Long-term Effects of Carboplatin Brainstem Infusions on Hearing Thresholds in Monkeys

Bradford J. May, PhD; Michael Guarnieri, PhD; Benjamin S. Carson, Sr, MD; Abdul Bachani, BS; George I. Jallo, MD

Objective: To isolate the central auditory neurotoxicity of carboplatin from its well-established ototoxic effects.

Design: The “best-case scenario” of targeted drug delivery to brain cancer was simulated by infusing carboplatin directly into the brainstem of cynomolgus monkeys with chronically implanted catheters. Because this manner of drug administration produced low levels of carboplatin in spinal fluid and blood, it was assumed that resulting deficits were dictated by the central auditory neurotoxicity of platinum compounds and not peripheral ototoxic effects. The magnitude of this hearing loss was estimated by comparing the auditory brainstem response thresholds of treated monkeys with results from normal controls.

Subjects: Six adult male cynomolgus monkeys (Macaca fascicularis) weighing 4 to 6 kg (3 received carboplatin treatment and 3 served as normal controls).

Intervention: Brainstem infusions of carboplatin.

Results: The average threshold of carboplatin-treated monkeys was elevated 8.8 dB (SD=7.3 dB) relative to normal controls 6 months after the termination of drug delivery and increased to 10.7 dB with less variation between subjects (SD=5.6 dB) 1 year after drug treatment. Although small in magnitude, the hearing loss was statistically significant (P<.05).

Conclusions: Brainstem infusions of carboplatin induced some degree of hearing impairment in all treated monkeys. These threshold elevations were modest compared with the ototoxic effects that have been reported after systemic doses of carboplatin. Our findings suggest that the neurotoxic sensitivity of cochlear hair cells is not shared by neurons in the central auditory pathways. As a result, methods for reducing the ototoxic effects of chemotherapy remain a viable strategy for preserving auditory function in patients with brain cancer.

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Platinum compounds represent the most effective chemotherapeutic agents for the treatment of solid tumors of the lung, ovaries, colon, and brain. The antitumor activity of these drugs is assumed to arise from the formation of platinum-DNA adducts, which interrupt RNA synthesis and ultimately trigger the death of cancer cells.1 Platinum-based chemotherapy is limited by toxic effects that preferentially target cochlear hair cells (ototoxicity) and renal tubular cells (nephrotoxicity).2,3 A common effect of platinum-based chemotherapy is the concomitant loss of cochlear hair cells. This ototoxicity is exacerbated in the treatment of brain tumors because chemotherapeutic agents must be administered in high systemic doses to deliver effective drug concentrations across the blood-brain barrier. Ongoing research efforts are exploring methods to preserve cochlear function by reducing the metabolic stress of drug treatment and improving the efficiency of drug delivery to the brain. While these approaches may enhance hair cell survival, the ultimate success of these strategies to preserve hearing depends on the largely unknown ability of central auditory neurons to withstand the local neurotoxic effects of platinum compounds.

Carboplatin-induced ototoxicity has been linked to a biochemical cascade that increases intracellular levels of toxic reactive oxygen species and free radicals.4 The eventual depletion of the native cochlear antioxidant system by this metabolic challenge is followed by progressive hair cell loss.5 Central auditory deficits also are implied by increased reactive oxygen species and depressed antioxidant enzyme activities in the inferior colliculus.6 Strategies to maintain cochlear function during platinum-based chemotherapy include temporary osmotic blood-brain barrier disruption to enhance drug delivery to the brain,7 and the local administration of chemoprotective agents to reduce oxidative stress.8 These treatments can be expected to preserve hearing only if central auditory neurons are...
more resistant to platinum toxicity than their cochlear counterparts. Because previous functional assays involve systemic injections of carboplatin, locally disordered processes of central auditory neurons cannot be separated from deficits that arise from a loss of peripheral inputs.

The Hunterian Brain Tumor Laboratory at Johns Hopkins University, Baltimore, Md, has been investigating chronic brainstem infusion as a method for circumventing the dose-limiting adverse effects of carboplatin in cynomolgus monkeys.9 Because very low levels of platinum are observed in plasma and cerebrospinal fluid with this system of drug delivery, their primate model represents a unique opportunity to examine the brainstem neurotoxicity of carboplatin in the absence of cochlear pathologic conditions. It is shown that auditory brainstem response (ABR) thresholds of carboplatin-treated subjects are only slightly elevated relative to normal counterparts. Because previous functional assays indicate that 80% of the drug solution is preserved in pumps at 37°C for periods up to 12 weeks,9,10 Blood and cerebrospinal fluid platinum levels were measured by inductively coupled plasma/mass spectrometry (National Medical Services, Willow Grove, Pa). The lower limit of platinum detection with this method is 2 µg/L. Measures were obtained biweekly during drug administration and with increasing intervals after the termination of treatment. Platinum levels in cerebrospinal fluid peaked at 49 to 89 µg/L during drug administration and had declined to undetectable levels 100 days after the termination of treatment. Platinum levels in blood did not exceed 6.1 µg/L at any time in any subject.

**AUDITORY BRAINSTEM RESPONSE**

Evoked potentials were recorded inside an electrically isolated sound-attenuating chamber while the monkey was anesthetized with ketamine hydrochloride. Difference electrodes were located on the vertex (noninverting input) and the ipsilateral mastoid (inverting input). The common electrode was placed on the back of the neck. The electrode signal was bandpass filtered with cutoff frequencies of 100 and 3000 Hz, amplified with a gain of ×30,000, and digitized at a sampling rate of 20 kHz. Auditory responses were evoked by free-field presentations of condensation and rarefaction clicks. The high-frequency speaker was calibrated at the beginning of each experiment to standardize overall stimulus levels and to confirm that the amplitude spectrum of the click deviated by less than ±5 dB at frequencies from 5 to 40 kHz. Recordings were made from only the left ear to minimize the length of anesthesia. Monaural stimulation was achieved by orienting the head so that the right (contralateral) ear faced down into acoustic foam and the left (ipsilateral) ear was directed toward the sound source. The average ABR waveform was based on 3000 click presentations. This number of stimulus repetitions exceeds typical sampling methods for large nonhuman primates. Additional averaging produced a reproducible waveform shape for capturing potentially subtle changes in response latency and magnitude. The clicks were delivered at a repetition rate of 30 presentations per second. The polarity of the clicks was reversed after each presentation to eliminate microphonic potentials. Response magnitude was derived from the average ABR waveform by measuring the maximum peak-to-peak voltage during a 5-millisecond (ms) interval that began 2.5 ms after the stimulus presentation. All procedures were approved by the Institutional Animal Care and Use Committee of the Johns Hopkins School of Medicine. Drug treatments and ABR recordings were performed in 3 adult male cynomolgus monkeys (Macaca fascicularis) weighing 4 to 6 kg. Because pretreatment ABRs were not available in this experimental group, the magnitude of drug-induced threshold shifts was estimated from control data from 3 untreated monkeys. The subjects were obtained from Biologic Research Farms (Houston, Tex) and showed good health and normal behavior at the inception of treatment.

**METHODS**

**SURGICAL IMPLANTATION OF DRUG DELIVERY CATHETER**

Surgical techniques for placing a drug delivery catheter into the brainstem of monkeys have been described in a previous publication. Briefly, a burr hole was made in the midline of the occipital skull 2.5 cm below the inion. The tip of the infusion catheter was manually inserted through the cerebellum to a midline medullar target with approximate stereotoxic coordinates of –6.0 mm anterior-posterior, –12.5 mm dorsoventral, and 0 mm mediolateral. The catheter was secured to the skull with cyanoacrylate. Postoperative computed tomographic scans confirmed the placement of catheters and revealed no evidence of pneumocephalus or hemorrhage within the cranial cavity. Carboplatin was delivered from a 1-mL Duros infusion pump (Durect Corporation, Cupertino, Calif). The body of the pump was implanted subcutaneously in the cervical/high thoracic region of the back and connected to the catheter with silicon tubing. The tubing was looped into a subcutaneous pocket that was created between the pump and catheter. The monkeys were active in their cages within 1 hour after the completion of the surgical procedure.

**DRUG CONCENTRATIONS**

Carboplatin was diluted in a 3% dextrose solution and delivered at a 0.42-µL/h infusion rate for 29 days. The drug concentration was adjusted to produce a total infused dosage of 694 µg (approximately 0.25 mg/kg). The cumulative exposure represents the maximum tolerated dose for brainstem infusions of carboplatin based on veterinary assessments and toxicity results from our previous studies of cynomolgus monkeys.9,10 The pumps were removed under ketamine anesthesia to stop the infusion and opened to verify drug delivery. Previous high-performance liquid chromatographic assays indicate that a 0.42-µL/h infusion rate for 29 days.
HISTOLOGICAL ASSESSMENT

Monkeys in the experimental group were killed by barbiturate overdose approximately 400 days after the termination of drug treatment and immediately perfused with a 4% formalin solution. The brainstem and cerebellum were cut into 5-µm coronal sections at intervals of 960 µm. Adjacent sections were processed with hematoxylin-eosin to describe conventional cytoarchitecture or the iron-sensitive Perls-diaminobenzidine stain to indicate accumulated hemosiderin from bleeding or necrosis.

Histological results from monkey 99-200 are representative of the tissue damage noted in all subjects. High levels of Perls-diaminobenzidine stain in serial sections 3 and 4 of Figure 1A reveal an infusion site in the ventromedial medulla near the somatosensory tract of the medial lemniscus. The intensity of the reaction product suggests localized bleeding and inflammation. Some necrosis is evident in section 4. Brainstem cytoarchitecture appears normal outside the region of degeneration.

The inferior colliculus was located approximately 10 mm dorsal to the infusion site and spanned sections 5 through 8. Background traces of Perls-diaminobenzidine labeling in the brainstem nuclei and cerebellar folia result from residual blood after perfusion. Hematoxylin-eosin stains showed no signs of tissue degeneration (Figure 1B). Additional auditory brainstem nuclei were lateral (cochlear nuclei) or rostral (superior olive) to the block of tissue that was submitted for histological processing.

RESULTS

Carboplatin-treated monkeys were monitored by veterinary staff for clinical signs of morbidity based on gait, interactions with caretakers, grooming habits, and food intake. Monkey 99-170 maintained normal weight and was judged to be bright, alert, and responsive throughout the experiment. Monkey 99-200 lost 9% of its initial weight and showed a transient lack of attentiveness that resolved during drug treatment. More pronounced behavioral impairments were noted in monkey 99-225. This subject lost 16% of its initial weight and developed a blunted affect with unsteady gait. Although the subject regained normal balance 2 to 3 months after treatment, it continued to display a general lack of focus. These results are predicted by our previous toxicology studies and provide further evidence that high doses of platinum can be delivered to the brain without inducing severe behavioral morbidity.

The effects of stimulus level on the magnitude, latency, and waveform shape of the ABR are shown in Figure 2. Compared with a control subject, the carboplatin-treated monkey (99-200) displayed no consistent differences in these basic response properties 6 months after the termination of drug treatment (post 1). The responses of the treated subject suggest a slight loss of sensitivity when ABR measures were repeated 6 months later (post 2).

The input-output functions in Figure 2 plot the peak-to-peak amplitude of the individual ABR waveforms. These values were calculated in the 2.5- to 7.5-ms time window after stimulus onset (0 ms). Background activity was measured during the 25- to 30-ms window of the corresponding stimulus off interval. The threshold criterion represents a response magnitude that is 2 SDs greater than the mean level of background activity in each data set. Recordings with background activity greater than 2 µV were not included in the threshold analysis (eg, the 33 dB SPL stimulus level for the 1-year session of the treated monkey).

The input-output functions in Figure 2 rise monotonically to a maximum ABR amplitude between 0.5 to 1.0 µV. Threshold is indicated by an abrupt increase in slope that corresponds well with the 2-SD criterion and visual inspection of the corresponding waveforms. Relative to the control data, the brainstem infusion of carboplatin did not radically elevate the hearing sensitivity of the treated monkey.
One year after drug treatment (post 1), experimental subjects produced an average evoked threshold of 40.0 dB SPL with little intersubject variability (SD=0.9 dB). Six months after drug treatment (post 2), the average threshold increased to 50.7 dB SPL and was accompanied by reduced variability (SD=5.6 dB).

The lower limits of the box plots reflect the sensitive thresholds of monkey 99-200 (Figure 2). Because this subject maintained normal hearing 6 months after treatment, the 8.8-dB elevation of the post 1 thresholds failed to attain statistical significance (unpaired t test, P>0.05). One year after treatment, the threshold of this subject had increased to 44 dB SPL, resulting in the statistically significant 10.7-dB elevation of the post 2 thresholds (unpaired t test, P=0.02).

Direct drug delivery has been shown to reduce the systemic effects of chemotherapy in animal models12,13 and in human patients with head and neck,14 ovarian,15 and brain cancer.16,17 Less is known about the localized toxicity of chronic drug infusion. The major finding of this study is that brainstem infusions of carboplatin elevate ABR thresholds by approximately 10 dB, which is substantially less than the hearing loss induced by high systemic doses. This result suggests that intracranial drug delivery minimizes the peripheral ototoxic effects of carboplatin without creating an equally debilitating pathologic condition in the central auditory system. Recent research efforts have attempted to enhance the delivery of these drugs to the brain by disruption of the blood-brain barrier. When left intact, this natural defense mechanism also has the capacity to restrict the circulation of chemical agents out of the brain and into sensitive cochlear and renal tissues.

The blood-brain barrier failed to prevent low levels of circulating platinum with chemoprotective agents.18 By withholding treatment, our results provide a conservative estimate of potential hearing loss. The observation of relatively normal auditory function in unprotected subjects is relevant for chemotherapy because it is not known...
how antioxidants and free radical scavengers degrade the oncological efficacy of platinum compounds.8

The ABR is assumed to reflect ascending synchronized activity in the auditory brainstem. Animal studies have identified the principal generator sites for the individual waves of this evoked potential by recording response latencies directly from the brainstem nuclei.19 The most direct correlation between structure and physiology is the auditory nerve contribution to the initial wave I of the ABR. The observation of normal thresholds, latencies, and magnitudes for this component supports normal cochlear function in our carboplatin-treated monkeys.

Multiple generator sites ranging from the cochlear nucleus to the lateral lemniscus are simultaneously active during waves II through V of the ABR. This mixed response prevents a straightforward interpretation of the functional integrity of specific nuclei based on changes in the individual waves of extracranial field potentials. Nevertheless, the absence of generalized differences in the ABR waveforms of our treated and control subjects makes it reasonable to conclude that these regions of the brainstem were functioning normally in carboplatin-treated monkeys.

Reduced antioxidant levels have been noted in the inferior colliculus shortly after systemic administration of carboplatin.6 Although the functional implications of this acute oxidative stress are not addressed by our present experimental design because ABR measures are not strongly influenced by activity in the inferior colliculus,20 similar metabolic changes may disrupt sound-evoked activity in the auditory brainstem. Our preliminary studies demonstrated transient threshold elevations at lower drug concentrations when ABRs were recorded during or immediately after a period of intracranial drug delivery (data not shown). Our long-term histological and electrophysiological assessments suggest that central auditory neurons survive this biochemical insult and ultimately return to normal function. A similar pattern of recovery is not observed in the cochlea, where oxidative stress is a precursor to widespread cell death.

This study evaluated the impact of carboplatin on hearing strictly in terms of ABR thresholds. While our approach may be adequate for confirming the stability of hearing sensitivity, it is clear that evoked potential measures fail to identify more subtle perceptual deficits. The ability to discern the location of a sound or understand the spoken word is based on information that must be faithfully encoded by the ear and then correctly interpreted by the brain. Because pathologic conditions may exist at any stage of auditory processing, more comprehensive animal behavioral models represent an important context for future assessments of the effects of platinum brainstem infusions on hearing.

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Correspondence: Bradford J. May, PhD, Johns Hopkins University, Department of Otolaryngology—Head and Neck Surgery, Traylor Bldg, Room 505, 720 Rutland Ave, Baltimore, MD 21205 (bmay@jh.edu).

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REFERENCES


