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A generic viral dynamic model to systematically characterize the interaction between oncolytic virus kinetics and tumor growth

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ABSTRACT

Oncolytic viruses (OV) represent an encouraging new therapeutic concept for treatment of human cancers. OVs specifically replicate in tumor cells and initiate cell lysis whilst tumor cells act as endogenous bioreactors for virus amplification. This complex bidirectional interaction between tumor and oncolytic virus hampers the establishment of a straight dose-concentration-effect relation. We aimed to develop a generic mathematical pharmacokinetic/pharmacodynamics (PK/PD) model to characterize the relationship between tumor cell growth and kinetics of different OVs. U87 glioblastoma cell growth and titer of Newcastle disease virus (NDV), reovirus (RV) and parvovirus (PV) were systematically determined *in vitro*. PK/PD analyses were performed using non-linear mixed effects modeling. A viral dynamic model (VDM) with a common structure for the three different OVs was developed which simultaneously described tumor growth and virus replication. Virus specific parameters enabled a comparison of the kinetics and tumor killing efficacy of each OV. The long-term interactions of tumor cells with NDV and RV were simulated to predict tumor reoccurrence. Various treatment scenarios (single and multiple dosing with same OV, co-infection with different OVs and combination with hypothetical cytotoxic compounds) were simulated and ranked for efficacy using a newly developed treatment rating score. The developed VDM serves as flexible tool for the systematic cross-characterization of tumor-virus relationships and supports preselection of the most promising treatment regimens for follow-up *in vivo* analyses.

1. Introduction

Oncolytic viruses (OV) have been intensely investigated for their potential to kill tumor cells and are considered as a promising new therapeutic concept for treatment of cancer. Already in 2005 the regulators in China approved the use of the first OV for treatment of head-and-neck cancer (Garber, 2006). Meanwhile the FDA approved in October 2015 the herpes simplex virus talimogene laherparepvec (T-VEC) for treatment of advanced melanoma (Ledford, 2015). Although, currently 60 studies are running which are investigating oncolytic viruses (Anon, n.d.) still, very little is known about the dose-concentration-effect relationships for oncolytic viruses and to our knowledge the analysis of OV biodistribution, clearance, shedding, and replication have been limited to preclinical studies (*e.g.* intralesion administration of T-VEC) (Vatsan, 2015). The biodistribution and shedding of T-VEC in humans is currently under investigation in a clinical study (Amgen, 2015).

OVs are replication-competent viruses, which are able to specifically infect and replicate in tumor cells and thus lead to rapid cancer cell death. Two types of OVs have been described: 1) naturally-occurring animal-hosted oncolytic viruses capable of inducing innate tumor lysis and with little or no human pathogenicity (Roberts et al., 2006) and 2) genetically engineered viruses, which replicate in tumor cells and remain avirulent to normal cells (Haseley et al., 2009). OVs achieve selective replication in tumor cells by exploiting tumor-specific alterations which are not manifested in non-transformed cells such as downregulation of the interferon pathway (Schirrmacher et al., 1999) or RAS mutation (Strong et al., 1998). Infected tumor cells become an endogenous bioreactor for virus production leading to a rapid tumor cell lysis and release of progeny virus particles which then infect surrounding cancer cells (Parato et al., 2005). This sequential process of repeated tumor infection, cell death and virus release augments the virus exposure, potentially enabling the application of low initial OV doses. The lysis of tumor cells by OVs concurrently self-limits the availability of bioreactors for further virus production. The pharmacokinetics (PK) of OVs are additionally influenced by the host immune system, which on the one hand mounts anti-viral responses to reduce OV titer (Ikeda et al.,

Abbreviations: MD, multiple dose; NDV, Newcastle disease virus; OV, oncolytic virus; PV, parvovirus; RV, reovirus; SD, single dose; VDM, viral dynamic model; VPC, visual predictive check.

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1999) whilst also eliminating tumor cell bioreactors necessary for virus propagation (Todo et al., 1999). These multiple processes influencing the *in vivo* lifecycle of OVs hinder the use of classical pharmacokinetic (PK)/pharmacodynamics (PD) models, where the fate of a compound (small molecule or antibody) is determined by distribution and elimination processes but no increase in the compound amount will be observed (Titze et al., 2016). In contrast, the quantity of OVs is dynamic and thus the generation of an *in silico* model factoring in these processes is of high value to understand the complex and nonlinear bidirectional interaction between tumor cell growth and virus replication. Depending on the question of interest the effect of OVs on tumor cells can be modeled by ordinary differential equations (ODEs) (Bajzer et al., 2008; Komarova and Wodarz, 2010), partial differential equations PDE (Jacobsen and Pilyugin, 2015) or spatial agent-based models (Bailey et al., 2013; Wodarz et al., 2012). While three-dimensional models are primarily used to describe the virus spread in solid *in vivo* tumors, ODE models can be applied to gain basic biological insights and to analyze particular treatment scenarios. So far, oncolytic virus dynamic models have been investigated on a theoretical basis (Karev et al., 2006; Novozhilov et al., 2006; Berezovskaya et al., 2007; Komarova and Wodarz, 2010) and applied to *in vivo* data (Dingli et al., 2006; 2009; Bajzer et al., 2008) but to our knowledge no oncolytic virus dynamic model has been utilized as a generic screening tool to investigate the virus dynamics in an *in vitro* setting.

In this article, we investigate the *in vitro* glioblastoma cell growth and oncolytic virus titer for Newcastle Disease Virus (NDV), reovirus (RV) and parvovirus (PV). We further describe the development of a generic ODE based mathematical model to systematically characterize the PK/PD relationship between the tumor cells and different OVs. The model was then employed as a functional application tool to compare the kinetics and tumor killing efficacy of different OVs. Finally, a treatment rating score was developed to predict and appraise different OV treatment regimens.

2. Material and methods

2.1. Cells and viruses

The human glioblastoma cell line U87 and the African green monkey epitheliar cell line Vero were obtained from the American Type Culture Collection (ATCC). The human kidney cell line NB-234K was obtained from Thermo Fisher Scientific. U87 and NB-234K was authenticated at the DSMZ (Braunschweig, Germany) using short tandem repeat (STR) markers. Alignment (DSMZ) of COI sequence of Vero revealed the sample to be species-specific for *Chlorocebus sabaeus* (African green monkey). All cell lines were propagated at 37 °C in a humidified atmosphere with 5% CO_2 in DMEM (Sigma) supplemented with 1% penicillin/streptomycin (life technologies) and 10% FCS (Sigma). Reovirus Type 3 Dearing was obtained from the American Type Collection. This virus was propagated in Vero cells (ATCC), then purified by ultracentrifugation at 40,000 rpm for 4 h at 4 °C on a 15%/25%/40% sucrose gradient. Parvovirus H1, kindly provided by Prof. Rommelaire (Deutsches Krebsforschungszentrum, Heidelberg), was propagated in NB-234K cells and purified by iodixanol gradient ultracentrifugation as described previously (Brown et al., 2002). The lentogenic NDV strain Hitchner B1 was kindly provided by Dr. Christian Grund (Friedrich-Loeffler-Institut, Riems, Germany) and was propagated in embryonated eggs from specific-pathogen free chickens. The allantoic liquid was harvested and virus purified by ultracentrifugation at 40,000 rpm for 4 h at 4 °C using a 20%/40%/60% sucrose gradient.

2.2. MTT-assay

Monolayers of 10,000 U87-cells were infected 24 h after seeding with RV, PV or NDV at 0, 1, 5, 10, 50 or 100 virus genome copies per cell (cp/c). Cells were then incubated for 24, 48, 72, 96 or 120 h after infection and then incubated for 2 h at 37 °C with 0.5 mg/ml Methylthiazoldiphenyl-tetrazolium bromide (MTT) (Sigma Aldrich, Germany). Cells were then lysed with Isopropanol (Biesterfeld AG, Germany) for 30 min at room temperature on a shaker, after which the absorbance was determined using a microplate reader at 550 and 620 nm. Each condition was performed six times resulting in 450 data points for infected and 90 data points for control cells.

2.3. Real-time PCR

Quantitative PCR was performed to determine virus titer in supernatants of infected cells and the amount of virus in the stocks. The number of virus particles in the supernatant can be used to characterize virus replication since virus particles accumulate in the supernatant. RNA from virus stocks and supernatants was isolated using NucliSens easy Mag kits (Biomerieux, France). Quantitative PCR was performed using a Light Cycler 480 II (Roche, Germany) using the following primer sequences: RV:5′-GAATGCAGAACATGATTCAGCAT-3′(sense) and 5′-TAGCAGTATGCTCAGTAGAGGTGG-3′ (antisense), PV: 5′-TCAATGCGCT CACCATCT-3′(sense) and 5′-TCGTAGGCTTCGTCGTGTTCT-3′(antisense), NDV: 5′-GAG AAT TCA GAA TCG TCC CGT TAC-3′ (sense) and 5′-TCT TGA TGT CGC AGA AGA TAG GTG AT-3′ (antisense). Cycling conditions were as follows: 15 min reverse transcription at 50 °C, initial denaturation at 95 °C, 40 cycles of denaturation at 95 °C for 8 s and annealing and extension at 60 °C for 45 s.

2.4. Modeling

A tumor growth model was developed to describe the growth of uninfected cells. The viral dynamic model (VDM) describing tumor cell growth under oncolytic virus therapy and at the same time virus kinetics was based on a viral dynamic model (Perelson et al., 1996) which has been used for modeling of various viral infections (Perelson, 2002). VDMs were initially developed separately for each virus strain and then subsequently combined and fitted to a common VDM. NONMEM V7.3.0 (Beal et al., 1998) was used for non-linear mixed-effects modeling analysis. The first order conditional estimation method with interaction was used for parameter estimation and the ADVAN 13 subroutine was used to express the models in ordinary differential equations. Standard goodness-of-fit plots, *p*-values, and visual predictive checks (VPCs) were used for VDM assessment (Akaike, 1974; Jonsson and Karlsson, 1999). In the case of nested models, *p*-values <0.05 indicated the superiority of one model over another. To account for diversity between the wells on the plate, interindividual variability (IIV) was modeled with an exponential variability model, tested on every structural parameter and subsequently included in the model if it led to significant model improvement, indicated by *p*-value <0.05. For a VPC the final model was simulated 500 times including interindividual and residual variability. The median and 90% confidence interval were plotted and overlaid with observations.

2.5. Simulations

Tumor cell growth and virus titer were simulated over 10 weeks to investigate the long-term dynamic interaction between tumor and virus. To model the optimal time for follow-up OV doses, a second OV dose of the same virus was applied at either day 10 or when a minimum cell or virus count (nadir) was reached. The effect of dual infection with two different OVs was tested by combining NDV with RV or PV at day 10. The effect of multiple doses (MD) was investigated by application of OV every 24 h starting at experiment begin or at virus or tumor nadir. The combination of OV with a hypothetical cytotoxic compound inducing 30% or 60% cell growth inhibition was modeled with compound dosing beginning at the onset of tumor or virus nadir. Further, the impact of tumor load (5000, 15,000 or 20,000 seeded cells) on response was investigated. To rank the treatment regimens for their