Stability of Melphalan Solution for Intravitreal Injection for Retinoblastoma

Intravitreal injections for the treatment of retinoblastoma have been gaining relevance among ophthalmologists, supported by reports on outcome and improvement of the administration technique. Currently, doses up to 30 μg per injection are used according to an extensively described technique for intravitreal injection that minimizes the risk of extraocular dissemination of tumor cells.

The commercial form of melphalan (Alkeran) contains 50 mg of melphalan to be reconstituted in 10 mL of vehicle, but each intravitreal injection consists of 30 μg of melphalan (its 1667th part). The rest of the vial is discarded. Specifically in countries with limited resources, this procedure may be optimized to prevent disposal of active agent and preserve it for future patients. The package insert states that after dilution (<0.45 mg/mL) of the reconstituted commercial formulation with sterile saline, the administration should be completed within 60 minutes of reconstitution because of the instability of melphalan. This degradation corresponds to a spontaneous hydrolysis to monohydroximelphalan and dihydroximelphalan. A solution of 400 μg/mL of melphalan in saline at 20°C lost 10% of its content in 4.5 hours; the same loss was attained after 2.4 hours at 25°C, a common room temperature in many clinical centers.

We evaluated the stability of melphalan solution after reconstitution of the commercial product and serial dilution with saline to a final concentration of 300 μg/mL. This concentration was chosen because in our practice we administer 0.1 mL, the volume that contains the most common clinical dose of 30 μg.

Methods | Reconstitution of melphalan was developed as stated in the package insert. After obtaining the 5-mg/mL solution, serial dilutions with saline were made to obtain a 300-μg/mL solution. This final solution was placed in different syringes to determine the stability of melphalan at different times in room conditions (25°C and laboratory light, solution A), stored in a refrigerator (5°C, solution B), and stored in a freezer (−20°C, solution C). Solutions A and B were quantified for melphalan immediately after reconstitution and after 1, 2, 3, 4, 6, and 24 hours. Solution C was analyzed after 6 months of storage by thawing unassisted at room temperature but protected from light. In all cases, 3 aliquots of each syringe were analyzed for melphalan using high-performance liquid chromatography and fluorescence as previously described.

Results | After 1 hour at room temperature, there was no loss of melphalan. However, the mean loss increased to 68.06% after 24 hours (Table). A 300-μg/mL solution of melphalan was stable at room conditions for 2 hours after reconstitution and dilution, as less than 5% of melphalan content (mean, 4.89%) was lost during this interval.

Similarly, melphalan loss from solutions stored at refrigeration was not significant up to 3 hours (Table). Thereafter, melphalan degraded by more than 10% of that which would be acceptable for patient injection.

Interestingly, melphalan solutions stored at −20°C for 6 months had a mean (SD) difference of 2.57% (1.22%) compared with the fresh solution.

Discussion | Our results show that in clinical practice after melphalan reconstitution and dilution with sterile saline at a concentration of 300 μg/mL for a 30-μg intravitreal dose, the solution should be kept no longer than 2 hours at ambient conditions (25°C) or 3 hours when refrigerated. Additionally, we showed that once a vial of melphalan is reconstituted and diluted to 300 μg/mL, it can be stored at −20°C for 6 months without loss of significant amounts of the drug. However, we emphasize the need to maintain sterile conditions at all times.

Emiliano Buitrago, Bsc
Eduardo Lagomarsino, PhD
Gabriel Mato, PharmD
Paula Schaiquevich, PhD

Author Affiliations: Clinical Pharmacokinetics Unit, Hospital de Pediatría J. P. Garrahan, Buenos Aires, Argentina (Buitrago, Schaiquevich); Department of Pharmacy, Hospital de Pediatría J. P. Garrahan, Buenos Aires, Argentina (Lagomarsino, Mato); National Scientific and Technical Research Council (CONICET), Hospital de Pediatría J. P. Garrahan, Buenos Aires, Argentina (Schaiquevich).

Corresponding Author: Paula Schaiquevich, PhD, Unidad de Farmacocinética Clínica, Hospital de Pediatría J. P. Garrahan, Comité de los Pozos 1881, C1245AAL, Buenos Aires, Argentina (paula.schaiquevich@gmail.com).

Published Online: July 31, 2014. doi:10.1001/jamaophthalmol.2014.2324.

Author Contributions: Dr Schaiquevich had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: All authors.

<table>
<thead>
<tr>
<th>Time, h</th>
<th>% of Initial Melphalan Concentration, Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ambient Temperature*</td>
</tr>
<tr>
<td>1</td>
<td>100.33 (1.09)</td>
</tr>
<tr>
<td>2</td>
<td>95.11 (0.90)</td>
</tr>
<tr>
<td>3</td>
<td>82.37 (0.40)</td>
</tr>
<tr>
<td>4</td>
<td>70.07 (0.34)</td>
</tr>
<tr>
<td>6</td>
<td>63.62 (0.45)</td>
</tr>
<tr>
<td>24</td>
<td>31.94 (0.47)</td>
</tr>
</tbody>
</table>

* Room conditions were 25°C and laboratory light.
Atypical Lymphocytic Angiitis of the Optic Nerve and Central Nervous System

Primary angiitis of the central nervous system (PACNS), also known as primary vasculitis of the central nervous system, is a rare, poorly understood, and often fatal disorder. Although the association of PACNS with optic neuropathy has been documented, this is the first report to our knowledge of visual loss with histopathologic involvement of the optic nerves.

Report of a Case | Our patient developed decreasing visual acuity and right-sided weakness in his 40s after falling from a height of 3 m. Neuroimaging revealed normal blood vessels on computed tomographic angiography and irregular large areas of hyperintensity in the brain and cervical spine on magnetic resonance imaging, consistent with demyelinating processes of different ages. He was treated for multiple sclerosis with interferon beta-1a (Avonex, Rebif) and natalizumab (Tysabri) for 4 years but then experienced altered mental status and left-sided weakness. He developed bowel and bladder incontinence, monoplegia of the left lower extremity, spasticity, and loss of vision in the left eye. During the last month of life, he was treated for multiple urinary tract infections and died of acute aspiration bronchopneumonia.

The brain was normal sized with multiple circular granular lesions ranging in diameter from 1 to 3 mm. The largest lesion was in the mid–corpus callosum at the level of the caudate nucleus, and multiple small lesions surrounded the calcarine fissure. Both eyes had indistinct borders of the optic nerve, suggestive of optic edema.

Microscopic examination of the brain revealed no multiple sclerosis plaques. Instead, there were prominent eosinophilic (Figure 1A), well-circumscribed nodules of fibrotic tissue (Figure 1B) in the white and gray matter of the right occipital lobe and medulla. The nodules had a central, cellular core containing a mixture of macrophages and CD3+ T lymphocytes (Figure 1C) and CD20+ B lymphocytes (Figure 1D), with T cells predominating. Lymphocytic vasculitis (angiitis) involved arterioles. Reactive astrocytosis surrounded arterioles within the nodules. Reactive microgliosis with a few macrophages surrounded the nodules. We interpreted the nodules as representing areas of fibrosis secondary to chronic inflammation, consistent with a partial response to immunosuppressive therapy.

Figure 1. Histopathologic Findings in the Brain

A, Section of the right occipital lobe shows well-circumscribed, eosinophilic nodules with demyelination indicated by loss of blue staining (hematoxylin-eosin and Luxol fast blue, scale bar = 500 μm). B, The nodules were fibrotic as highlighted by the green staining using Masson trichrome stain (scale bar = 500 μm). The nodules had a central, cellular core containing a mixture of macrophages and CD3+ T lymphocytes (C) and CD20+ B lymphocytes (D), with T cells predominating. The lymphocytes surrounded and infiltrated the wall of an arteriole.