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SHORT COMMUNICATION

POLYMER THERAPEUTICS FOR TREATMENT OF VIRAL INFECTIONS SUCH AS EBOLA – HOW TO TEACH NEW TRICKS TO AN OLD DOG? A HYPOTHESIS.

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Summary

Polymer drug delivery systems were during last few decades proven to be efficient potential therapeutics for cancer treatment, especilly for the treatment of solid tumors, where they may take advantage of the Enhanced Permeability and Retention (EPR) effect for tumor-specific passive accumulation. Controlled release of anticancer drugs in cancer cells may be triggered by. e.g., cathepsin B activation after endocytosis. Endosomal proteases, especially cathepsins B and L, are known to be one of the key factors influencing some viral infections. For instance Ebola virus requires partial proteolysis of its surface glycoprotein for efficient endosome escape within its life cycle. We hypothesize that polymeric cathepsin B and L inhibitors may utilize advantages of polymer delivery systems for more effective treatment of viral infections with cathepsin inhibitors reducing systemic toxicity and increasing efficacy by targeted delivery of these inhibitors.

Key words: Ebola virus; cathepsin; polymer; inhibitor

Nanosized drug delivery systems were during last few decades proven to be efficient potential therapeutics for cancer treatment, some of them reaching use in clinical practice or being in clinical trials [1-3]. They may be based, e.g., on soluble biocompatible polymers such as poly[*N*-(2-hydroxypropyl)methacrylamide], nanoparticles, micelles or liposomes [3, 4]. The nanosized drug delivery systems share some common features. If they are biocompatible, they may have prolonged blood circulation time (hours to days) because of having hydrodynamic diameter larger than pores in glomerular membrane. This means that they can be only hardly eliminated by kidneys [5] and thus far slower hepatobiliary excretion route must take place.

Nanoparticles with limited biocompatibility or with certain structural motives such as DC-SIGN-attacheable D-mannosylated surface are rapidly scavenged by reticuloendothelial system [6-9], i.e., they end up in liver, spleen and lymph nodes. Biocompatible nanoparticles may take advantage of the Enhanced Permeability and Retention (EPR) effect (see Figure 1) for tumor-specific passive accumulation [10]. Solid tumors cannot grow above relatively small diameter typically around 1 mm without forcing the organism to form neovasculature into them.

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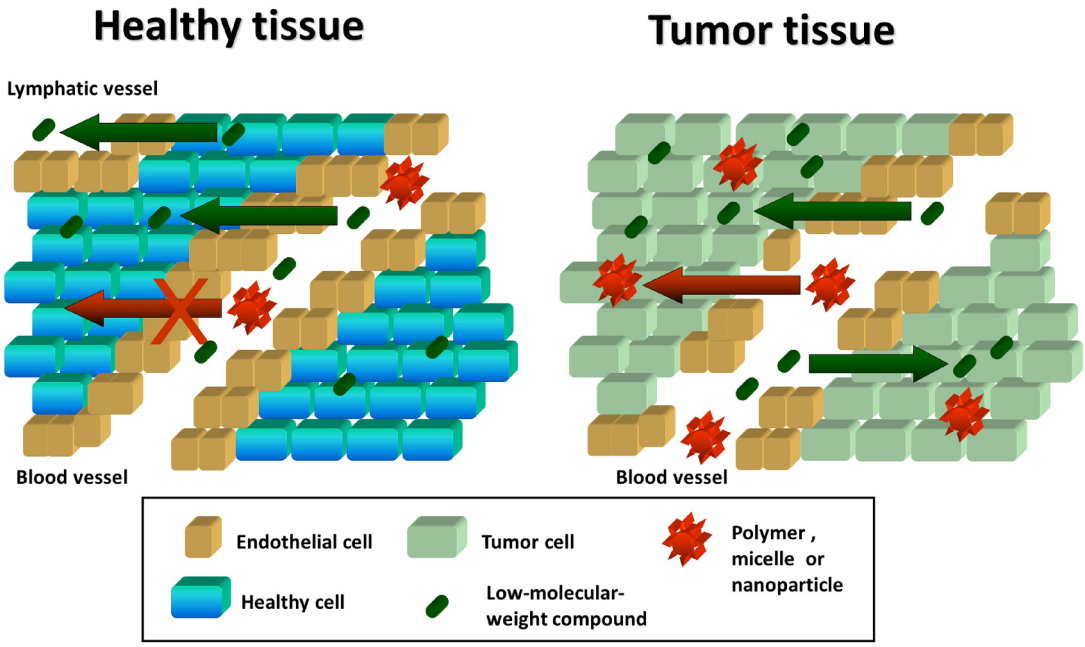

Figure 1. Scheme of the Enhanced Permeability and Retention (EPR) effect.

The newly formed vessels are grown rapidly and have different anatomy being permeable for particles up to hydrodynamic diameter ca 200 nm. The nanomedicines may therefore penetrate into solid tumor tissue and in addition, tumor tissue is generally lacking lymphatic drainage, so they are retained there. Inflammated/damaged tissues posses similar properties as solid tumor according to permeability for nanoparticles, because access of immune system is to be facilitated into such areas [11].

In a typical anticancer nanomedicine concept, the polymer carries the drug (e.g., doxorubicin) in nonactive form which should be stable as much as possible in bloodstream while releasing the drug in tumor cells. Drug release may be triggered by a drop in pH due to acidic environment in hypoxic tumor interstitial space (pH ca 6.5) or in endosomes of cells after internalization (wher pH gradually drops to ca 5) [12], e.g., by hydrolysis of acidolabile hydrazone bond [13]. Another mechanism for endosome-localized drug release may be utilizing cathepsins, endosomal acid proteases. In this way, cathepsin B-cleavable tetrapeptide linker Gly-Phe-Leu-Gly was introduced in 1983 by dr. Kopecek´s group for doxorubicin delivery [14] and later used by other research groups [13,15].

The way how viral particle enters the cell to be infected greatly differs according to virus type, however the general scheme is attachment to receptor on the target cell, receptor-mediated endocytosis, endosome escape into cytoplasm and further steps including capside disassembly, replication of viral nucleic acid etc. Principially all steps may be therapeutic targets for treatment of viral infections.

Viruses from the family *Filoviridae*, e.g. Ebola virus, require after endocytosis partial proteolysis of the viral surface glycoprotein GP1 by endosomal cathepsins for efficient endosome escape, crucial for further steps of viral life cycle [16-19]. Proteolysis makes the surface glycoprotein membrane-active in acidic (pH ca 5) endosomal environment and also exposes binding sites facilitating endosome escape, assumed to be with binding Niemann-Pick C1 protein (NPC1), a late endosomal and lysosomal protein [16-19]. For Ebola virus, causing deadly haemorrargic fevers, it is thought that the key activating proteases are endosomal cathepsins B and L[17] (different for various Ebola strains [16]). Acidification of extracellular space together with extracellular activation of digestive cathepsins where they are normally not active is thought to be also the key factor in drastical damage of tissues, typical for *Filoviridae* viral infections and in particular for Ebola [20]. Recently it was discovered that also numerous other viruses, e.g. caliciviruses [21], noraviruses [21] or coron-aviruses such as SARS virus [22,23] are cathepsins-dependent in their endosomal escape, especially cathepsin L is the key enzyme in these cases.

Not surprisingly, several patents as well as scientific articles recently emerged [23-29] on antiviral agents based on cathepsin inhibitors, all of them low-molecular-weight small molecules mostly based on peptide-analogic nitriles, hydrazides or alkylating agents [23-26]. This approach has one extra advantage that the target is not a viral protein, but human enzyme, which is not subject of mutation and subsequent viral parasite evolution leading to resistance. Although a number of them are efficient in nanomolar scale in vitro, they are generally cytotoxic (they decrease cell viability and may induce apoptosis), because cathepsins are of high importance for normal function of the cells and the agents under considerations are not infected cell-specific. *We hypothesize, that polymer drug delivery systems may circumvent this problem and increase intivirotic efficacy due to targeted delivery of these substances.*

Polymer drug delivery systems (both nanoparticulate and soluble polymer-based) should make these agents more specific, because they penetrate much faster into inflammated tissues via „EPR-like" effect than into normal tissues and also their permeability in tissues is considerably more enhanced by tissue damage (typical for *Filoviridae*) than for low-molecular-weight agents [11,20]. Their way of cell entry is via endocytosis [30,31] and they should therefore be colocalized in endosomes with virions, which should greatly enhance their antivital efficacy. Additional targeting may be implemented, e.g. with viral capside-specific ligands or with D-mannose [7-9] to target macrophages and dendritic cells, the cell types typically damaged by *Filoviridae* viral infections. Macromolecular carriers with molecular weight above renal threshold have also prolonged blood circulation times [5]. The inhibitor may be bound to polymer carrier by a stable bond or site-specifically released in endosome. Another way may be designing polymer drug delivery systems combining drugs with different mode of action, e.g., polymer delivery systems combining cathepsin inhibitors with favipiravir. Such system would have additional benefit in direct delivery of favipiravir to target cells due to restriction in permeability to health tissue. Macromolecular protease inhibitors were published to, e.g., treat osteoporosis by cathepsin K inhibition [32], *but to best of our knowledge not as antiviral agents.*

In conclusion, our hypothesis is that a polymer carrier with cathepsin inhibitors will efficiently block the development of Ebola virus with significantly reduced systemic toxicity. Tailored design of such macroinhibitors is underway in our group, we are currently searching for appropriate partners for biological testing.

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