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Original article

Influence of method of systemic administration of adenovirus on virus-mediated toxicity: Focus on mortality, virus distribution, and drug metabolism

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ABSTRACT

Introduction: Doses of 2×10^{12} virus particles/kilogram (vp/kg) and higher of recombinant human adenovirus serotype 5 (HAdV-5) given via the tail vein induce significant toxicity and mortality in the rat. This was not observed when doses of 5.7×10^{12} vp/kg were given through a surgically implanted jugular catheter. Here we assess how the manner by which HAdV-5 is introduced into the systemic circulation affects biodistribution, transgene expression, toxicity and mortality 0.25, 1, and 4 days after treatment in the rat. Animals were given 5.7×10^{12} vp/kg of HAdV-5 expressing beta-galactosidase or saline through a jugular catheter or by direct tail vein injection. **Results:** All animals survived after jugular vein dosing. Tail vein injection of HAdV-5 increased the mortality rate to 42% ($p\leq0.01$). All deaths occurred within 4 h. Animals dosed through the jugular vein had significantly higher levels of transgene expression in the liver and spleen and significantly more viral genomes in these tissues and kidney and lung within the first 24 h of viral infection compared to those dosed by tail vein injection ($p\leq0.01$). There was no significant difference between the groups thereafter. Samples from animals that died contained even higher levels of viral genomes and serum transaminases were elevated on average by a factor of 4 at the time of death. There was no significant difference between the two dosing methods with respect to changes in hepatic cytochrome P450 expression and activity throughout the study. **Conclusion:** These findings suggest that the method of systemic administration should be carefully considered when assessing toxicity data and other parameters at early time points after virus administration in the rat and possibly other animal models.

1. Introduction

Recombinant adenoviruses are currently under development for the treatment of various monogenetic, hereditary and infectious diseases. Although adenoviruses have been used clinically since the 1970s (Gooch & Mogabgab, 1972; Top, 1975), continual use of their recombinant counterparts in successful therapeutic regimens for the treatment of several cancers (Cattaneo, Miest, Shashkova, & Barry, 2008; Vähä-Koskela, Heikkilä, & Hinkkanen, 2007; Yang et al., 2007), is hindered by a narrow, non-linear therapeutic threshold dictated by an acute, innate immune response when they are introduced into the systemic circulation (Seiler, Cerullo, & Lee, 2007). Significant work in the area of adenovirus biology has revealed that this effect is largely due to the ability of the one component of the virus that makes it so attractive as a gene delivery vector, the protein capsid, to directly interact with and activate blood-borne factors (complement proteins C3 and C4b and clotting factors IX and X) and antigen presenting cells (macrophages, Kupffer cells and dendritic cells) (Baker, Mcvey, Waddington, Di Paolo, & Shayakhmetov, 2007; Hartman, Appledorn, & Amalfitano, 2008). This response, which can have severe manifestations within several minutes to hours after administration, is characterized by thrombocytopenia and elevated liver enzymes in response to the release of large amounts of cytokines (IL-1, IL-6, TNF-α) and chemokines (MIP-2, MCP-1, IP-10 and RANTES) into the general circulation by antigen presenting cells in the liver, spleen and peritoneum. The dose as well as the route of administration can often dictate whether this resolves quickly or has a fatal outcome (Ben-Gary, McKinney, Rosengart, Lesser, & Crystal, 2002; Bessis, GarciaCozar, & Boissier, 2004).

Development of physical and biochemical methods to prevent interaction of the virus with components of the immune system to reduce its toxicological profile without compromising transduction efficiency is currently an intensely active area of research. Direct injection of the virus into target tissues, use of immunosuppressive reagents prior to administration of virus, genetic modification of the capsid to include proteins from other adenovirus serotypes and covalent attachment of molecules to target specific organs and shield

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the virus from recognition by the immune system represent only a few of the current approaches to alter the therapeutic index and improve the utility of adenovirus-mediated gene transfer (Campos & Barry, 2007; Kreppel & Kochanek, 2008; Wu & Curiel, 2008; Zaldumbide & Hoeben, 2008). As with any novel biological therapeutic, pre-clinical testing of these vectors includes characterization of the pharmacological and toxicological effects of a series of doses after administration by different routes in rodents prior to testing in higher species.

Research in our laboratory involves the development of recombinant adenoviruses capable of evading the immune response and the mechanistic study of how viral infection affects the expression and function of key cytochrome P450 enzymes in the liver, kidney and intestine (Callahan, Boquet, Ming, Brunner, & Croyle, 2006; Callahan, Ming, Lu, Brunner, & Croyle, 2005; Callahan, Wonganan, & Croyle, 2008; Le, Boquet, Clark, Callahan, & Croyle, 2006). Discussion of our results with others in the field has revealed that doses of 2×10^{12} virus particles/kilogram (vp/kg) and higher of recombinant adenovirus serotype 5 given via the tail vein induce significant toxicity and mortality in the rat (A. Byrnes and J. Smith, personal communication, June 6, 2003). This was not observed in any of our studies when 5.7×10^{12} vp/kg was given through a surgically implanted jugular catheter which allows for easy, relatively stress-free serial blood sampling in the rat (Cocchetto & Bjornsson, 1983; Thrivikraman, Huot, & Plotsky, 2002). Animals given this much virus did not exhibit any signs of toxicity and survived for up to 14 days when they were sacrificed at this predetermined time point. Careful evaluation of the literature revealed that documented reports of adenovirus-induced deaths in rodents were limited and were not the primary subject of any given study. We did find some reports, however, describing marked signs of virus-induced toxicity including bloody urine, black ocular discharge, weight loss, lethargy and subsequent mortality when similar viruses were given by direct injection in the tail vein at much lower concentrations in healthy animals and those with pre-existing liver disease (Garcia-Banuelos et al., 2002; Morrissey, Horvath, Snyder, Patrick, & MacDonald, 2002; Smith, Tian, Muller, & Byrnes, 2004; Smith, Tian, Lozier, & Byrnes, 2004).

Given this information, we initiated a series of experiments designed to determine how slight changes in the manner by which recombinant adenoviruses are administered could influence the toxicological profile associated with the virus, something that is of great concern in and out of the clinic. We also tested the hypothesis that long-term effects associated with the virus such as transgene expression or changes in the expression and function of hepatic cytochrome P450 enzymes would not be affected by the different methods of virus administration. Male Sprague–Dawley rats were given 5.7×10^{12} vp/kg of a first generation adenovirus serotype 5 expressing the *Escherichia coli* beta-galactosidase transgene by either direct tail vein injection or through a cannula implanted in the jugular vein. Animals were closely monitored for the first 8 h for visible signs of distress and toxicity. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured 0.25, 1, and 4 days after administration. Distribution of viral genomes and transgene expression were also measured in key tissues (liver, spleen, kidney, lung and heart) at the same time points. Changes in hepatic CYP3A2 and 2C11 over the course of 4 days after administration of virus by either route is also discussed.

2. Materials and methods

2.1. Amplification and production of recombinant adenovirus

First generation E1/E3 deleted adenovirus serotype 5 containing the *E. coli* beta-galactosidase transgene under the control of a CMV promoter was amplified in HEK 293 cells (CRL-1573, ATCC, Manassas, VA) and purified from cell lysates by two rounds of cesium chloride density gradient ultracentrifugation according to established protocols (Altaras et al., 2005). Viral bands isolated after the final centrifugation step were desalted on Econo-Pac 10DG disposable columns (BioRad, Hercules, CA) equilibrated with phosphate buffered saline (pH 7.4, Sigma Aldrich, St. Louis, MO). Fractions containing virus were collected and the number of virus particles determined using the method of Maizel et al. with the following formula (Maizel, White, & Scharff, 1968):

$$\text{Virus particles/ml} = (\text{absorbance at 260 nm}) \times (\text{dilution factor}) \times 1.1 \times 10^{12}.$$

All animals were treated with freshly purified virus.

2.2. Plaque assay

The amount of active virus in a given preparation was determined by plaque assay according to an established protocol (Graham & van der Eb, 1973). Assays were performed on the day animals were treated. Plaque forming units (pfu) were calculated according to the following formula:

$$\text{pfu/ml} = \text{average number of plaques} \times \text{dilution factor} \times 4(\text{a conversion factor from } \mu\text{l to ml}).$$

The particle to pfu ratio was calculated by dividing the number of particles obtained from the absorbance reading of a preparation at 260 nm by the number of active particles (pfu/ml) detected by the plaque assay. The average virus particle to pfu ratio for the virus preparations used in these studies was 53:1.

2.3. Assay for detection of replication competent adenovirus (RCA)

A two-cell line bioassay was performed on each preparation to determine the presence of RCA as described previously (Murakami et al., 2002). One RCA was detected for every 3×10^{12} virus particles tested.

2.4. Administration of recombinant adenovirus

All procedures were approved by the Institutional Animal Care and Use Committee of The University of Texas at Austin and are in accordance with the guidelines established by the National Institutes of Health for the humane treatment of animals. Male Sprague–Dawley rats (7–8 weeks, Charles River Laboratories, Wilmington, MA) were housed in individual cages and given unrestrained access to standard rodent chow (Harlan Teklad, Indianapolis, IN) and tap water. A single intra-muscular injection of a 1:1:1 (v/v/v) ratio of ketamine (100 mg/ml, Wyeth, Fort Dodge, Animal Health, Overland Park, KS), xylazine (20 mg/ml, Sigma Aldrich), and acetopromazine (10 mg/ml, Sigma Aldrich) achieved deep plane anesthesia for placement of catheters into the right jugular vein. Aside from a single dose of heparinized saline (0.3 ml, 20 U/ml, Heparin Sodium, Baxter Healthcare, Deerfield, IL) to maintain the cannula immediately following surgery, animals in this study did not receive any additional medications prior to or after administration of recombinant adenovirus. Twenty-four hours after surgery, rats were placed in polycarbonate plastic restraining devices (Braintree Scientific, Braintree, MA) and given a single intravenous dose of either 5.7×10^{12} viral particles per kilogram (vp/kg), bacterial lipopolysaccharides (LPS, 1 mg/kg, from *E. coli* serotype 0127:B8, Sigma Aldrich) or phosphate buffered saline each in a 0.5 ml volume followed by 0.5 ml of saline to ensure that each reagent was effectively flushed from the catheter and into the bloodstream.

A separate group of animals that did not undergo jugular cannulation was placed in polycarbonate restraining devices and given each of these agents in the same volume (0.5 ml) by direct injection in the lateral tail vein according to established procedures (Cocchetto &