Study of the toxicity, the ecotoxicity and the genotoxicity of polymers and nano-polyplexes with biomedical applications in the gene therapy field

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FINAL REPORT
Nanoparticles have gained a huge interest in recent decades covering a large variety of applications and industries. In the research field of biomedical applications, these particles have been widely applied and studied. One of the main advantages of nanoparticles within this field is their very limited size. Nanoparticles are typically smaller than 100 nm and are thus expected to be easily taken up by cells. This opens up a huge range of interesting applications including the development of drug and gene delivery systems.

Gene delivery has become an increasingly important strategy for treating a variety of human diseases, including infections, genetic disorders and tumors. To avoid the difficulties of using viral carriers, more and more non-viral gene delivery nanoparticles are developed, among them, the polyethylene imine (PEI) is considered as one of the most effective polymer based solution. Polyethylene imines have been shown to be capable of delivering DNA into various cell lines and in various in vivo applications. PEI was, for example, successfully used as a carrier for the delivery in a tumor of the suicidal thymidine kinase gene of the Herpes simplex virus, and the subsequent suppression of the tumor PEI was also successfully used as a carrier for siRNA, for which there is many potential therapeutic applications and the delivery is the biggest challenge. Another polymer, the Poly(2-N,N-dimethylamino ethyl methacrylate) (PDMAEMA) is investigated using as a alternative because it is expected to have a better biocompatibility. The polymer:DNA polyplexes form nano sized particles in the solution. They can therefore be included in a broader class of materials with the nanomaterials, and they can be considered for research to answer questions typical of the emerging field of nanotoxicology. Indeed, the development of new materials at the nanoscale raises several questions on the interaction of these materials with the living world. At this scale, the size matters as well as the chemical properties when it comes to describe the properties of the material and its interactions with living cells. On one hand, the nanoparticles and the nanoscaled materials show a huge surface area and a related higher activity. On the other hand, the small size of the nanoparticles facilitates their uptake by cells and transcytosis across the epithelial or the endothelial barrier into the blood and lymph circulation where they can reach sensitive organs.

Given the expectations for the applications of these polymers, a series of questions can be raised about their hazards for human beings and the environment. Therefore we started this study with the aim to describe the toxic response induced by different forms of the polymers and their nano-particulate form when associated with nucleic acids (polyplexes). The toxicological properties of PEI and PDMAMA and their nucleic acid associated nano-particulate derivatives were considered using a large variety of test.

PEI was used as its commercially available form, while PDMAEMA was produced by one of the partners specifically for this study. PDMAEMA produced for this study could be obtained in high yield and purity, allowing all the groups to work with material coming from the same batch. Both polymers can fully condense the nucleic acid used for this study when mixed with a polymer:DNA charge ratio of 2:1 or of 4:1.

The ecotoxicological side of this study was studied trough the effects of the polymers and the polyplexes on aquatic unicellular alga (Pseudokirchneriella subcapitata), on the embryogenesis of an aquatic tetrapod (Xenopus laevis) and on the gene profiling of a bacterium (E. coli) and a mammalian cell line (human hepatocytes, HepG2) The characterization of the human toxicity of the polymers and the polyplexes was done with different monoculture of diverse cell types (HepG2 hepatocytes, keratinocytes, A549 epithelial cells and THP-1 monocytes) , co-culture of epithelial cells and macrophage differentiated monocytes (A549 and THP-1) and reconstituted epidermis. Genotoxicity of the polymers and polyplexes was assed on A549 cells.

In the different system tested, the PEI polymer is more toxic than the PDMAEMA polymer. The same difference is seen for the polyplexes made with PEI and PDMAE, and the higher the charge ratio, the more toxic are the polyplexes. Also, the polymeric "pure" form is in general more toxic than the polyplex formed by a polymer:DNA complex. The picture is not as straightforward and some variations from this simplistic summary are observed, however, a general conclusion is that the effects of the polymers and their related polyplexes on biological material is mainly driven by the amount of positive charges they present. In this perspective, the more the charges are equilibrated in-between the DNA and the polymer (1:1), the less the polymer should be able to cause damages.

Considering the nano-particulate form of the polyplexes, it is difficult to conclude to effects typical to nanoparticles with the results we obtained during this project. To see if there are effects specific to the nano-particulate state of the polyplexes, it is probable that studies going deeper in the description of the interactions between the cells and the nano- polyplexes will be needed in the future.

In summary, this study establishes a rough picture of the toxicity of two cationic polymers and their nano-particulate derivatives with different charge ratios. It is difficult to draw definitive conclusions from such a short collaborative experience, but tracks are laid for a more in deep study of the effects of the cationic polymers and/or the nano-particulate polyplexes on living cells, bacteria are reconstituted tissues.

SUMMARY

Gene delivery has become an increasingly important strategy for treating a variety of human diseases, including infections, genetic disorders and tumors. To avoid the difficulties of using viral carriers, more and more non-viral gene delivery nanoparticles are developed, among them, the polyethylene imine (PEI) is considered as one of the most effective polymer based solution. Polyethylene imines have been shown to be capable of delivering DNA into various cell lines and in various in vivo applications. PEI was, for example, successfully used as a carrier for the delivery in a tumor of the suicidal thymidine kinase gene of the Herpes simplex virus, and the subsequent suppression of the tumor PEI was also successfully used as a carrier for siRNA, for which there is many potential therapeutic applications and the delivery is the biggest challenge. Another polymer, the Poly(2-N,N-dimethylamino ethyl methacrylate) (PDMAEMA) is investigated using as a alternative because it is expected to have a better biocompatibility. The polymer:DNA polyplexes form nano sized particles in the solution. They can therefore be included in a broader class of materials with the nanomaterials, and they can be considered for research to answer questions typical of the emerging field of nanotoxicology. Indeed, the development of new materials at the nanoscale raises several questions on the interaction of these materials with the living world. At this scale, the size matters as well as the chemical properties when it comes to describe the properties of the material and its interactions with living cells. On one hand, the nanoparticles and the nanoscaled materials show a huge surface area and a related higher activity. On the other hand, the small size of the nanoparticles facilitates their uptake by cells and transcytosis across the epithelial or the endothelial barrier into the blood and lymph circulation where they can reach sensitive organs.

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