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Evaluation of neurapheresis therapy in vitro: a novel approach for the treatment of leptomeningeal metastases

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Abstract

Background. Leptomeningeal metastases (LM), late-stage cancer when malignant cells migrate to the subarachnoid space (SAS), have an extremely poor prognosis. Current treatment regimens fall short in effectively reducing SAS tumor burden. Neurapheresis therapy is a novel approach employing filtration and enhanced circulation of the cerebrospinal fluid (CSF). Here, we examine the in vitro use of neurapheresis therapy as a novel, adjunctive treatment option for LM by filtering cells and augmenting the distribution of drugs that may have the potential to enhance the current clinical approach.

Methods. Clinically relevant concentrations of VX2 carcinoma cells were suspended in artificial CSF. The neurapheresis system's ability to clear VX2 carcinoma cells was tested with and without the chemotherapeutic presence (methotrexate [MTX]). The VX2 cell concentration following each filtration cycle and the number of cycles required to reach the limit of detection were calculated. The ability of neurapheresis therapy to circulate, distribute, and maintain therapeutic levels of MTX was assessed using a cranial–spinal model of the SAS. The distribution of a 6 mg dose was monitored for 48 h. An MTX-specific ELISA measured drug concentration at ventricular, cervical, and lumbar sites in the model over time.

Results. In vitro filtration of VX2 cancer cells with neurapheresis therapy alone resulted in a 2.3-log reduction in cancer cell concentration in 7.5 h and a 2.4-log reduction in live-cancer cell concentration in 7.5 h when used with MTX. Cranial–spinal model experiments demonstrated the ability of neurapheresis therapy to enhance the circulation of MTX in CSF along the neuraxis.

Conclusion. Neurapheresis has the potential to act as an adjunct therapy for LM patients and significantly improve the standard of care.

Key Points

- Neurapheresis is a promising adjunctive therapy for leptomeningeal metastases.
- CSF filtration and intrathecal drug circulation may be effective in reducing tumor burden.

Importance of the Study

There are 30 000 cases annually of leptomeningeal metastases (LM) with an average survival of 3–6 months with current therapies. The incidence of LM is estimated at 3–5% of cancer patients and has been increasing due to their longer overall survival. LM presents a difficult challenge in metastatic cancer treatment plans because of the lack of effective access and therapies. Systemic therapy with anticancer drugs including methotrexate cytarabine and thiotepa is not effective enough due to poor penetration of the blood-brain barrier. Although intrathecal (IT) drug delivery systems, including Ommaya reservoirs, have been associated with longer overall survival, they rely on passive diffusion to distribute IT drugs. Future therapies, such as neurapheresis filtration, could enhance the distribution of IT drugs and therefore further improve survival. Investigation into the in vitro use of neurapheresis filtration serves as the first step in determining its applicability as an adjunctive clinical approach to systemic chemotherapy and radiation, allowing for personalized treatment by disease severity.

Leptomeningeal metastases (LM), also known as carcinomatous meningitis or leptomeningeal carcinomatosis, are a severe complication arising often late in the course of cancer progression. LM occurs when cells from a primary solid or hematologic malignancy invade the subarachnoid space (SAS), spread throughout the cerebrospinal fluid (CSF), and seed the arachnoid and pia mater, collectively known as the leptomeninges.[1–6] LM patients can present with multifocal neurological findings including cranial nerve deficits, motor deficits, altered mental status, visual disturbances, seizures, headaches, and radicular pain, as well as symptoms of obstructive hydrocephalus.[7–11]

As novel cancer therapies have extended survival, the incidence of LM has increased, with an estimated 110 000 diagnoses per year in the United States.[11,12] Despite advances in therapy, however, the prognosis for patients with LM remains dismal, with survival from time of diagnosis ranging from 3 to 6 months.[11] Left untreated, patients with LM can die from neurologic deterioration within just 4–6 weeks.[13] One of the primary goals of the standard of care is to reduce tumor cell burden in the CSF in order to palliate symptoms, such as pain from bulky metastases and obstruction of CSF flow.[11,14] Existing therapies for LM remain hampered by several limitations. While targeted radiation is quite effective in reducing bulky, symptomatic lesions, eradication of tumor cells throughout the leptomeninges would require craniospinal irradiation, which carries significant bone marrow toxicity.[11] Additionally, systemic therapies with anticancer drugs such as methotrexate (MTX), cytarabine, and thiotepa fail to penetrate the blood-brain barrier effectively unless delivered in high-dose regimens, which carries a risk of systemic toxicity.[8,15] Intrathecal (IT) chemotherapy, the mainstay treatment for LM, via Ommaya reservoirs or lumbar punctures has previously been shown to reduce tumor cells in the CSF and produce positive responses,[16,17] but these rely on passive drug diffusion throughout the CSF.[18–23] This can yield unequal distribution and accumulation of chemotherapy, high rates of neurotoxicity, and dose limitations.[24] Future approaches that enhance the distribution of IT drugs and lead to the greater clearance of cancer cells in the CSF are needed to improve the efficacy and tolerability of treatment for patients with LM.

Herein, we propose a dual lumen IT catheter-based extracorporeal filtration system as an additional mechanical intervention for the removal of tumor cells in CSF. This filtration device forms a closed loop to, in effect, dialyze tumor cells from the CSF, aspirating CSF from the lumbar region and returning filtered CSF to the mid-thoracic SAS via a dual lumen catheter. This process is referred to as neurapheresis therapy. Preclinical testing of the neurapheresis filtration system has demonstrated promise in cryptococcal meningitis by reducing fungal burden in the CSF.[25] Additionally, clinical testing of neurapheresis filtration has demonstrated its ability to safely remove red blood cells from the CSF in subarachnoid hemorrhage patients.[26,27]

Since the neurapheresis system will alter CSF flow dynamics, it may also improve the circulation of IT chemotherapeutic drugs, such as MTX, throughout the neuraxis. With increased mixing of cancer cells and chemotherapeutics, the positive results could be 2-fold: neurotoxicity risks may decrease or be completely mitigated because the drug will not be able to accumulate in the site of administration, and the drugs may have a greater cytotoxic effect due to greater exposure to the cancer cells in the CSF. As such, here we discuss the development and in vitro testing of the neurapheresis system for tumor cell removal and chemotherapy circulation as a potential adjunctive treatment for LM.

Methods

Tumor Cell Culturing

The VX2 carcinoma cell line used in this study is an aggressive anaplastic carcinoma composed of adherent fibroblast-like cells that have an average diameter of 10–20 μm (Supplementary Figure 1). The cell line originated from the Shope cottontail rabbit papillomavirus-induced

carcinoma.[28] The Division of Cancer Treatment and Diagnosis of the National Cancer Institute provided the cell line originally from Dr Solomon Praveen Samuel. Prior to the receipt, the cell line was tested by IDEXX Bioresearch for major cell culture pathogens including Ectromelia, EDIM, Hantaan, LCMV, LDEV, MHV, MNV, MPV, MVM, *Mycoplasma pulmonis*, *Mycoplasma* sp., Polyoma, PVM, REO3, Sendai, TMEV by PCR. All test results were negative. The VX2 carcinoma is not listed on the ICLAC database of commonly misidentified cell lines. The VX2 carcinoma was grown in vitro in DMEM:HAMS F12 (1:1) containing 10% fetal bovine serum, 1% L-glutamine, and 1% antibiotic/antimycotic. The cultures were maintained at 37°C in a humidified incubator containing 5% CO_2. Cells were continuously passaged in T150 culture flasks (Corning). For experiments, cells were harvested in the logarithmic growth phase by 0.05% trypsin at 37°C for 10 min. Cells were suspended in artificial CSF (aCSF) with 3 mM EDTA in an Erlenmeyer flask to achieve the desired concentration for each experiment. aCSF was prepared according to the recipe available from Cold Spring Harbor without the addition of gaseous CO_2 and O_2.[29]

In Vitro Filtration: Dead-End Filter Capacity

As a point of comparison to the neurapheresis tangential flow filter system, dead-end filters were tested as well to measure the pressure they can withstand in conjunction with cell concentration. A peristaltic pump was used to pass VX2 cells (1.7×10^6 cells/mL) suspended in 150 mL aCSF through dead-end polyethersulfone filters with a pore size of 5 μm (40 mm diameter, Sterlitech). Experiments were conducted in a heated room at approximately 37°C. The flow rate ranged from 0.25 to 1.0 mL/min, and a pressure sensor was attached to the system to monitor filter pressure throughout the experiment. Samples were collected for every 10 mL and were processed in order to determine when cells began to break through the filter membrane, denoting that the filter's capacity was reached.

Cranial–Spinal Model

A model of the cranial–spinal SAS was created (polycarbonate; internal construction) for experimental testing with the neurapheresis filtration system (Figure 1). The model roughly approximates the volume and distribution of CSF in the human SAS. Ports were built into the ventricular, cervical, and lumbar sites of the model to allow for sample collection and insertion of the neurapheresis catheter into the lumbar SAS extending to the mid-thoracic area. Two side ports were built into the ventricular site to allow for the infusion of fluid and release of passive waste.

For all experiments, the model was filled to near-maximum capacity with phosphate-buffered saline (PBS) (~136 mL). An external reservoir of PBS introduced fresh fluid at a rate meant to be equivalent to that of the waste rate of the neurapheresis system (0.225 mL/min) into a side port in the ventricular site of the model, though the actual infusion rate across experiments varied between 0.19 and 0.30 mL/min. To maintain a constant volume of fluid in the model, a passive waste port in the ventricular site allowed for any excess fluid to flow out of the model, thus simulating natural production and removal of CSF over time from the system.

Control experiments (n = 5) consisted of a bolus injection of MTX into the ventricular site of the model to simulate standard of care drug delivery via an Ommaya reservoir. Experiments with neurapheresis filtration (n = 4) involved the same bolus injection of MTX to the ventricles, but the dual lumen neurapheresis catheter was inserted in the model as well. A peristaltic pump set at 2.0 mL/min aspirated fluid in the model from the

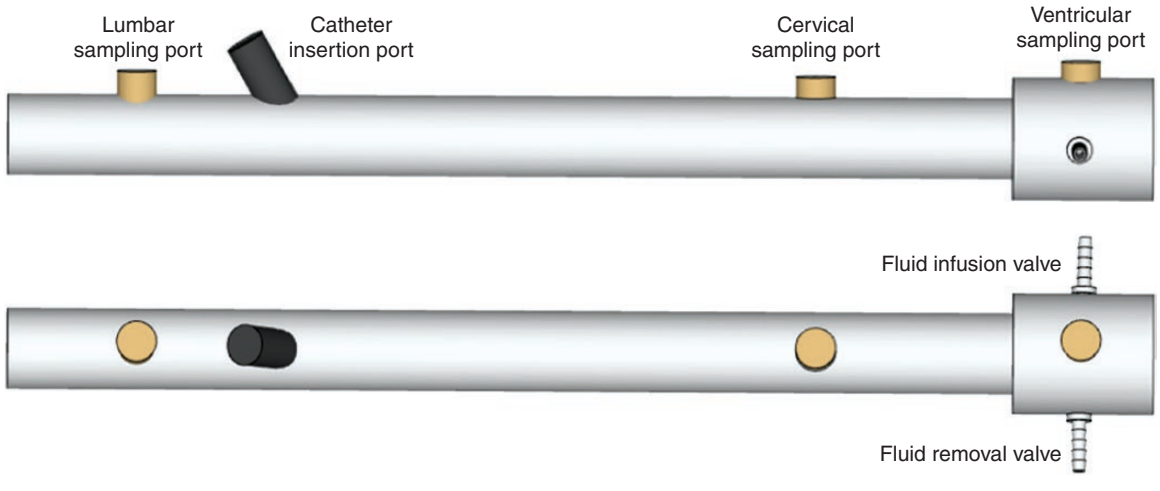

Figure 1. Schematic of the human cranial–spinal model. Samples were collected using 1 mL syringes and 22 G needles from the 3 sampling ports (yellow). The neurapheresis catheter was placed in the model through the insertion point (gray); the upper tip (return port) of the catheter reached approximately halfway between the cervical sampling port and the ventricular sampling port. The aspiration port of the catheter rested just below the "Catheter insertion port" of the model. Methotrexate was infused at the ventricular sampling port. The fluid infusion valve indicates where fluids (PBS) were infused back into the model. The fluid removal valve indicates where fluids were passively removed from the model.

lumbar catheter inlet port and returned filtered (via 2 tangential flow filters [TFFs]) fluid to the cervicothoracic catheter outlet port of the dual lumen catheter at a rate of approximately 1.775 mL/min. The system maintained a waste rate set at 0.225 mL/min. The model was covered with either aluminum foil or a blackout curtain throughout the duration of the experiment to prevent degradation of the drug from light.

In Vitro Neurapheresis Filtration: Tangential Flow

The neurapheresis filtration system was assembled using two 100 kDa TFFs (Millipore Sigma) connected in a closed loop using silicone tubing to an experimental stock flask, representing the CSF of an LM patient. The fluid was passed through the system at a programmable flow rate via a peristaltic pump. The cellular debris, or "retentate," was collected in a waste reservoir at a rate of 0.2–0.25 mL/min, modulated by an adjustable backpressure valve. The clean fluid, or "permeate," was returned to the experimental flask to close the loop. Sensors within the system were connected electronically to a computerized controller capable of logging flow rate, waste rate, and pressure data. Output was continuously measured to ensure the proper functioning of the system.

The neurapheresis system was first primed with preservative-free aCSF using a syringe to eliminate the dead volume and air bubbles. VX2 cells (3×10^5 cells/mL) were suspended in 150 mL aCSF with 3 mM EDTA in a 250 mL Erlenmeyer flask to represent the approximate total human CSF volume. Cells were kept in a heated room at approximately 37°C throughout the duration of the experiment. The stock flask was placed on a magnetic stir plate with constant mixing to maintain a homogeneous suspension. The flow rate was set at 0.5 mL/min at the start of filtration and gradually increased to 2.0 mL/min. Samples from the experimental flask were taken following the passage of every full CSF volume (150 mL) through the system, designated as a complete cycle of filtration. For each sample, the pump was stopped briefly to collect triplicate 300–500 µL samples from the stock and permeate sites, and the stock flask was replenished with aCSF to return to a total volume of 150 mL to mimic the regeneration of CSF in a patient. For comparison, a control flask was also prepared with the same starting concentration of VX2 cells under all the same conditions and without any experimental intervention. Singular samples were taken from the control flask following each complete cycle of filtration. Cell concentration was measured within the first hour post-sampling to ensure quality results.

In Vitro Neurapheresis Filtration in the Presence of MTX

Methotrexate Hydrate (Sigma-Aldrich; cat# A6770-100MG) was reconstituted with 1 N NaOH and frozen in 12 mg (clinical dose)[30] aliquots at –20°C. Aliquots were thawed and diluted in 2.76 mL of normal saline for experiments. A syringe pump (Harvard Apparatus) infused 3 mL of MTX at a rate of 0.2 mL/min into the stock flask of VX2 cells undergoing neurapheresis filtration. A 12 mg bolus dose of MTX was also administered to a separate control flask of VX2 cells without filtration. For comparison, 2 more flasks were prepared; a flask of VX2 cells that underwent neurapheresis filtration without MTX and a control flask of VX2 cells that was prepared under all the same conditions but without any experimental intervention (Figure 2). After each cycle of filtration, triplicate samples of cells in aCSF were taken from the flask receiving neurapheresis filtration and MTX infusion, while singular samples were taken from the 2 control flasks. All samples were counted manually using Trypan Blue Solution to measure cell concentration. Previous testing has also shown that on average 75% of MTX remains in circulation during the filtration process.

In Vitro Intraventricular MTX Infusion With Neurapheresis Filtration in a Cranial–Spinal Model

A series of experiments were also completed to test the ability of neurapheresis therapy to circulate, distribute, and maintain MTX levels throughout the SAS in a cranial–spinal model of the SAS. For these experiments, Methotrexate (Sigma-Aldrich; cat# M9929-100MG) was reconstituted from powder with 1 N NaOH, diluted in 1 mL PBS, and frozen in 6 mg aliquots at –20°C. Based on preliminary data which suggested 6 mg of MTX could sustain the minimum cytotoxic concentration of the drug in the model through 24 h, a 6 mg dose of MTX was used for injection. On the day of experiments, aliquots were thawed and further diluted in 1.88 mL of PBS for a total dose volume of 3 mL.

Approximately 300–500 µL of fluid were withdrawn from each of the ventricular, cervical, and lumbar sites of the model via needle injection ports for sampling, which was conducted over a 48-h period ($t = 0.5, 4, 8, 12, 24$, and 48 h) following drug administration. Samples from the spine model were analyzed by a methotrexate-specific enzyme-linked immunosorbent assay (ELISA) kit according to its instruction manual (ENZO).

Cell Counting

VX2 cell concentration was quantified after every full CSF volume cycle (150 mL) processed across the filters. For filtration studies without MTX infusion, total cell concentration was calculated with the samples using a 1:1 dilution with Acridine Orange on the Nexcelom Cellometer Auto T4 Bright Field Cell Counter. For filtration studies with MTX infusion, Trypan Blue Solution (0.4%; Thermo Scientific) was used to assess live and dead cell concentrations using a hemocytometer. All counts in which no cells were observed were reported as a concentration of 1×10^3 cells/mL, the approximate limit of detection of the Nexcelom cell counter and hemocytometer.

VX2 Cytotoxicity After Exposure to MTX

The Pierce Lactate Dehydrogenase (LDH) Cytotoxicity Assay (Thermo Scientific) was used to assess the viability of VX2 cells following MTX administration. Results from preliminary experiments measuring baseline levels of VX2 cytotoxicity in vitro after exposure to MTX and staurosporine, an apoptosis inducer, validated the use of

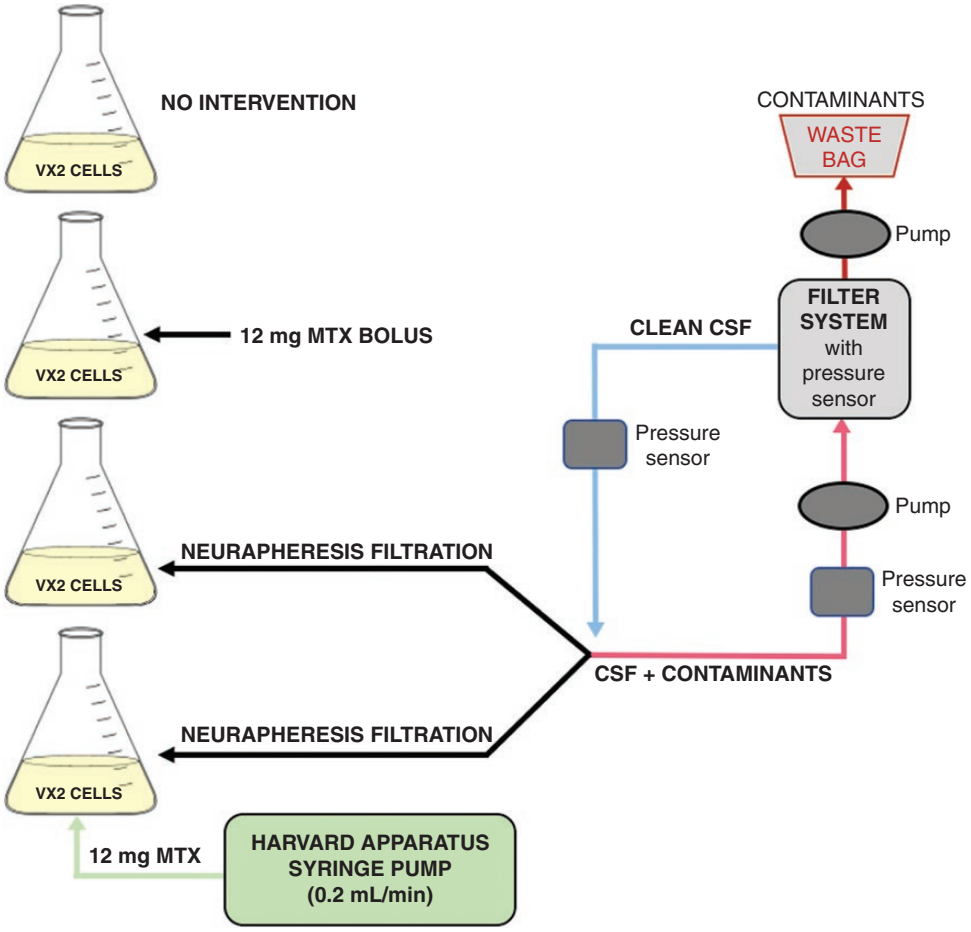

Figure 2. In vitro setup for VX2 cell interventions. For the first experimental flask, a syringe pump infused 12 mg MTX into the stock flask of VX2 cells, which then also received neurapheresis filtration. A 12 mg bolus dose of MTX was also administered to a separate control flask of VX2 cells without filtration. A flask of VX2 cells that underwent neurapheresis filtration without MTX and a control flask of VX2 cells without any experimental manipulation were also created for comparison. All cells were suspended in aCSF that flowed through the inlet line (pink) and passes through the filter system. Filtered particulates (cells) then enter a waste reservoir and clean aCSF returns to the "patient" (flask) through the permeate line (blue). Pressure and flow metrics are recorded by computer software through the attached sensors.

this assay for neurapheresis experiments. LDH levels were measured for cells undergoing neurapheresis filtration with a 12 mg MTX infusion, cells given a bolus of 12 mg MTX, and untreated cells without filtration. Triplicate samples of 50 μL were taken at each filtration cycle to perform the Chemical Compound-Mediated Cytotoxicity Assay. Percent toxicity was calculated using Eq. (1).

$$\%\ \text{Cytotoxicity} = \left(\frac{\begin{array}{c}(\text{compound treated}\\ \text{LDH activity})\\ -(\text{spontaneous}\\ \text{LDH activity})\end{array}}{\begin{array}{c}(\text{maximum}\\ \text{LDH activity})\\ -(\text{spontaneous}\\ \text{LDH activity})\end{array}} \right) \times 100 \quad \text{Eq. (1)}$$

Cytotoxicity measurements were compared to the maximum LDH activity control, spontaneous LDH activity control, and aCSF.

Statistical Analysis

Statistically significant differences between groups were determined using a 2-factor ANOVA test without replication at a confidence level of 95% ($\alpha = 0.05$). All significance testing was performed with Microsoft Excel.

Results

In Vitro Neurapheresis Filtration With MTX Infusion Demonstrates a 2.4-Log Reduction (>99%) in Live-VX2 Cell Concentration in 6 Cycles

First, dead-end filters were used to remove high concentrations of VX2 cells in vitro (Supplementary Figure 2). Due to the limitations of dead-end filtration, 100 kDa TFFs were instead chosen for use for all experiments due to their ability

to continuously operate without clogging at relatively high solid loads while effectively filtering biological particles at the given diameter.

The neurapheresis system, which has 2 TFFs, was then tested to determine how many cycles of filtration were required to clear a clinically significant concentration (approximately 3×10^5 cells/mL in 150 mL aCSF) of VX2 cells to the limit of detection. In order to assess the impact of neurapheresis filtration and MTX compared to an MTX bolus alone, live cell counts were reported.

When neurapheresis alone was used, it resulted in a 2.3-log reduction in live-VX2 cells after 6 cycles of filtration (7.5 h). This condition was compared to a flask that underwent both neurapheresis filtration and MTX administration. MTX was infused into the neurapheresis filtration loop to assess the adjunctive effect of drug intervention in addition to filtration. After the sixth cycle, the VX2 cell concentration dropped to the limit of detection (1×10^3 cells/mL) and exhibited a 2.4-log reduction. The 2 neurapheresis filtration interventions were also compared to a control flask with VX2 cells that was given a bolus of 12 mg MTX without neurapheresis intervention ($P < .01$) and a control flask that received no treatment ($P < .01$) (Figure 3). The concentration of live cells in the control flasks stayed approximately constant for the duration of the experiment ($P < .001$).

Cell concentration measurements throughout the duration of filtration indicated that neurapheresis filtration played a substantial role in tumor cell clearance. Compared to the neurapheresis therapy and MTX condition, the control flask that only received the MTX bolus showed a minimal decrease in cell burden. Results from an LDH cytotoxicity assay showed increased levels of cytotoxicity in the flask of VX2 cells that received the MTX bolus, confirming the drug's cytotoxic effects on VX2 cells. Cells undergoing neurapheresis filtration in combination with MTX infusion showed negligible levels of LDH following MTX infusion due to the removal of dead or damaged tumor cells and the presence of free LDH over time (Figure 4).

In Vitro Neurapheresis Filtration Demonstrates Faster Drug Distribution

Following a 6 mg bolus dose of MTX to the ventricles in the cranial–spinal model, neurapheresis therapy overall demonstrated an increased and faster distribution of MTX throughout the entire neuraxis, specifically to the cervical and lumbar sites, when compared to the control experiments without neurapheresis therapy (Figure 5A and B).

The MTX concentration trended higher in the cervical and lumbar sites in the neurapheresis experiments at all time points between 4 and 24 h compared to the control experiments. Concentrations during neurapheresis were also maintained above the minimal cytotoxic drug concentration for MTX (0.454 μg/mL).[31] However, the only time point in which this difference achieved statistical significance was at the lumbar site at 8 h ($P = .0074$). At the 8-h time point, the average concentration of MTX across the control experiments was 9.05 ± 11.14 μg/mL in the cervical region and 5.53 ± 5.61 μg/mL in the lumbar region, compared to 17.61 ± 5.79 and 17.01 ± 2.69 μg/mL for the neurapheresis experiments, respectively (mean ± 1 SD). The neurapheresis system was also able to rapidly decrease the high and potentially neurotoxic concentration

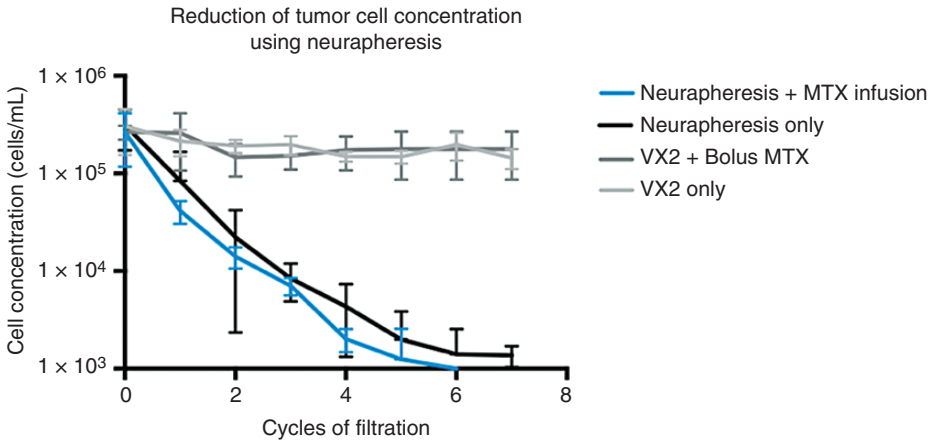

Figure 3. Reduction of tumor cell concentration using neurapheresis with MTX drug delivery: a comparison. Data shown in gray represent VX2 cells that did not undergo neurapheresis filtration ($n = 4$). The cells that are represented by the dark gray line received a bolus dose of 12 mg MTX, while the cells represented by the light gray line received no drug intervention. Concentrations for VX2 cells that underwent neurapheresis without drug intervention are shown in black as a direct comparison to cells that were given a 12 mg MTX infusion in addition to filtration shown in blue. Throughout the duration of filtration, cells that received MTX infusion and underwent neurapheresis filtration are cleared at a comparable rate to that of neurapheresis without MTX. In vitro neurapheresis filtration with MTX infusion accomplished a 2.4-log reduction in cell concentration in 6 cycles (7.5 h), and neurapheresis alone accomplished a 2.3-log reduction in live cell concentration. Plotted points represent the mean cell concentration at each cycle for each experiment. Error bars show ±1 SD.

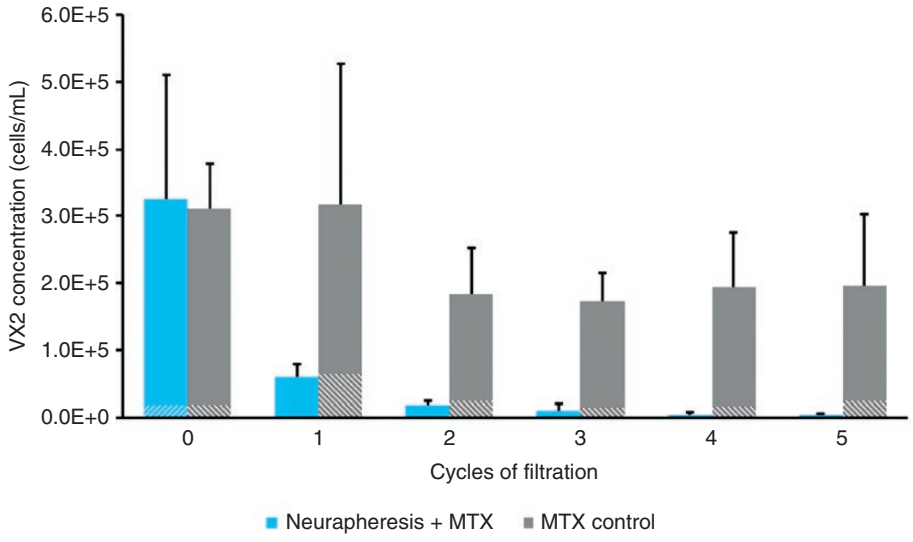

Figure 4. Cytotoxicity levels following exposure to MTX. Data shown above compare total VX2 concentration (solid bars) and cytotoxicity (striped bars) in aCSF at each Nneurapheresis filtration cycle ($n = 3$). Cells treated with bolus MTX (gray) experience increased levels of cytotoxicity compared to those that underwent neurapheresis and treatment with infused MTX (blue). The low cytotoxic levels exhibited by the neurapheresis group over the 3 cycles were due to the lower overall concentration of viable cells present in aCSF due to the ability of neurapheresis to filter out the VX2 cells. As a result, negligible amounts of LDH were present as filtration progressed. Error bars show +1 SD.

of MTX in the ventricles compared to the control experiments immediately following intra-ventricular drug delivery and up through 12 h post-injection (Figure 5C). Without neurapheresis therapy, the distribution of MTX to the lumbar and cervical regions was inconsistent, slower, and reached these sites in lower concentrations.

Discussion

Neurapheresis therapy represents a novel, dual-approach method that has the potential to reduce tumor burden in patients with LM. Current therapies are limited in terms of drug distribution and localization, but neurapheresis therapy may provide direct and tightly controlled access to the SAS, rapid clearance of circulating tumor cells below the limit of detection, and controlled circulation of chemotherapeutics within the SAS.

Rapid clearance of circulating tumor cells was shown here in vitro using the neurapheresis system. The structure of TFFs allowed for effective filtration of cells without clogging, despite the relatively high solid load. As a result, the tangential flow was deemed more appropriate for our purposes compared to a traditional dead-end filter. With TFFs, tumor burden was reduced below the limit of detection after 6 cycles (7.5 h) of filtration without any drug intervention and without any pressure buildup due to the aggregation of cells on the filter membrane.

Experiments assessing changes in live-VX2 cell concentration showed that the infusion of MTX in addition to neurapheresis filtration clears live-VX2 cells close to the limit of detection after 5 cycles (6.25 h) of filtration. Based on our data, one can reasonably expect the benefits of IT MTX with neurapheresis therapy to materialize over longer periods of filtration in clearing both live and dead cancer cells and their debris within the CSF. Additionally, the neurapheresis system maintained full functionality in the presence of MTX. Preliminary compatibility tests have demonstrated minimal loss of drug in components of the neurapheresis filtration system, although further in-depth material compatibility testing will be conducted prior to clinical testing.

In treating LM, the spread of MTX and maintenance of a concentration above the minimum cytotoxic concentration across the entire neuraxis are essential for managing disease progression. Without neurapheresis filtration, intra-ventricular delivery of MTX runs the risk of significantly limited diffusion throughout the SAS beyond the site of injection. Taken together, the in vitro intra-ventricular drug injections in a human cranial–spinal model demonstrated an increased and faster distribution of MTX to the lumbar and cervical regions when neurapheresis therapy is used. The improved circulation of the chemotherapeutic drug along the neuraxis of LM patients with neurapheresis therapy thus may enhance the cytotoxic effects of the drug on malignant cells in the CSF and contribute to further decrease of tumor burden within the SAS.

Furthermore, enhanced circulation of the drug via neurapheresis filtration may help to mitigate treatment-related neurotoxicity from IT drug injections for patients with LM. Without neurapheresis therapy, removal of MTX from the CSF is solely limited to passive diffusion. Experiments with the human cranial–spinal model showed that neurapheresis filtration helps to decrease and diffuse the high concentration of MTX at the ventricular site of

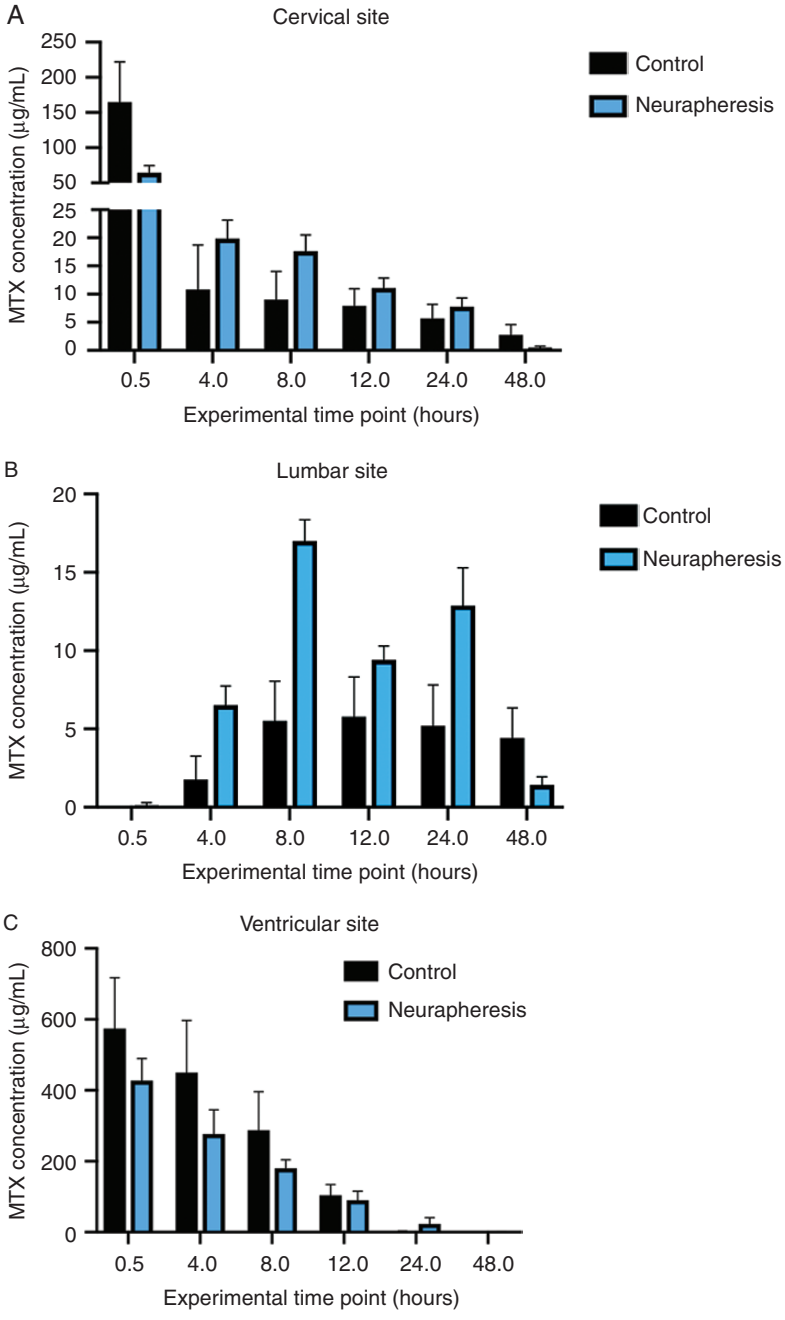

Figure 5. Methotrexate distribution in the cranial–spinal model. MTX (6 mg in 3 mL saline) was delivered to the ventricular sampling port at T0. Samples were collected at hours 0.5, 4, 8, 12, 24, and 48. Control experiments (n = 5) consisted of solely the bolus MTX, while neurapheresis experiments (n = 4) had the neurapheresis catheter inside the model with a flow rate of 2.0 mL/min. Error bars show +SEM. (A) MTX concentrations in the cervical region, (B) MTX concentrations in the lumbar region, and (C) MTX concentrations in the ventricular region. Neurapheresis filtration allowed for a greater and quicker distribution of MTX to the cervical and lumbar regions of the model and reduced the high, neurotoxic concentration of MTX in the ventricular site immediately following drug injection.

injection over the course of 12 h following drug administration. This effect is modulated by the waste rate of the neurapheresis system and can be reduced if necessary.

Future studies will focus on pharmacokinetics (PK) work to further evaluate the ability of the neurapheresis system flow loop to enhance the distribution of MTX throughout the SAS. Intraventricular boluses of MTX coupled with the neurapheresis system could drastically improve the circulation of the drug in the SAS while simultaneously removing circulating tumor cells, both live and dead. PK experiments in both animals and an advanced 3D human cranial–spinal model are currently in progress.

Conclusions

Neurapheresis therapy may be a potential approach to enhance the current treatment of LM. Our data suggest that neurapheresis therapy has the ability to significantly reduce the viable CSF tumor burden in vitro and greatly enhance the circulation of IT chemotherapy throughout the neuraxis of a cranial–spinal SAS model. Moving forward, in vivo preclinical and eventual clinical research is warranted to refine the therapy in an animal and human model, respectively. Neurapheresis filtration has the potential to be used, in addition to radiation and systemic therapy, to improve the standard of care methods for patients with LM.

Supplementary Data

Supplementary data are available at *Neuro-Oncology Advances* online.

Keywords

leptomeningeal metastases | methotrexate | Neurapheresis | subarachnoid space | VX2

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