A new strategy for the treatment of viral infections - Virus like particles loaded with antiviral drugs for antiviral therapy and description of a new method to obtain genome free viruses as an alternative to virus like particles

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Abstract

Intact viruses, containing no viral genome but which are loaded with a therapeutic drug or virus like particles loaded with antiviral drugs are potentially selective drug carriers for the intracellular delivery of therapeutics.
These virus constructs could be used as therapeutics for the therapy of viral infections. For example HI-viruses could be designed, that are loaded with antisense oligonucleotides that avoid replication of the wild type HI virus. The advantage of such antisense oligonucleotide loaded viruses could be the fact that they enter cells and deliver the therapeutics to the host cells of the wild type HI virus. It should be possible to treat all viral infections with this method. Wild type viruses are therapied by a modified virus of the same type or a virus like particle containing a antiviral drug – e.g. an antisense oligonucleotide. This method should work for all viruses.
Additionally, a new method is described how intact viruses, containing no viral genome but which are loaded with a therapeutic drug could be obtained. The major obstacle is that the viral genome and viral proteins must be present for virus assembly on the one hand, on the other hand it must be removed subsequent to virus assembly in order to obtain a safe drug carrier. The idea is: Virus assembly takes place in the presence of the viral genome with subsequent removal of the viral genome. Firstly the viral genome is immobilized on a solid support – comparable to DNA chips. Virus assembly proceeds at the solid support. After virus assembly and drug loading the immobilized viral genome is removed. Viral proteins could be obtained from a packaging cell line. The lysate of such a packaging cell line containing the viral proteins is incubated with the immobilized viral genome to allow virus assembly. The lysate is removed after succesful assembly. The next step would be the loading of the virus with a therapeutic drug. As the viral proteins contain amino and carboxy groups they can be chemically modified by a therapeutic drug. Finally the drug loaded virus is removed from the solid support and with it from the viral genome. This could be a strategy to obtain drug loaded, intact viruses lacking the viral genome.

Introduction

A virus consists of an envelope (proteins), a number of other functional proteins and a nucleic acid (DNA or RNA) [1]. Some virus types – such as the influenza virus – additionally contain a lipid layer [1].
They specifically recognise the surface of the host cells. Subsequent to cell surface binding, the viral genome is internalized into the host cell [2]. Viruses can be seen as high specific carriers for the intracellular delivery of nucleic acids.
Despite of their high molecular weight and their negative charged groups nucleic acids can not enter cells [3]. Gene technique utilizes viruses as carriers of nucleic acids into cells. The virus genome is modified by a non-viral polynucleotide.

In order to obtain an intact virus all components it consists of – proteins and genome – must be

present during virus assembly [4, 5]. Viral proteins and viral genome recognise each other [4]. This recognition is essential for the survival of the specicif virus [4]. Without this specific recognition other nucleic acids than the one of the virus could be encapsidated. The nucleic acids must have a specific size and elelctrostatic interactions between the negative charged sugar-phosphate backbone and the positive charged residues of the virus proteins play an important role in this recognition process [4, 6, 7, 8]. In retroviruses a specific region of the genome – the so called packaging signal – is responsible for encpasidation of the genome [10].
By coupling ricin A to a bacteriophage RNA the selective delivery of ricin to host cells was possible [11]. However, this procedure is ineconomic.
There are several reports about virus like particles. Capsid proteins are able to agglomerate to particles in the absence of the viral genome [12, 13, 14, 15, 16]. They can be used as vaccines [12,13,15]. HI viruses agglomerate in the presence of so calles crowding agents – such as polyethylenglycol), however the decay of these virus like particels occurs quickly when the concentration of the crowding agents decreases [14].
Virus like particles, consisting of the VP1 (papilloma virus) are able to adsorb foreign DNA [17, 18]. Additionally they show affinity to sialyl residues being displayed on the surface of mammalian cells [17, 18, 19, 20]. A tissue specific targeting would not be possible with these viruses. The decay of virus like particels can be avoided by performing virus assembly under mild oxidative conditions to form disulfide bonds among different capsid proteins [20]. The incorparation of tissue specific ligans to VLPs would lead to tissue specificity [20].
The nucleocapsid of influenza viruses could can be removed from the lipid bilayer which contains cell specific lipids and viral proteins in order to obatin empty influenza envelope [21, 22].

In this research proposal I want to present an idea how viruses could be obtained that are loaded with drugs instead of the viral genome. The major advantage of such viruses would be their ability for a selective intracellular drug delivery. Additionally and the major point, is that either genome free viruses loaded with antiviral drugs or tissue specific virus like particles could be used as specific therapeutics against viral infections caused by the same virus type as they should have the same tissue distirbution.
Antisense oligonucleotides are drugs as well, they are synthetic oligoribonucleotides that are complementary to a specific m-RNA and they consists of 16-20 base pairs [23]. They are tools for the specific inhibition of the intracellular synthesis of a specific protein [23]. However, their intracellular bioavailability is low [24]. By coupling antisense oligonucleotides to carrier molecules they can be delivered into the cell [25, 26]. In contrast to viruses - most carriers are not very specific, and some – such as somatostatine shows affinity only to a small number of exotic cell types [26].
Whereas the utilization of viruses in gene technique is more or less routine – they are not used as specific drug carries. In order to use the potential of antisense oligonucleotides it would be a promising approach so load viruses with therapeutic antisense oligonucleotides instead of the native viral genome. Such viruses could be used as specific therapeutic antiviral approach against viruses of the same type.

Antisense or drug loaded viruses as antiviral therapeutics

Virus infections could be treated with modified viruses or virus like particles. Whereas the infectious wild type virus contains the viral genome, the antiviral viruses or virus like particles would contain antiviral drugs instead of the viral genome. These artifical virus constructs are suggested to show the same biodistribution as the wild type virus.
For example HI-viruses could be designed, that are loaded with antisense oligonucleotides that avoid replication of the wild type HI virus. The advantage of such antisense oligonucleotide loaded viruses could be the fact that they enter cells and deliver the therapeutics to the host cells of the wild type HI virus. It should be possible to treat all viral infections with this method. Wild type viruses are therapied by a modified virus of the same type or a virus like particle containing a antiviral drug – e.g. an antisense oligonucleotide.
Alternatively antisense oligonucleotides or other antiviral drugs (reverse transcriptase inhibito, proteinase inhibitors) could be adsorbed to virus like particels. After the infection of a cell line with viruses, the cells are incubated with the modified drug loaded viruses or the drug loaded virus like particles. The replication efficacy and cell

viability of the the cells treated with drug loaded viruses or virus like particels should be compared with control cells that were only infected.
This strategy could lead to an improved drug targeting and therapy of virus infections.

Hypothesis for the assembly of viruses loaded with drugs and lacking the viral genome

To obtain genome free viruses (an alternative to virus like particels) is difficult as genome and proteins are necessary for virus assembly. The idea is to allow virus assembly in the present of the viral genome with subsequent removal of the viral genome in order to obatin a safe, non-infectious drug. The trick is to fix the viral genome on a solid support – comparable to DNA chips – and let virus assembly take place at the solid support. After assembly and drug loading the virus is removed from the solid support. The immobilized viral genome could be incubated with a lysate form a packaging cell line. The drugs should be covalently coupled to the assembled viruses with a linker that contains a group for intracellular cleaavage.

The idea is to immobilize the viral genome to allow virus assembly at the solid support. This makes the isolation of the viral genome easier.

Immobilisation of the viral genome to a solid support

As virus assembly is supposed to take place at a solid support, the viral genome must be immobilized. This can be realized by techniques used for DNA chip building. It is planed to covalently couple the viral genome to the solid support. In order to show that virus asembly works at the solid support the solid support loaded with viral genome must be incubated with the viral proteins which could be obtained from a packaging cell line.

Viral proteins from a packaging cell line for solid phase virus

A packaging cell line contains a copy of a defect retorviral genome [10]. All information for the intracellular synthesis of viral proteins are present, but the genome cannot be encapsidated as the packing signal is missing [10]. Such a cell line delivers all virus components necessary for virus assembly at the solid support.
For this proposal only the packaging cell line lysate is necessary. The immobilized genome is incubated with the lysate. Afterwards the lysate is removed and the drug is covalently coupled to the immobilized virus.

Separation fof the drug loaded viruses from the immobilized genome. Stability and functionality tests,

Subsequent to drug loading of the immobilized viruses, the viral genome must be removed. The condititons must be evaluated. The separation could be realized under the conditions used in affinity chromatography by adding capsid proteins in a flow chamber. Furthermore the stability of the genome free viruses should be determined. The viruses could be stabilized by disulfide bond formation using the conditions described in literature 20.
The host specifity and the intracellular delivery of the drug must be shown in cell culture.
If this concept works antiviral drugs should be used as drugs. Paralell to this virus like particels should be loaded with antiviral drugs.

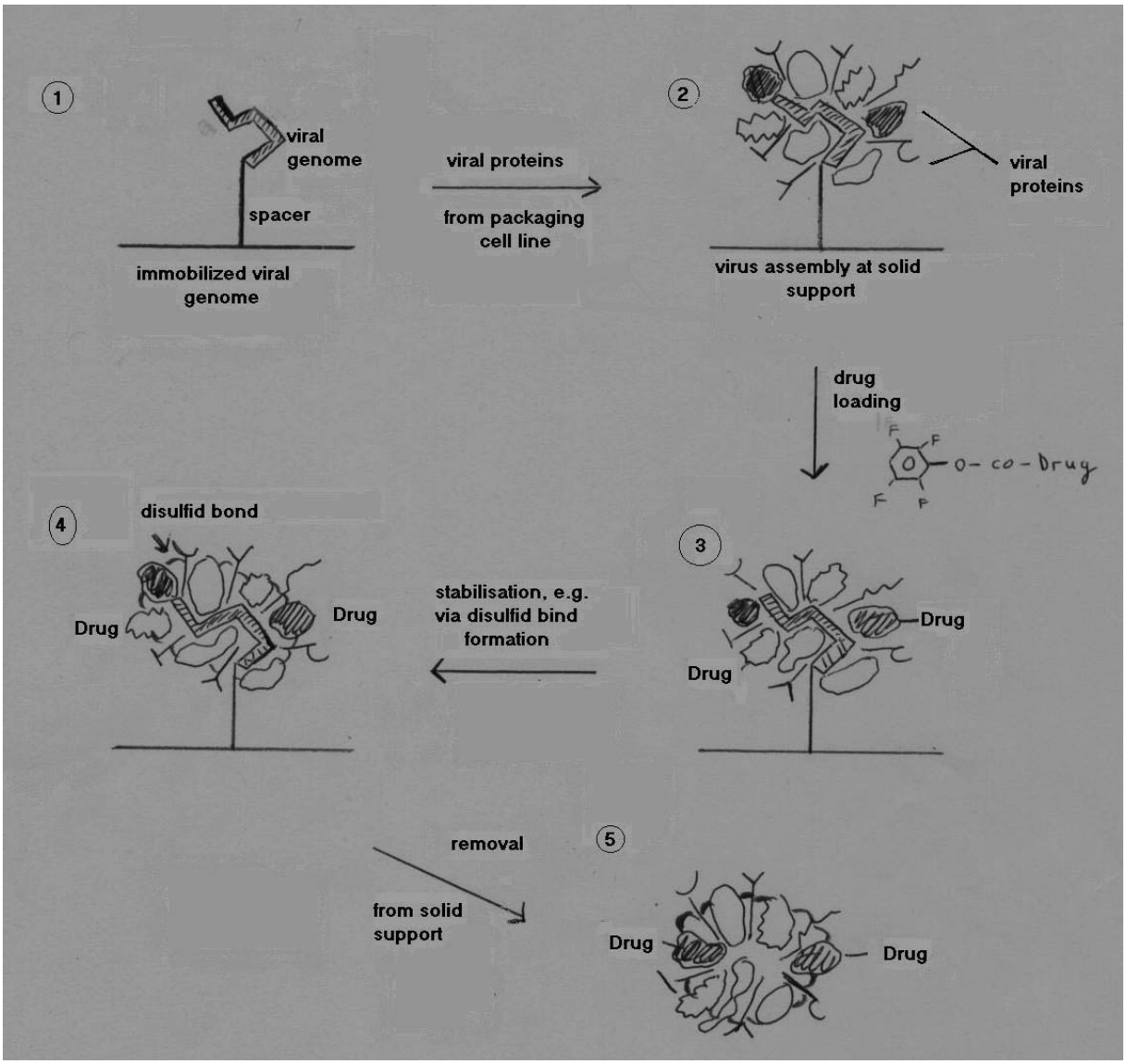

Fig 1. Solid phase virus assembly.

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