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Effects of ocular surface strontium-90 beta radiotherapy in dogs latently infected with canine herpesvirus-1

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ABSTRACT

Latent canine herpesvirus-1 (CHV-1) infections are common in domestic dogs, but stimuli causing viral reactivation and recrudescent disease are poorly understood. Immunosuppressive pharmaceuticals are currently the only experimentally established triggers for recurrent ocular CHV-1 infection in dogs; however, ocular CHV-1 shedding has been reported clinically following strontium-90 beta radiotherapy of the ocular surface and it has been speculated that radiotherapy can directly induce viral reactivation. Strontium-90 is used as a beta radiation source for the treatment of a variety of neoplastic and immune-mediated canine ocular surface diseases. In the present study, the effects of ocular surface strontium-90 beta radiotherapy in dogs latently infected with CHV-1 were evaluated. Ten mature dogs with experimentally induced latent CHV-1 infections were randomly divided into two groups: one group received a single fraction 50 Gy radiation dose in one application from a strontium-90 ophthalmic applicator and the second group received sham radiotherapy. Dogs were then monitored for 45 days for recurrent ocular CHV-1 infection using clinical and virological outcome measures. Clinical ophthalmic examinations, ocular sample CHV-1 PCR assays, and serum CHV-1 virus neutralizing antibody assays were performed at specified intervals. No abnormalities suggestive of recurrent CHV-1 ocular disease were observed on clinical examination in any dog during the study. Ocular viral shedding was not detected and CHV-1 virus neutralizing titers remained stable in all dogs. A single fraction 50 Gy radiation dose administered to the ocular surface by strontium-90 beta radiotherapy did not result in detectable recurrent ocular CHV-1 infection in mature dogs with experimentally induced latent infection.

1. Introduction

Canine herpesvirus-1 (CHV-1) is a member of the genus *Varicellovirus* and the subfamily Alphaherpesvirinae (Rémond et al., 1996). Canine herpesvirus-1 has a host range restricted to canids, but is closely related to several alphaherpesviruses that infect other host species and cause recurrent ocular disease, including herpes simplex virus-1 (HSV-1) of humans (Manning et al., 1988). Infection with CHV-1 causes fetal death, abortion, and mummification in naïve pregnant dams and acute viremia, often resulting in death, in neonates. Subclinical infection or mild respiratory, genital, or ocular disease is typical of infection in immunocompetent dogs older than 3 weeks of age (Evermann et al., 2011). Ocular CHV-1 infection in older dogs manifests clinically as conjunctivitis, ulcerative or non-ulcerative keratitis, and blepharitis (Ledbetter, 2013). Conjunctival petechiation and ulceration are common clinical findings with ocular CHV-1 infection (Ledbetter et al., 2009a).

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Ocular disease associated with CHV-1 infection can occur in dogs with primary or recurrent viral infection. During primary infection latency is established via retrograde axonal transport of virus to regional sensory ganglia, most commonly the trigeminal ganglia for ocular infections (Miyoshi et al., 1999). Following reactivation, virus is transported anterograde along sensory neurons back to peripheral tissues where viral replication and shedding occur (Diefenbach et al., 2008). Reactivation of latent CHV-1 can occur with administration of systemic immunosuppressive pharmaceuticals (Ledbetter et al., 2006, 2009b). Other causes of CHV-1 reactivation have been postulated including chemotherapeutics, topical immunomodulatory agents, and radiation therapy (Ledbetter, 2013). Grüning et al. (2001) reported a conjunctival biopsy specimen positive for CHV-1 by polymerase chain reaction (PCR) assay from a dog with chronic superficial keratitis following strontium-90 beta radiotherapy and a causal relationship was suggested.

Strontium-90 emits a low-energy beta radiation as it decays which has limited tissue penetration and is useful in the treatment of superficial tissue conditions where surgical resection would be challenging (Farrelly and McEntee, 2003). Strontium-90 radiotherapy is used for the treatment of ocular surface and adnexal neoplastic and inflammatory conditions in both humans and dogs (Banks et al., 1972; Nishimura et al., 2000; Stannard et al., 2013). In humans, squamous cell carcinoma and malignant melanoma are the most common malignant tumors of the conjunctiva and are both routinely treated with strontium-90 radiotherapy in combination with surgical excision, cryotherapy, or chemotherapy (Cohen et al., 2013; Stannard et al., 2013). Reported ocular surface complications of strontium-90 therapy in humans include idiopathic corneal ulcers, superficial punctate keratitis, and conjunctivitis (Hughes, 1952). A causal link between megavoltage external beam radiation therapy and HSV-1 reactivation in humans is widely reported (Oakley et al., 1997). The mechanism of HSV-1 reactivation associated with external beam radiation in humans is unclear, but is speculated to involve the cumulative effects of several factors including local prostaglandin production, sensory nerve stimulation, local immunosuppression, and the direct effect of radiation on ganglia harboring latent virus (Oakley et al., 1997). In addition, excimer laser irradiation of the cornea is implicated in the reactivation of HSV-1 (Deai et al., 2004), but viral reactivation and recurrent herpetic disease are not currently established complications of strontium-90 therapy in humans.

In dogs, strontium-90 treatment is reported for various ocular surface tumors (e.g., hemangiosarcoma, melanoma, papilloma, and squamous cell carcinoma) and inflammatory conditions (e.g., chronic superficial keratitis, idiopathic corneal vascularization, and pigmentary keratitis), but the risk of inducing CHV-1 reactivation is not established (Candlin and Levine, 1952; Silver and Cater, 1965; Bernays et al., 1999; Höcht et al., 2002; Donaldson et al., 2006a,b; Busse et al., 2008; Nevile et al., 2014). Reported adverse effects in dogs associated with strontium-90 plesiotherapy include conjunctivitis and keratitis (Donaldson et al., 2006a; Busse et al., 2008), which could represent undocumented CHV-1 infection. The purpose of the present study was to determine if administration of a single fraction of strontium-90 radiotherapy induces recurrent ocular CHV-1 infection in latently infected mature dogs.

2. Materials and methods

2.1. Animals, study design, strontium-90 radiotherapy

All protocols were approved by the Animal Care and Use Committee of Cornell University and were conducted in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. Ten 3-year old specific pathogen-free sexually intact Beagles with experimentally induced CHV-1 latent infection were used. The study dogs included six males and four females. Dogs were individually housed and strict biosecurity was maintained throughout the study. Latent CHV-1 infection was experimentally induced by topical ocular inoculation 12 months prior to the beginning of the present study using previously described methods (Ledbetter et al., 2009b,c, 2012). Briefly, dogs seronegative for CHV-1 were topically inoculated in both eyes, using the ocular drop method, with 2×10^5 $TCID_{50}$ of a field strain of CHV-1 isolated from corneal samples of a dog with dendritic ulcerative keratitis treated at the Cornell University College of Veterinary Medicine Hospital for Animals, Ithaca, NY, USA. The dogs were then maintained in the isolation facility until the present study.

A randomized, placebo surgery-controlled, parallel study design was used. Dogs were randomly assigned into two groups; group 1 ($n = 5$ dogs) received a 50 Gy beta radiation dose by strontium-90 radiotherapy and group 2 ($n = 5$ dogs) received sham radiotherapy. Block randomization, with gender as the blocking factor, was used to assure an even distribution of male and female dogs between the study groups. On study day 1 all dogs were sedated with intravenous hydromorphone (0.1 mg/kg), acepromazine (0.1 mg/kg), and glycopyrrolate (0.004 mg/kg). A Barraquer eyelid speculum was placed in the right eye to retract the eyelids and expose the ocular surface. A 50 Gy radiation dose was administered to the right eye of dogs in group 1 using a handheld strontium-90 ophthalmic applicator (Amersham International 55 mCi strontium-90 pterygium applicator, Arlington Heights, IL, USA). The applicator end was placed in contact with the ocular surface and the center of the 8.5 mm diameter active source was placed directly over the temporal limbus, covering both cornea and bulbar conjunctiva. The applicator tip was cleaned between each dog by thoroughly wiping the contact surface with 10% betadine solution and sterile balanced salt solution, then allowing it to dry. The precise treatment time to deliver the required radiation dose was calculated by referring to the strontium-90 ophthalmic applicator surface dose rate measurement certificate and source decay table (applicator dose rate when the study conducted = 36.7 cGy/s). Dogs in group 2 were sedated and an eyelid speculum was placed for the same period of time as group 1, but no radiation dose was