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The pharmacokinetics of rituximab following an intravitreal injection

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Abstract

Rituximab is a monoclonal antibody directed against the CD20 B-cell antigen and is approved for the treatment of B-cell lymphoma. We investigated the pharmacokinetics of rituximab following intravitreal administration to assess the feasibility of treating primary intraocular lymphoma. Intravitreal injections of rituximab 0.1 ml (1 mg) were performed in rabbits. Drug concentrations in the aqueous and vitreous humor were measured at intervals from 2 to 17 days after administration. The half-life of the total amount of rituximab in the two compartments was calculated to be 4.7 days. The aqueous and vitreous humor drug levels decayed in parallel maintaining an average ratio of approximately seven. Fitting the data to a two-compartment model yielded a clearance from the aqueous humor of 1.2 μl/min. The clearance was less than the reported rate of aqueous humor outflow indicating that elimination by this route could have been sufficient to account for the disappearance of the drug from the eye. The duration of time over which sustained levels of rituximab were achieved suggest that intravitreal administration warrants further investigation as an approach to treating vitreous and anterior chamber infiltrates in patients with primary intraocular lymphoma.
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Keywords: intravitreal injection; pharmacokinetic analysis; rituximab; b-cell lymphoma

1. Introduction

Primary central nervous system lymphomas (PCNSL) are almost always of diffuse large B-cell lymphoma (DLBCL) histology and originate in the brain, spinal cord, or eyes (DeAngelis, 2001; Hochberg and Miller, 1988; Maher and Fine, 1999; Paulus, 1999). Ocular involvement with PCNSL, also known as primary intraocular lymphoma (PIOL), occurs in approximately 25% of patients with PCNSL, and can cause blindness (Hochberg and Miller, 1988). Treatment with systemic and intrathecal chemotherapy and/or radiotherapy can frequently achieve clinical remissions; however, disease relapse in the brain or eye is common and patients frequently die from disease progression (Bataille et al., 2000; Cassoux et al., 2000; Chan and Wallace, 2004; DeAngelis, 2001; Nasir and DeAngelis, 2000). Intravitreal methotrexate has been used successfully in some patients to treat local recurrences, but disease relapse is common when therapy is discontinued (Fishburne et al., 1997; Smith et al., 2002; Velez et al., 2002a,b). To improve the durability of treatment responses for recurrent PCNSL, rituximab (Rituxan®, Genentech, Inc., South San Francisco, CA), a monoclonal antibody directed against CD20 B-cell-specific antigen on malignant lymphoma and normal B-cells, has been used in open-labelled trials (Harjunpaa et al., 2001; Pels et al., 2002; 2003; Raizer et al., 2000; Ruhstaller et al., 2000). To date, there has been limited efficacy of intravenous rituximab for PCNSL, since

Abbreviations C_A, concentration of rituximab in the aqueous humor [μg/ml]; C_V, concentration of rituximab in the vitreous [μg/ml]; k, rate constant [day^{-1}]; M_V, total amount of rituximab in the vitreous [μg]; M_A, total amount of rituximab in the aqueous humor [μg]; k_1, rate constant for transfer of rituximab from the vitreous into the aqueous humor [day^{-1}]; k_2, rate constant for elimination of rituximab from the aqueous humor [day^{-1}]; $t_{1/2}$, half-life of rituximab [day].

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monoclonal antibodies are too large to effectively penetrate the intact blood–brain barrier (Neuwelt et al., 1985) and blood-retinal barriers (Mordenti et al., 1999). We examined the ocular pharmacokinetics of rituximab following an intravitreal injection in the rabbit eye to determine the feasibility of local therapy for recurrent PIOL.

2. Methods

2.1. Ocular pharmacokinetics

A total of 16 New Zealand White (NZW) rabbits of either sex weighing 2–3 kg (Covance Laboratories, Inc., Vienna, VA) were used, and all procedures adhered to the guidelines from the Association for Research in Vision and Ophthalmology statement for the use of animals in ophthalmic and vision research. Animals were anesthetized with ketamine hydrochloride (Fort Dodge, Inc., Fort Dodge, IN; 35 mg/kg) IM and xylazine (Phoenix Scientific, Inc., St Joseph, MO; 5 mg/kg) IM; proparacaine 1% ophthalmic drops (Allergan America, Hormigueros, PR) were used topically on the eye. The pupils were dilated with one drop each of phenylephrine hydrochloride 2.5% (Akorn, Inc., Decatur, IL) and tropicamide 1% (Alcon, Inc., Humacao, PR). A baseline eye examination including fundoscopy with an indirect ophthalmoscope and intraocular pressure measurement was performed. Rituximab (Rituxan™, Genentech, South San Francisco, CA) is commercially available and a single 500 mg vial (10 mg/ml concentration) was obtained for our studies. The intravitreal injection was performed using a 1-ml tuberculin syringe with a 30-gauge needle containing 100 μl of drug taken directly from the vial. After adequate anesthesia and akinesia, a lid speculum was placed and the right eye was injected 4 mm behind the surgical limbus in the superotemporal quadrant into the mid-vitreous. Following a 100-μl injection into the vitreous cavity of a rabbit, the intraocular pressure increased to >50 mmHg. As a result, our standard procedure included a 50–75 μl anterior chamber paracentesis immediately following the intravitreal rituximab injection which lowered the intraocular pressure to <20 mmHg. The paracentesis was performed using a self-sealing, shelved corneal incision with an I-Knife (Alcon Surgical, Fort Worth, TX) 3 mm from the limbus temporally. The left eye of each rabbit was not treated. Four rabbits were euthanized on each date; 2 days, 7 days, 11 days, and 17 days post intravitreal injection. Euthanasia was performed with an intracardiac pentobarbital overdose (Beuthanasia-D Special, Schering-Plough Animal Health Corp., Kenilworth, NJ), and both eyes were enucleated and immediately frozen at -70 °C for later drug extraction. The eyes were dissected while frozen, the vitreous humor and the aqueous humor isolated separately using previously described methods (Velez et al., 2002), and rituximab concentration was determined by an immunoassay. A 96-well microtiter plate was coated with polyclonal goat anti-IDEC-2B8 antibody. The test sample was subsequently added and serially diluted. The rituximab (IDEC-C2B8) present in the sample bound to the coating antibody on the plate. Known amounts of rituximab were used to make a standard curve on each plate. Goat anti-human IgG conjugated with Horseradish peroxidase was used as a detector. Color was developed by adding substrate to the wells and absorbance was read by photometric colorimetry. Absorbance was directly proportional to concentration of analyte. The lower limit of detection was 6 ng/ml.

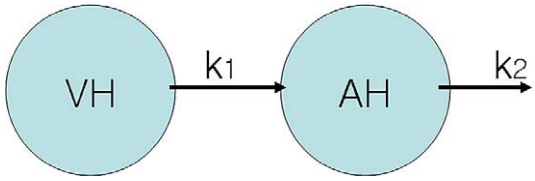

Fig. 1. The schematic of a two-compartment model for the rituximab. VH and AH represent the vitreous and the aqueous humor, respectively. The rate constants for transfer of rituximab from the vitreous into the aqueous humor and for elimination of rituximab from the aqueous humor are denoted by k_1 and k_2, respectively.

All concentration experimental data were fit to the single exponential decay expression in Eq. (1):

$$C(t) = C_0 \exp(-kt) \tag{1}$$

where C [μg/ml] and C_0 [μg/ml] denote concentration at any time, t [day] and at $t=0$, respectively. k [day^{-1}] represents a rate constant.

The half-lives of rituximab in the vitreous and the aqueous humor were calculated with the following equation:

$$t_{1/2} = \frac{0.693}{k} \tag{2}$$

A two-compartment pharmacokinetic model was employed to further analyze the pharmacokinetics of rituximab (Fig. 1). Mass balances on the two compartments yielded two first-order ordinary differential equations that were solved simultaneously to determine transfer rate constants with the SAAM II software (Version 1.2.1, Saam Institute, Seattle, WA).

$$V_V \times \frac{dC_V}{dt} = -k_1 \times V_V \times C_V \tag{3}$$

$$V_A \times \frac{dC_A}{dt} = k_1 \times V_V \times C_V - k_2 \times V_A \times C_A \tag{4}$$

where C_V and C_A are the concentrations of rituximab in the vitreous and the aqueous humor [μg/ml], respectively, and V_A and V_V are the compartment volumes [ml]. k_1 and k_2 denote the rate constant for transfer of rituximab from the vitreous into the aqueous humor and for elimination of rituximab from the aqueous humor [day^{-1}], respectively.

3. Ocular toxicity

Four NZW rabbits were anesthetized and injected in the right eye with 1 mg of rituximab in the same manner as described above, and the left eye was not treated. Clinical examinations were performed weekly to assess for signs of inflammation. Rabbits were euthanized and both eyes were enucleated 4 weeks following the injection and fixed in 10% formalin. Paraffin sections through the pupillary-optic nerve