problemas inherentes al empleo de estructuras víricas, como son las reacciones inmunológicas. La polietilenimina ha emergido como el polímero más prometedor en este ámbito por múltiples razones las cuales serán ilustradas en el presente trabajo.

Los objetivos de la presente revisión son otorgar al lector un conocimiento sobre qué es la polietilenimina, porqué suscita tanto interés hoy en día en terapia génica, y cuáles son algunas de sus aplicaciones, haciendo especial hincapié en el tratamiento del cáncer.

PALABRAS CLAVES: Cáncer, Polietilenimina, Terapia génica.

ABSTRACT

Therapy is a branch of health science that deals with the means used and the manner of application in the treatment of diseases in order to relieve symptoms or produce a cure. Today there are many therapeutic tools available to combat the diverse health problems faced daily. With this evolution of medicine, it is inevitable that more and better strategies emerge leading to a more innovative therapeutic spectrum. Within the new strategies, we find that gene therapy is currently one with most potential. This type of therapy is based on using genetic material as an active substance against various pathologies and it is on which this literary review is focused. With the high demand for systems that vehiculize genetic material, diverse forms of carrying said material have emerged in the past decade. One of these forms consists of the use of non-viral vectors. The non-viral vectors act as transfectants that evade problems inherent to the use of viral structures such as immunological reactions. Polyethylenimine has emerged as the most promising polymer in this field for many reasons which will be illustrated in this paper.

The objectives of this review are to give the reader an understanding of what Polyethylenimine is, why it has produced so much interest today in gene therapy, and what its applications are, with special emphasis on cancer treatment.

KEYWORDS: Cancer, Gene therapy, Polyethylenimine, .

INTRODUCTION

Therapy advances, reinvents itself and looks for new ways to address health problems. Nanotechnology, that could dominate as the therapy used to carry out nanoscale systems, has lead to significant progress in recent years[1]. In this new therapeutic field multiple systems necessary for the application and development of said technologies have appeared such as solid lipid particles, polymeric nanoparticles or liposomes[2]. Each one of these presents its own specific advantages and disadvantages that allow us to chose the most suitable vehiculization for each different situation and therapy. However, the choice does not stop there. These different types of systems, in turn, can be composed of different materials in order to optimize, even more, its use in more specific applications[3]. The materials used are very diverse, such as magnetite4or maghemite iron oxides[5], and control the location of the system thanks to an external magnetic field gradient; fatty acids, elements fundamental to the formation of liposomes that help the vehiculization of soluble substances or system functionalization and, finally, cite the polymers, a large group of substances with diverse applications in which we find the focus of this paper, Polyethylenimine (PEI).

PEI is an organic polymer that has experienced growing interest in recent years in medicine. This is due to its ability to complex DNA and protect itself once inside the cell, being the base of the majority of non-viral vectors used in transfection. Research into non-viral vectors is a booming field given the promise of gene therapy and the problems inherent in using the same viruses as they are, among others, immune reactions.

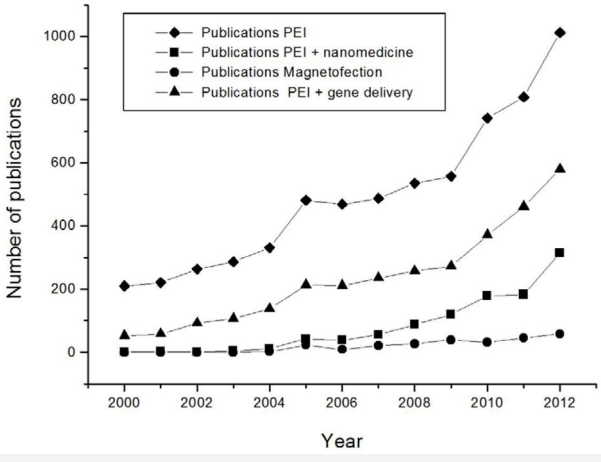
In this paper, we delve into the literature of the polymer thanks to major bibliographic search tools with the intent of clarifying what the position of PEI is in the field of new therapies, specifically in gene therapy, and how it can be used in the treatment of malignancies.

BIBLIOGRAPHIC OVERVIEW

The first literary work that makes reference to PEI, data from 1965, analyses its capacity as a protecting agent against the flocculation of colloidal suspensions of gold particles yielding values much lower than the average of other substances used for this purpose[6]. It wasn't until 1998 when references began to appear in the field of nanotechnology and directly in nanomedicina[7]. From that year, references, as well as article citations referring to the same, began to increase significantly. In order to get an overview of the importance and the state in which PEI is found in research, a bibliographic search was done between 2000 and 2012 in an important scientific biomedical database with four different search criteria: PEI, PEI + nanomedicine, magnetofection and PEI + gene delivery. Such criteria were considered because they not only allow the appreciation of the volume of work on the polymer but also illustrated how the works were distributed in its most relevant applications; one of them being gene delivery. The addition of a criterion such as magnetofection allowed us to detect how new terms within gene therapy continue appearing for increasingly specific processes that look for an improvement in genetic material transportation. The results, classified by year, are shown in figure 1.

As one can see in the figure, there is no denying the growing importance PEI has each passing year in research. It can be seen that from the year 2000 PEI has been used in gene delivery and the involvement of said polymer has been such that it is now the protagonist in more than half of the works in this field. Equally, terms like nanomedicine and magnetofection have gradually been incorporated into the surroundings of PEI. More specifically, nanomedicine has also become a field with multiple literary works relating to it. If we carefully analyze the figure, we can see that not all the publications on PEI encompass gene delivery and nanomedicine; this is because the polymer presents many other uses such as the creation of synthetic enzymes or the functionalization of other systems. Likewise, not all the publications on gene delivery correspond to the field of nanomedicine and magnetofection. This is because gene delivery is not a process exclusive to nanomedicine or gene therapy but can also be used in transfection processes in other fields such as industrial or microbiological. In conclusion, gene delivery is the field where PEI is finding more prominence and application in this decade and, as one can see, it is still a very current issue that has yet to find its limits.

Figure 1. Number of publications dealing with PEI as a function of time



TYPES OF POLYETHYLENIMINE

PEI is an organic polymer in which the chain of carbon atoms that has a nitrogen heteroatom for every two carbon atoms. The presence of those nitrogen atoms confers a *strong positive surface charge* from which most of its properties and uses are derived. A sample of this surface charge can be found in the coating of magnetic cores of magnetite whose surface charge, once treated with citric and according to results obtained in its own research, generated values of -40.1 ±3.7 mV. After its polymer coating those values shifted between 33.7±1 mV and 74.4±4.8 mV depending on conditions used to obtain it.

Before talking about the different properties and how they are conditioned by mainly two variables, such as its degree of cross-linking and its size, different types of PEI and how to obtain it will be exhibited in order to better understand the concepts later on.

There are two distinct types of PEI; lineal PEI (IPEI) and cross-linked PEI (bPEI). Both differ in structure and the degree of branching of the chains, IPEI being strictly linear and bPEI being cross-linked. Both structures can be seen in figure 2.

One of the methods of obtaining bPEI consists of the controlled polymerization reaction of aziridine or, more commonly known, ethyleneimine. This reaction produces PEI with a high degree of branching as the new ethyleneimine monomers have a higher probability of joining the more substituted nitrogen atom.

On the other hand, we have the IPEI that can be obtained through a reaction of ring-opening polymerization of N (2-tetrahydropyranyl) aziridine. The chemistry of the reaction makes the monomers from this opening incorporate and form a chain, contrary to the previous reaction, and produces long lineal chains of PEI.

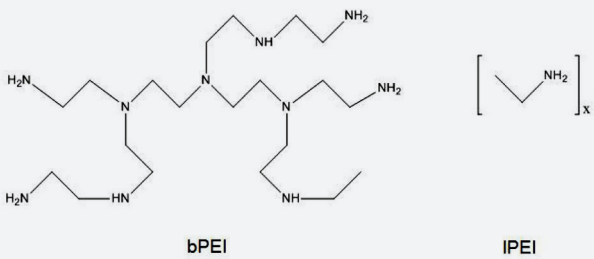
In addition to the degree of branching of the polymer, another determining property is the size of the polymer itself. The range of PEIs regarding molecular weight (MW) is also very high and can oscillate from 11 KDa to 800 KDa describing the PEIs as having low, intermediate or high MW[8].

Concluding, we can find six types of PEI: IPEI of high, intermediate and low MW and bPEI of high, intermediate and low MW.

MAIN CHARACTERISTICS OF PEI

The two main characteristics to consider of PEI are the degree of cross-linking and it's MW, especially if we are going to talk about its relation with genetic material and its capacity to vehiculize it. Below, small sections are devoted to speak about each one.

Figure 2. Chemical structure of PEI.



Degree of Branching

How the degree of cross-linking influences the behavior or the polymer is a fact that continues rediscovering itself. Currently, it can be said that the higher the degree of cross-linking, the higher quantity of tertiary and primary amines appears in the chemical structure of the polymer, with a consecutive loss of secondary amines, reaching the extreme case of comparing IPEI, which only contains secondary amines, with bPEI, which contains approximately 25% of secondary and tertiary amines and 50% of primary amines. These different proportions of amines significantly influence several of the properties required in evaluating gene therapy such as cell viability, the ability and method of complexing DNA, the ability of transfection, and nuclear promotion, etc. Because of this, we can have PEIs significantly different from each other depending on the degree of cross-linking[9]. As it may be deemed crucial in the vehiculization of genetic material, how the degree of cross-linking influences the ability and method of complexing DNA as well as the differences that can be found between IPEI and bPEI will be explained.

bPEI is capable of complexing DNA in small spherical vesicles of nanometric sizes and in a wide variety of environments. However, in this environment, IPEI forms much larger micrometric complexes and must resort to a salt-free medium and elevate the N/P ratio, consisting of the ratio of nitrogen atoms belonging to PEI compared to those of phosphorous of the genetic material, in order to obtain complexes of similar sizes and shapes to those obtained with bPEI. Apart from this, it has been observed that the IPEI complexes presented, in general, increased cell viability, greater capacity to promote cellular localization and, ultimately a greater power of transfection[8,10]. Much has been discussed about this fact because, in principle, one would expect a greater capacity for transfection of bPEI due to its greater ease ("proton sponge") of maintaining the pH of the endocytic vesicle environment when the

cell attempts to destroy its contents downloading a large amount of protons on it to lyse it. This property of bPEI is justified by its wide variety of amines and it is because of this fact that it is considered more efficient because it protects the genetic material once inside the cell. Returning to transfection efficiency, some authors argue that the disparity in the results obtained in the field for IPEI and bPEI find their explanation in a fact that may be overlooked, such as the cell cycle phase where the cells are transfected. Those authors have presented papers as evidence that IPEI shows less dependency on the cell cycle than bPEI at the time of the transfection of target cells. This means that when testing on cell lines or cultures that exhibited low or no growth rate, IPEI will show greater efficiency while if testing on cell lines or cultures with higher growth rate, bPEIs will be found[11].

Size

The variety of MW of PEI is huge. The interval of sizes oscillates between a few KDa and 800 KDa. With the intention of using the most suitable PEI for each type of biomedical use, many authors try to relate the size with different behaviors of the polymer and the system to be used. Discussing gene delivery, a pair of well-known relationships is the influence of the size of PEI on the *in vivo* toxicity of the system and in the transfection efficiency. PEIs with greater MW, for example, 800 KDa, have been described as being more toxic than those with a lower MW, for example 25 KDa. However, in contrast, the former are much more efficient if we talk in terms of transfection. This has been a dilemma since the potential of PEI in gene therapy was discovered and research has been done to find the most suitable size for gene delivery obtaining, in an acceptable range of toxicity, the greatest possible transfection efficiency. This search finally gave the weight of 25 KDa, reason for which it is the most used PEI MW in gene vehiculization[12].

OTHER USES NOT RELATED TO THE TRANSPORT OF GENETIC MATERIALS.

Although we will focus primarily on the uses of PEI as a gene carrier, one cannot ignore that other significant parts of the polymer exists, regarding uses that do not correspond to gene therapy. Some of the most frequent uses of PEI as a polymer outside of gene therapy are:

1. The electric surface properties, markedly positive characteristics of PEI, provide it with a unique ability to capture different molecules. This capability is used in the industry to create very specific chemical sensors in complex chemical environments. In these environments it is more difficult to discriminate between the molecule of interest and the rest of the molecules and it's more complicated to obtain a good sensor. The synthesis and use of carbon nanotubes functionalized with PEI to be used as a method of rapid analysis that detects the degree of rancidity of soybean oil could be an example of great interest in the food industry[13].

2. Another application is the creation and study of synthetic enzymes. In them, bPEI is the enzyme body on which necessary chemical groups are incorporated to provide it with the desired form and reactive capacity. bPEI is used in this field for several reasons: its cross-linked structure, its high solubility in water and its large number and variety of amino groups ready to be functionalized. A set-up and characterization of how bPEI can be used for obtaining various enzymatic molecules has been described by Junghun Suh et al. in some of their publications[14]. In them, they studied how enzymes with a bPEI base have different behaviors versus pH ranges such as the conformational behavior or its ability to continue to attract some ligands.

3. PEI also can be used as a catalyst for the synthesis of star polymers, a special formation consisting of many polymer chains that converge at a single point. This paper[15] describes a hybrid system with two polymers, PEI and poly(ε-caprolactone), where the core from which the poly(ε-caprolactone) chains start is formed by PEI. PEI acts not only as an initiator in the synthesis of polymerization but also as a part of the star polymer allowing the use of both as a nanocarrier that can hold and transport lipophilic substances from within thanks to the hydrophilic PEI.

4. Another possibility within the pharmaceutical field is the use of the polymer as part of drug carrier systems. The possibilities are numerous and the polymer can be part of magnetic nanoparticles[16], nanogels[17], liposomes[18], and micelles[19] among others. Regarding the latter, to give an example, Li Yan Qui et al. vehiculize an active antitumor, such as doxorubicin, in a micellar system formed by PEI and poly(ε-caprolactone).

5. Recently, a new way of applying magnetic cores for obtaining magnetic resonance imaging (MRI) of tumors *in vivo* has appeared. This new technique consists of using small molecules known as aptamers capable of concentrating specifically on tumor cells of the tissue *in vivo*. This permits them to act as probes that carry magnetic cores required for obtaining magnetic resonance imaging (MRI). However, the use of these molecules consisting of small nucleic acid sequences, presents certain difficulties. One of these

difficulties is its rapid degradation when circulating in the bloodstream. The use of PEI to protect these systems formed by aptamers and magnetic cores has demonstrated that it ensures that the vehicle remains in the blood longer and the technique can be applied to avoid the half-life problems of the aptamers[20].

POLYETHYLENIMINE AND GENE THERAPY

DNA complexing process

Before delving into the uses of PEI in gene therapy to treat cancerous pathologies, it´s important to know how it interacts with genetic material to form nanoparticles (NP) that can act as a non-viral vector. For this, how the N/P ratio and the environment where the synthesis of the non-viral vector is carried out influence the formation thereof and how this can influence the efficiency of transfection will be briefly commented on.

N/P ratio

The NP ratio is probably the condition to take into most account when forming the non-viral vector. It is fundamental to choose the right proportion of nitrogen atoms belonging to PEI compared to the phosphorous genetic material because it will determine the overall charge of the complex as well as how and with what intensity PEI will be joined with the genetic material[21]. It is necessary to take into consideration that the overall charge of the vector should be predominantly positive, since the interaction with the cell surface depends on it. However, it cannot be excessive because a cationically elevated complex is cytotoxic[22]. Something else to take into account is that the "proton sponge" capacity should not be altered by an excess of nitrogens occupied with the phosphates of the genetic material. This would result in an excess of amines available for buffering the proton discharge made by the cell when the nanoparticle is internalized into it causing the consequent destruction of genetic materials. Finally, the interaction established between PEI and genetic material should not be too strong because it would prevent dissociation, and therefore, the release of the genetic material of the system after overcoming the intracellular obstacles. There are many researchers who have worked on this and it is important to note that, in view of the results, a specific N/P ratio exists for each PEI-genetic material pair. This makes the parameter an objective that should be studied, since it not only determines the viability of target cells but also, through the previously described mechanisms, determines the transfection efficiency of the NP[23,24].

Environment

As for the composition of the medium where the entrapment of genetic material by PEI takes place, the reaction conducted between them is quite simple. Normally a saline solution of NaCl undergoes mechanical agitation with the genetic material, while another solution of PEI is added little by little[25,26,27]. In this way, PEI fuses together with the genetic material, which can be anything from plasmids to small sequences of RNAi, forming the NPs. However, over time, there has been an increase in exploring ways of trying to control the process by varying the conditions under which it is performed. This is interesting because the way that the NP is formed is going to be crucial for the transfection process. Numerous studies exist that demonstrate how the use of appropriate conditions can positively influence the transfection efficiency, such as the presence of surfactants[28] or the use of glucose in the medium instead of NaCl[29].

Use of PEI in gene therapy

Gene therapy uses two types of vehicles to facilitate the delivery of genetic material to the site of action for therapeutic purposes. Both types of vectors are classified according to their nature as follows:

1. Viral vehicles: more traditional in gene therapy. Its main characteristics are its high power of transfection, natural tropism towards specific cell types, intrinsic ability to escape the endolysosomes and natural mechanisms to direct the genetic material towards the core. On the contrary, they are immunogenic capable vehicles, which can produce chromosomal insertions or activate proto-oncogenes. Additionally, they have a limited capacity of genetic material and in turn can produce toxic reactions or may be contaminated by live viruses[30,31].

2. Non-viral vehicles: arise in response to the growing interest in gene therapy and the need to overcome the main problems of viral vectors. While they can achieve immunogenic vectors, without the risk of insertions and with a higher capacity of genetic material, the transfection efficiency is significantly lower. Here is where PEI ranks as the leading exponent of non-viral vectors in gene therapy[30,31,32].

Non-viral vectors have a prominent role in numerous studies focused on the treatment of multiple types of diseases such as cancer[33], infections[34], circulatory disease[35] and autoimmune or recessive genetic defects like cystic fibrosis[36]. Below, we will describe the main cancer treatment strategies based on gene therapy and the decisive role non-viral vectors formed by PEI play in some of them:

1. Immunogenic therapy: this type of therapy aims to improve and focus an individual's immune response against malignant cells. The idea of this strategy consists in using genes that modify target cells so that they acquire the ability to attract and enhance the immune response against them. This can be achieved either by providing them the ability to synthesize chemotactic molecules or allowing them to behave as antigen presenting cells[30]. The IL-2 is a known immunogenic response stimulant and its use in the treatment of cancer is being researched. However, due to problems in its stability *in vivo* and numerous adverse affects, its administration to the patient does not constitute a good therapeutic strategy. The use of PEI to specifically transfect tumor cells in such a way that it overexpresses the molecule allowing a selective localization thereof is a promising way to avoid the problems mentioned above[37].

2. Suicide gene therapy: in this type of therapy the transfection of target cells is produced in a way that they acquire the ability to produce a non-native enzyme. Previously, a patient was administered a harmless substance, or as harmless as possible, that acted as a prodrug. This, to reach the transfected cells, will be modified by an induced enzyme converting itself into an active substance. In this review[38], Springer, C. J. et al. explain step by step the implementation of suicide therapy in cancer treatment, with emphasis on the most used non-native enzyme prodrug pairs. Some of these pairs are CB 1945, bacterial nitroreductase and 6-methoxypurine arabino nucleoside**,** viral thymidine kinase. In this other article[39], Bing Liang et al. use a non-viral vector formed by polyethylene glycol, folic acid and PEI (PEG-AF-PEI) to induce the production of a non-native enzyme and an immunogenic agent. These are the 5-fluorocytosine, cytosine deaminase (5-FC/CD) and the inducing ligand of TNF-related apoptosis, respectively. Applying the prodrug, in this case 5-fluorocytosine, a synergistic relationship develops between both types of therapy, suicide gene therapy and immunogenic therapy, thereby achieving greater tumor efficacy with the combined use of both therapies as opposed to the use of just one.

3. Antiangiogenic Therapy: Heightened growth and metabolic activity are common features of most tumors. In order to carry out these processes, the tumor mass requires a large supply of nutrients and energetic sources from the blood. It is because of this that an angiogenesis process starts to supply the tumor according to its metabolic needs. This is where antiangiogenic therapy comes into play, thanks to genetic modification directed towards the endothelial cells near the tumor, acquiring the ability to synthesize substances that prevent the development of new controlled vessels and stopping excessive growth of the tumor. Some of the molecules used as antiangiogenics are angiostatin, batimastat or SU5416. However, this type of therapy is being studied as an adjunct to other more widespread therapies like chemotherapy or radiotherapy resulting in the combination of therapies such as cyclophosphamide, anti-cancer agents and TNP-470, an inhibitor of the migration and proliferation of vascular endothelium[40]. The use of PEI for obtaining non-viral vectors that transport these types of genetic materials has not only allowed the possibility of vehiculization but has also, in some cases, improved the transport over the use of other types of vectors [41].

4. Tumor suppressor genes: the process of transformation from normal cell into a tumor is almost always mediated by two different genetic events: the alteration of proto-oncogenes leading to an abnormal cell division cycle or a mutation of the tumor suppressor genes that determines the protein synthesis incapable of controlling the cell cycle. One of the possibilities of gene therapy lies in restoring the original genetic charge of the tumor cell with the subsequent reconversion to a healthy cell. Although in theory this is possible, this type of approach has some issues that are still to be overcome. It is possible that the most notable of these is the need for complete restitution or the genetics of each and every one of the cells. Otherwise, the continued growth of the tumor would not be prevented and it could return even if a single cell was not restored[32].

CONCLUSIONS

After reading this work, it is clear why interest has aroused in PEI research and, more specifically, in biomedical research. Its characteristics and, mainly, its strong, positive surface electric charge, qualify it as a promising polymer for obtaining polymeric nanoparticles that act as non-viral vectors in gene delivery. This use, in turn, puts the spotlight on gene therapy and makes it participate in the treatment of many pathologies where it has made room for itself such as cancer, infections or cardiovascular diseases, among others. Due to the strong presence of cancer in our society and the impact it has on it, this paper aims to illustrate how PEI similarly has a fundamental role in the fight against it.

However, not everything is said about the polymer

and, currently, numerous works are emerging about the improvement of gene delivery through the functionalization of PEI with different molecules yielding promising results[42,43].

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