341a Purification of Aav Gene Therapy Vectors by Selectively Inactivating Helper Adenovirus Using High Hydrostatic Pressure

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Recombinant adeno-associated virus (AAV) is a highly promising gene therapy vector for numerous reasons, including its lack of pathogenicity and its ability to induce long-term expression of a transgene in multiple types of target cells *in vitro* and more importantly *in vivo*. In practice, AAV is produced in cell cultures and requires complementation with so-called "helper" virus gene products, which can be accomplished either by infecting the producer cells with a helper virus, such as adenovirus, or by transient plasmid transfection to induce expression of specific helper virus genes. This requirement complicates production for therapeutic applications, because plasmid transfections do not scale up well, and although the method of helper virus infection is well-suited to large cultures of suspended cells, the helper virus must eventually be inactivated and/or removed in order to avoid helper virus-induced toxicity or immunogenicity. One widely employed procedure uses adenovirus type 5 (Ad5) infection during the production phase and subsequent heat-inactivation via incubation at 56°C. Though this process inactivates most of the helper adenovirus, this procedure also results in a concomitant loss of ~50% of the active AAV particles. Other commonly used separations processes, including affinity chromatography and density-equilibrium centrifugation, accomplish separation of AAV from adenovirus, but they are costly and do not necessarily inactivate trace adenovirus contaminants. Therefore, from both process optimization and product safety standpoints, it would be desirable to develop alternative rapid, robust, and economical methods that selectively inactivate undesired viruses (including adenovirus helper virus) while leaving AAV particles intact.

We have developed a method for using high hydrostatic pressure to selectively inactivate adenovirus helper virus (Ad5) without causing any detectable loss of activity in an AAV serotype 2 (AAV-2)-based gene therapy vector. High hydrostatic pressure has previously been shown to effectively inactivate microorganisms and viruses, including HIV-1, poliovirus, and adenovirus. Here, we report the inactivation kinetics of AAV-2 under a range of pressurization conditions, including precise control for incidental thermal effects. We thereby determined a range of high-pressure treatment conditions that leave AAV-2 fully active while rendering adenovirus essentially fully inactivated. AAV-2 inactivation kinetics would also be useful in determining whether high pressure might be used to inactivate other undesired viruses, such as those that might contaminate the producer cell lines, during the production of clinical-grade viral therapeutics. In addition, we are investigating the mechanisms by which AAV-2 is inactivated under high pressure in order to gain biological insights into the structure of AAV-2 particles, which would be useful for engineering gene therapy vectors for enhanced properties, such as delivery efficiency and stability. Finally, we are investigating whether high pressure treatment can be used with complementary separations processes to achieve enhanced product purity.