Short Communication

Impaired neutralising antibody formation and high transduction efficacy after isolated hepatic perfusion with adenoviral vectors

B van Etten1, AMM Eggermont*,1, G Ambagtsheer1, ST van Tiel1 and TLM ten Hagen1

1Erasmus University Medical Centre-Daniel den Hoed Cancer Centre, Department of Surgical Oncology, PO Box 5201, 3008 AE Rotterdam, The Netherlands

Local adenoviral gene transfer can be performed by means of isolated hepatic perfusion (IHP). This methodology is a very effective and safe way to deliver adenoviral vectors. We studied the immune response after IHP. A decreased neutralising antibody formation was observed, offering possibilities for further research in the field of gene therapy in isolated perfusion settings.

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The main reason for failure of adenoviral gene delivery is insufficient transduction efficacy in vivo. A method, which improves in vivo efficacy, is locoregional administration. Previously, we and others demonstrated successful transduction and subsequent tumour response after isolated limb perfusion (ILP) and isolated hepatic perfusion (IHP) (de Roos et al, 1997, 2000; de Wilt et al, 2001; van Etten et al, 2002).

Adenoviral mediated gene transfer results in transient gene expression. Loss of the therapeutic gene requires readministration to achieve prolonged gene expression, which however is precluded by formation of neutralising antibodies (Yang et al, 1994; Kaplan et al, 1997). Since IHP is a technique with minimal systemic exposure and washout possibilities after the perfusion, immune response after adenoviral treatment may be impaired.

We performed IHP in rats with a recombinant adenoviral vector and investigated the production of neutralising antibodies and leukocytes compared to systemic intravenous (i.v.) treatment.

MATERIALS AND METHODS

Animals

Male inbred immunocompetent WAG/Rij rats, weighing 250–300 g (Harlan-CPB, The Netherlands) were used. The rats were fed a standard laboratory diet and were housed under standard conditions of light and accommodation. The ethical committee for animal research of the Erasmus University Medical Center approved the protocol. The experimental protocols adhered to the rules outlined in the Dutch Animal Experimentation Act of 1977 and the published UKCCCR Guidelines for the Welfare of Animals in Experimental Neoplasia (Workman et al, 1998).

Recombinant adenovirus construct

The viral constructs were provided by Aventis-Pharma (Vitry-sur-Seine, France) and are described previously by us in detail (van Etten et al, 2002). AV1.0CMV is a recombinant replication-deficient adenovirus vector. AV1.0CMV.LacZ expresses the Escherichia coli derived β-galactosidase protein that can be detected by X-gal histochemistry.

Routes of administration

Isolated hepatic perfusion We have described the rat isolated liver perfusion model in detail earlier (van IJken et al, 2000). Rats were perfused for 10 min with oxygenated and heated (38–39°C) colloid fluid (Haemaccel, Behring Pharma, Amsterdam, Netherlands) with 1.0 × 10^11 virus particles (vp). This dose was previously determined as the maximum tolerated dose (MTD). Afterwards, a washout procedure was performed to remove all nonbound viruses by perfusing with 10 ml Haemaccel.

Intravenous injection A volume of 200 µl of PBS containing 2.5 × 10^11 vp (MTD) was slowly injected into the penile vein.

Blood and tissue sampling

Blood samples were taken via the tail vein at day 0, 3, 6, 9, 16, 23, and 30 after treatment. Serum was collected after centrifugation (16 000 × g) and stored at −80°C until further analysis. At 24 h after treatment animals were killed. Liver was taken out and snap frozen in liquid nitrogen. Cryosections of tissue samples were stained according to the X-gal staining protocol (van Etten et al, 2002).

Measurement of neutralising antibodies

Adenovirus type 5 specific neutralising antibodies were measured by the virus neutralisation (VN) test as previously described (Schrader and Wigand, 1981; De Jong et al, 1999). The presence of cytopathic effects of Hep 2 cells caused by the virus was scored.
under the microscope. Neutralising antibody titres were expressed as the highest serum dilution showing no cytopathological effects.

**Measurement of leukocyte count, liver and renal functions**

Leukocyte numerations were determined with a microcell counter (Sysmex; Kyoto, Japan). Liver functions (alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, total bilirubin and γ-glutamyl transpeptidase) and renal functions (creatinine and urea) were measured by spectrophotometric analysis (ELAN-analyzer; Eppendorf-Merck, Hamburg, Germany).

**Statistical analysis**

Results were evaluated for statistical significance with the Kruskal–Wallis and Mann–Whitney U tests with SPSSv10.0 for Windows 2000. A significance level of \( P < 0.05 \) was used.

**RESULTS**

We observed augmented transfection of cells after isolated perfusion \((n = 3)\) (transduction efficacy of 80–90%) compared to systemic therapy \((n = 3)\) (transduction efficacy around 5%) (Figure 1).

**DISCUSSION**

Isolated hepatic perfusion is a more effective and safe method to deliver adenoviral vectors towards liver or tumour cells compared to i.v. injection (de Roos et al, 1997; van Etten et al, 2002). A transduction efficacy up to 45% of hepatocytes by means of IHP combined with a chelating agent has been described (de Roos et al, 1997). Here we demonstrate that this highly selective delivery method can result in even higher transduction rates and, importantly, is accompanied by an impaired neutralising antibody formation and leukocyte proliferation. Several studies have been conducted to influence immune response upon adenoviral gene therapy, including incorporation of immunosuppressive genes into the vector or manipulation of the immune system during administration (Qin et al, 1997; Nakagawa et al, 1998; Kuzmin et al, 2001). To our knowledge this is the first report demonstrating an impaired immune response using isolated perfusion methodology with a ‘regular’ adenoviral vector in an immunocompetent animal model.
The liver is known for its ability to induce immune tolerance (Calne, 2000). Both in patients and animal models host-to-graft tolerance was observed (Kamada et al, 1981; Mazariégos et al, 1997). Liver sinusoidal endothelial cells (LSECs), which clear the antigen from the blood can function as an antigen-presenting cell (Limiter et al, 2000). Naïve T cells, which are activated by the LSECs, do not differentiate into effector T cells thereby inducing antigen-specific T cell tolerance (Knolle and Limmer, 2001). This mechanism might play a role in the lesser immune response we observed.

It is known that the height of neutralising antibodies titres is correlated with the dose of virus administrated (Yang et al, 1996). Since most circulating viruses are washed away at the end the perfusion, only a relative low viral load is left behind in the liver. Worgall et al (1997) demonstrated that the acute innate immune mechanism eliminated 90% of the circulating adenoviral vectors within 24 h. So, if hardly any circulating viruses are left after IHP and the majority is cleared rapidly, only an extremely low amount of viruses can induce the humoral immune response, likely resulting in low neutralising antibody production.

Retrograde infusion of adenoviruses in the common bile duct of mice resulted in increased hepatic restricted gene transfer, combined with lower neutralising antibody titres (Peeters et al, 1996). It has been reported that the route of administration strongly determines the humoral immunity to the transgene in experiments with adeno-associated virus vectors in mice. Delivery via the hepatic artery resulted in higher transgene expression and an absent immune response to the transgene product (Nathwani et al, 2001). These results and our current findings suggest that loco-regional liver directed gene therapy offers advantages at gene transfer efficacy level as well as at immune response level. We recently performed pilot experiments with repeated administration. Primary adenovirus treatment by IHP followed by i.v. challenge 30 days later. These preliminary data showed no advantage with respect to transduction efficacy after the i.v. administration. Since repeated IHP in rats is not possible due to technical reasons we cannot provide data on this issue at this moment. In pigs we previously developed leakage free and potentially repeatable balloon catheter based IHP technique (van IJken et al, 1998), offering possibilities for future experiments with repeated IHP.

In conclusion, our findings are a strong argument for further research on delivery of viral vectors in isolated perfusion settings (limb, kidney, lung, liver).

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