Comparative Neurotoxicity of Oxaliplatin, Cisplatin, and Ormaplatin in a Wistar Rat Model¹

Jennifer Holmes,* Jason Stanko,* Maria Varchenko,* Hong Madden,† Victoria J. Madden,‡ C. Robert Bagnell,§ Steven D. Wyrick,¶ and Stephen G. Chaney*

*Department of Biochemistry and Biophysics, Curriculum in Toxicology and Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599; †Inorganic Chemistry Laboratory, Analytical and Chemical Sciences, Research Triangle Institute, Research Triangle Park, North Carolina; §Microscopy Services Laboratory, Department of Pathology and Laboratory Medicine, School of Medicine, University of North Carolina, Chapel Hill, North Carolina 27599; and ¶Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599

Received March 30, 1998; accepted August 7, 1998


Oxaliplatin (4 mg/kg), cisplatin (2 mg/kg with 20 mg/kg mannitol) and ormaplatin (2 mg/kg) were administered IP twice weekly for 4.5 weeks. Lactose injections (0.9%) were used as a control for oxaliplatin and 0.9% saline injections were used as a control for cisplatin and ormaplatin. Morphometric changes to dorsal root ganglia L4–L6 were quantitated as a measure of neurotoxicity. Drug treatment resulted in a decrease in cell and nuclear area and an increase in the percentage of cells with eccentric nuclei for neuronal cell bodies in the DRG. Immediately following treatment the order of morphometric changes was ormaplatin > cisplatin ≥ oxaliplatin. The accumulation of platinum in the DRG was measured by inductively coupled plasma mass spectrometry. The order of accumulation was cisplatin > oxaliplatin > ormaplatin. Following an 8-week recovery period the order of morphometric changes to the DRG was ormaplatin = oxaliplatin > cisplatin. This correlated with a greater retention of platinum by the DRG for ormaplatin and oxaliplatin than for cisplatin. The results suggest that ormaplatin is uniquely neurotoxic immediately following treatment in the Wistar rat model. However, following an 8-week recovery period both ormaplatin and oxaliplatin are more neurotoxic than cisplatin and this neurotoxicity correlates with a greater retention of platinum by the DRG. © 1998 Society of Toxicology.

Oxaliplatin ((trans-1,2-diaminocyclohexaneoxalatoplatium(II))² is a second-generation platinum anticancer agent which has shown efficacy against metastatic colorectal cancer (de Gramont et al., 1997; Bertheault-Cvitkovic et al., 1996; Machover et al., 1996; Levi et al., 1992, 1993, 1994) and cisplatin-resistant ovarian cancer (Chollet et al., 1996). It has been approved for the treatment of colorectal cancer in France and has recently entered phase II/III clinical trials in the United States. Because neurotoxicity is dose-limiting for oxaliplatin (Machover et al., 1996; Levi et al., 1993; Extra et al., 1990), it is important to compare its neurotoxic potential with that of other platinum anticancer complexes.

Of the platinum complexes in use or in clinical trials, cisplatin has been best characterized with respect to neurotoxicity. As doses of cisplatin have escalated in recent years, neurotoxicity has emerged as dose-limiting (Mollman et al., 1988; van der Hoop et al., 1990). The initial symptoms of neurotoxicity are paraesthesias and numbness (Mollman et al., 1988; van der Hoop et al., 1990). At cumulative doses of 300–600 mg/m², a peripheral sensory neuropathy develops which is characterized by loss of vibratory sensation, loss of deep tendon reflexes, and marked proprioceptive sensory ataxia (Mollman, 1990; Mollman et al., 1988; van der Hoop et al., 1990). Neurophysiological studies have shown decreased sensory nerve conduction velocity but normal motor conduction velocity (Hamers et al., 1991; Mollman, 1990). Morphometric studies have shown preferential loss of large myelinated fibers (Mollman, 1990). Finally, biodistribution studies have shown that the platinum concentrations are greatest in the dorsal root ganglia, followed by the dorsal root and peripheral nerves (Gregg et al., 1992). Platinum levels are very low in the spinal cord. Neurotoxicity may continue to develop for several months after cisplatin treatment is discontinued. The neurotoxicity is generally at least partially reversible, but recovery may take many months (Mollman, 1990).

Cisplatin neurotoxicity has also been extensively studied in the Wistar rat model. As in humans, chronic cisplatin administration results in small, but statistically significant decreases in sensory nerve conduction velocity (McKeage et al., 1994; van der Hoop et al., 1988, 1990; Hamers et al., 1991, 1993), but
not in motor nerve conduction velocity (van der Hoop et al., 1990). Morphometric studies have suggested that the dorsal root ganglia are the primary site of cisplatin neurotoxicity (van der Hoop et al., 1994; Cavaletti et al., 1991, 1992; Muller et al., 1990; Tomiwa et al., 1986). The most prominent morphometric changes in the dorsal root ganglia are neuronal shrinkage (characterized by decreases in both cell and nuclear area) (Cavaletti et al., 1991, 1992; Tomiwa et al., 1986). However, fragmentation and eccentricity of the nuclei in neuronal cell bodies (Cavaletti et al., 1991, 1992; Tomiwa et al., 1986) is also observed, presumably reflecting damage to DNA which was sufficient to interfere with nucleolar organization and/or rRNA synthesis. Morphometric changes in peripheral nerves are more subtle. Small decreases have been observed in the number of large myelinated fibers (van der Hoop et al., 1994; Cavaletti et al., 1991, 1992). Ultrastructure studies have reported axonal and adaxonal accumulations indicative of axonopathy in some of the largest myelinated fibers (Cavaletti et al., 1991, 1992). No morphometric changes have been observed in spinal cord neurons (Cavaletti et al., 1991). Thus, the rat model system appears to accurately reflect the pattern of neurotoxicity seen in humans. The major site of damage appears to be the dorsal root ganglia, which is consistent with the platinum accumulation studies in humans (Gregg et al., 1992). The damage to the dorsal root ganglia appears to result in axonopathy of peripheral nerves, especially in the large myelinated fibers responsible for sensory nerve conduction.

In comparison, much less is known about the neurotoxicity of other platinum complexes. Ormaplatin, a platinum complex which is similar to oxaliplatin in that it has the 1,2-diaminocyclohexane carrier ligand, also produces dose-limiting neurotoxicity. The neurotoxicity associated with ormaplatin is primarily a peripheral sensory neuropathy, with symptoms very similar to the neuropathy produced by cisplatin (Schilder et al., 1994; O’Rourke et al., 1994; New et al., 1993). Morphometric studies in rat dorsal root ganglia have shown neuronal shrinkage, with subsequent vacuolization and cell death (New et al., 1993). The sciatic nerves showed marked axonal atrophy and a decrease in the number of the large sensory axons, while the motor axons remained unaffected (New et al., 1993). While the neurotoxicity associated with ormaplatin was similar in nature to the neurotoxicity associated with cisplatin, it prevented the clinical development of ormaplatin because it occurred at low total cumulative doses (165–200 mg/m²), the symptoms were severe, the onset of symptoms was delayed, and reversibility was incomplete (Schilder et al., 1994; O’Rourke et al., 1994).

There are two distinct types of neurotoxicity associated with oxaliplatin (de Gramont et al., 1997; Bertheault-Cvitkovic et al., 1996; Machover et al., 1996; Levi et al., 1993, 1994; Misset et al., 1991; Extra et al., 1990). There are cold-sensitive paresthesias which are unique among the platinum complexes studied to date. These paresthesias are acute, occur at low total cumulative doses, are always reversible, and do not require discontinuation of therapy. However, there is also a peripheral sensory neuropathy with symptoms similar to those seen with cisplatin and ormaplatin. This neuropathy occurs at total cumulative doses >800 mg/m². It is generally reversible, but may last for several months.

Thus, cisplatin, ormaplatin, and oxaliplatin all result in a peripheral sensory neuropathy with very similar characteristics. Neurotoxicity is not normally associated with carboplatin administration. However, very little effort has been made to quantitatively and qualitatively compare the neurotoxicity of these different platinum complexes in a single model system. The current study was designed to obtain a qualitative and quantitative comparison of oxaliplatin, cisplatin, and ormaplatin neurotoxicity in a Wistar rat model system. Because the dorsal root ganglia have previously been shown to be the most severely affected neural tissue for both cisplatin and ormaplatin, we focused on the morphological changes in this tissue. In order to distinguish between differences in the neurotoxic potential of the adducts formed and simple differences in the biodistribution of these platinum complexes, we also determined the platinum content of the dorsal root ganglia by inductively coupled plasma mass spectrometry (ICP-MS). Because both delayed onset of neurotoxicity and reversibility of neurotoxicity are important clinical issues, we included an 8-week recovery phase following platinum treatment.

MATERIALS AND METHODS

Materials. Ormaplatin (tetraplatin, (trans-d,l)-2-diaminocyclohexanetetrachloroplatium(IV)) was prepared by Dr. Steven D. Wyrick, Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina at Chapel Hill, as described previously (Wyrick and Chaney, 1987; Wyrick and Chaney, 1990). Oxaliplatin (trans-d,l)-1,2-diaminocyclohexaneoxalatoplatinum(II) was kindly provided by Dr. Martine Bayssas (Debiopharm, S. A., Lausanne, Switzerland). Cisplatin and carboplatin were obtained from Sigma (St. Louis, MO). Trace metal grade nitric acid was obtained from Fisher Scientific (Pittsburgh, PA). ULTREX ultrapure hydrogen peroxide was obtained from J. T. Baker (Phillipsburg, NJ). All other general laboratory chemicals were reagent grade or better and were used without additional purification.

Animals. Male Wistar adults rats (200–225 g) were obtained from Charles River Breeding laboratories (Raleigh, NC) and housed in clear plastic cages on a 12-h light/12-h dark cycle with access to water and Purina rodent Chow ad libitum. Room temperature was maintained at approximately 22°C. Animals were allowed a 1-week acclimatization period before use in experiments. The animal protocols were approved by the University of North Carolina Institutional Animal Care & Use Committee and were conducted in accordance with the Guiding Principles in the Use of Animals in Toxicology of the Society of Toxicology.

Methods: Drug preparation and administration. Oxaliplatin was supplied in 100-mg vials containing 900 mg of lactate. It was dissolved in water to give a stock solution of 1 mg/ml oxaliplatin in 0.9% lactate. Carboplatin stock solutions were prepared at 5 mg/ml in 0.9% lactate. The oxaliplatin and carboplatin stock solutions were divided into aliquots and frozen until use, since control experiments showed no loss of drug upon freezing and thawing. Cisplatin and ormaplatin stock solutions were prepared fresh for each injection. Cisplatin solutions were prepared at 0.167 mg/ml cisplatin and 1.67 mg/ml mannitol in 0.9% saline. Preparation of cisplatin stock solutions required heating at 60°C in the dark for 10–15 min with gentle stirring. Ormaplatin stock solutions were prepared at 0.5 mg/ml in 0.9% saline. This also required heating the solution at 60°C in the dark for 10–15 min.

For both of the experimental studies, oxaliplatin was administered ip at 4
mg/kg, lactose at 0.9%, cisplatin at 2 mg/kg with 20 mg/kg mannitol, ormaplatin at 2 mg/kg, and saline at 0.9%. Injections were given ip twice per week (Mondays and Thursdays) for 4.5 weeks (a total of 9 injections). The first experimental study was performed with 8 animals/group. Morphometric analyses were performed following dose 9. The second experimental study was performed with 16 animals/group. Morphometric analyses were performed on 6 animals following dose 9 and on the remaining animals at the end of the recovery period. For all three drugs, the dose chosen represented the maximum tolerated dose under these treatment conditions. For oxaliplatin the dose was based on a pilot study in which 1 of 10 animals died during the 4.5 week treatment at 4 mg/kg and 4 of 10 animals died during treatment at 6 mg/kg. For cisplatin and ormaplatin the dose was based on previously published studies in Wistar rats (van der Hoop et al., 1988, 1990; Hamers et al., 1991, 1993; McKeage et al., 1994; New et al., 1993; Scrence et al., 1997). No morphometric evidence of neurotoxicity was observed for carboplatin at 20 mg/kg in a pilot study so it was not included in the experimental studies.

**Morphometric determinations.** At the end of the 4.5-week injection period and again following an 8-week recovery period, six animals from each group were anesthetized with ether and perfused with 2% paraformaldehyde and 4% glutaraldehyde in 0.15 M sodium phosphate buffer, pH 7.4. Livers and kidneys were removed and stored individually at −80°C. Lumbar dorsal root ganglia L4–L6 were removed and stored in perfusion fluid at 4°C. Ganglia from the left side of the animals were used for ICP-MS determination of Pt levels, while ganglia from the right side of the animals were used for morphometric analysis.

For morphometric analysis, the ganglia were washed with 0.15 M sodium phosphate buffer, postfixed in 1% osmium tetroxide, dehydrated in ethanol series and propylene oxide, and embedded in Spurr epoxy resin. Serial thin sections (1 μm) were cut from the left and right sides of each ganglia at intervals of 25 μm, mounted on a glass slide and stained with 1% toluidine blue in 1% sodium borate for 30 s at 60°C. Light microscopy was performed using a Nikon Microphot-FXA microscope at 40× magnification. Images were scanned with an Optronics TEC-470 CCD camera and captured with a Scion LG3 Image Capture Card. Morphometric analyses were performed on a Macintosh 840AV computer using the public domain NIH Image Program, Version 6.2. All cells in which at least one nucleolus was visible in the section were evaluated for cellular area, nuclear area, percentage of cells with more than one nucleolus, and percentage of cells with eccentric nucleoli (defined as cells in which the nucleolar center was in the outer half of the radius of the nucleus).

Statistical comparisons were made by analyzing each dependent variable separately by a univariate ANOVA. In those cases where the p value was ≤0.05, pairwise comparisons among treatment groups were performed using Tukey’s HSD multiple comparison procedure. Statistical analyses were performed using Systat 5.0 for Windows.

**Atomic absorption analysis of Pt levels in liver and kidney.** The frozen liver and kidney from each animal were ground to a fine powder with a glass mortar and pestle and stored individually at −20°C until analysis. Aliquots (100 mg) were digested with concentrated nitric acid/30% H2O2 (Blisard et al., 1991). Platinum determinations were performed on a Perkin-Elmer Zeeman 5100 atomic absorption spectrometer with a HGA-600 graphite furnace. Statistical comparisons were performed as described for the morphometric analyses.

**Inductively coupled plasma mass spectrometry (ICP-MS) analysis of Pt levels in the ganglia.** Three ganglia from each animal were pooled, weighed, and digested as described above. To obtain the low backgrounds required for ultratrace metal analysis, trace metal grade nitric acid and H2O2 were used (see Materials). In addition, a separate set of digestion instruments and glass hydrosolysis tubes was obtained and used exclusively for the experiments involving ICP-MS determinations. The glass tubes were cleaned between hydrosytheses by heating with trace metal grade nitric acid at 90°C overnight. Finally, the digestion, weighing and hydrosylation steps were all performed in neighboring laboratories which did not use platinum compounds in their research.

The Pt determinations were performed on a VG PQ-XR ICP-MS unit equipped with a concentric nebulizer and a Scott double-pass spray chamber. Continuous sample introduction was achieved with a Gilson 222 autosampler and a peristaltic pump. The ICP-MS operating conditions were 1350 W forward power, 12.5 liters/min argon coolant flow, 1.3 liters/min auxiliary flow, 0.84 liters/min nebulizer flow, and 4.5°C spray chamber temperature. Samples were diluted fivefold and analyzed with iridium and bismuth internal standards. Minimum matrix interference was observed under these conditions. The method detection limit was 0.008 ng/ml of Pt, which corresponded to 4 pg of Pt in 100 μg of neuronal ganglia. The standard deviation in the tissue matrix was 5.7% or better. The conditions and controls for these determinations are described in more detail elsewhere (H. Ding et al., manuscript in preparation). Statistical comparisons were performed as described for the morphometric analyses.

**RESULTS**

The first experimental study was performed to obtain morphometric evaluation of neurotoxicity immediately following drug treatment. This study compared 2 mg/kg cisplatin plus 20 mg/kg mannitol and 2 mg/kg ormaplatin with a 0.9% saline control and 4 mg/kg oxaliplatin with a 0.9% lactose control. Decreased weight gain was observed in all drug treatment groups with cisplatin > oxaliplatin > ormaplatin (Fig. 1). No deaths were observed in any treatment groups. The morphometric data are shown in Fig. 2 and Table 1. There was a trend towards decreased neuronal cell size in dorsal root ganglia L4–L6, as measured by both decreased cell area and decreased nuclear area, in all three groups of drug-treated animals. However, this reduction in cell size was statistically significant only for the ormaplatin-treated group. There was also a trend toward an increased percentage of cells with multiple nucleoli in all of the drug-treated animals. This change was statistically significant in the cisplatin- and ormaplatin-treated groups. No significant differences were seen in the percentage of cells with eccentric nucleoli between the control animals and the drug-treated groups. Pairwise comparisons showed that the decrease in cellular area for ormaplatin-treated rats was significantly different than that for cisplatin- or oxaliplatin-treated rats, while cisplatin- and oxaliplatin-treated rats were not significantly different from each other. The atomic absorption analyses of platinum levels in liver and kidney are summarized in Tables 2 and 3. As expected, platinum accumulation was greater in the kidney than in the liver for all the drugs. In both the kidney and the liver the order of accumulation was cisplatin > oxaliplatin > ormaplatin. ICP-MS analyses of platinum levels in the spinal ganglia are shown in Table 4. Platinum accumulation in spinal ganglia was slightly less than in either the kidney or the liver and was in the order cisplatin > oxaliplatin > ormaplatin.

Because both delayed onset of neurotoxicity and reversibility of neurotoxicity are important clinical issues, the second experimental study included both a treatment phase and an 8-week recovery period. The experimental conditions and treatment groups were the same during the treatment phase as those in the first study. Decreased weight gain was seen during the treatment phase in all of the drug-treated groups with cisplatin = oxaliplatin > ormaplatin. No animals died during the treatment phase. However, several animals died during the recovery phase and a number of animals developed severe bloating of the peritoneal cavity (Table 5). Both mortality and
peritoneal bloating were more severe in the oxaliplatin and ormaplatin groups. Furthermore, the pattern of mortality was different for the cisplatin group than for the oxaliplatin and ormaplatin groups. Most of the deaths in the cisplatin-treated group occurred during the first 3 weeks of recovery, while most of the deaths in the oxaliplatin and ormaplatin-treated groups occurred during the last 2 weeks of the recovery period. One animal each from the oxaliplatin- and ormaplatin-treated groups was sacrificed for necropsy. The necropsy results showed inflammation and/or fibrosis of the peritoneal surfaces, including the liver, kidney, and splenic capsules. The peritoneal cavity of both animals was found to contain an abundant milky pink fluid mixed with blood. All drug-treated animals showed weight gain during the recovery period, but it was not considered a reliable indicator of recovery due to the peritoneal bloating.

Morphometric data immediately following drug treatment were unavailable due to problems with the perfusions. Morphometric data at the end of the recovery period are shown in Table 6. There was a significant decrease in neuronal cell and nuclear area for both the oxaliplatin and ormaplatin-treated animals. This appeared to be primarily due to a decrease in the number of large (>1500 μm²) neuronal cell bodies (Fig. 3). Changes in both parameters were smaller and were nonsignificant for nuclear area in the cisplatin-treated group. No significant changes were seen in eccentricity or number of nucleoli for any of the drug-treated groups compared to control. Pairwise comparisons showed that the reductions in cell and nuclear area for both ormaplatin and oxaliplatin were significantly different from cisplatin, but that ormaplatin and oxaliplatin were not significantly different from each other.

Tissue platinum levels were determined both at the end of the treatment period and at the end of the recovery period. Atomic absorption analysis of platinum levels in the kidney and liver are shown in Tables 2 and 3, respectively. Platinum levels in the kidneys of oxaliplatin-treated rats were slightly higher and platinum levels in the livers of ormaplatin-treated rats were slightly lower than those in the first experiment. Following the recovery period, about 42% of the initial platinum was retained in the liver of oxaliplatin-treated rats. Very little platinum remained in the liver of cisplatin- and ormaplatin-treated rats, but about 42% of the initial platinum was retained in the livers of oxaliplatin-treated rats. ICP-MS analyses of platinum levels in spinal ganglia L4–L6 are shown in Table 4. In this experiment platinum accumulation in the ganglia was slightly greater (125–160%) than that in the first experiment and the order of accumulation was cisplatin = oxaliplatin > ormaplatin. Following the recovery period 31% of the platinum was retained in the ganglia for the cisplatin-treated rats compared to 56–59% in the oxaliplatin- and ormaplatin-treated rats.
FIG. 2. Sections of dorsal root ganglia from control and Pt-treated rats immediately following drug treatment. The treatment conditions were as described in the legend to Fig. 1. The preparation and morphometric analyses of the dorsal root ganglia sections were performed as described under Materials and Methods. Sections are shown at 600× magnification. E, examples of eccentric nucleoli; M, examples of multiple nucleoli.
Our study represents the first quantitative comparison of the extent of neurotoxicity caused by cisplatin, ormaplatin, and oxaliplatin in a single model system. The Wistar rat is widely used as a model for studying the neurotoxicity of platinum anti-cancer agents (Cavaletti et al., 1991, 1993; Gao et al., 1995; Hamers et al., 1991, 1993; McKeage et al., 1994; Muller et al., 1990; Screnci et al., 1986; Tredici et al., 1994; van der Hoop et al., 1988, 1994). It should be noted that this study and previous studies in rats (Cavaletti et al., 1991, 1993; Gao et al., 1995; Hamers et al., 1991, 1993; McKeage et al., 1994; Muller et al., 1990; Screnci et al., 1997; Tomiwa et al., 1986; Tredici et al., 1994; van der Hoop et al., 1988, 1994) have used the ip route of administration, while platinum drugs are usually given iv in the clinical setting. However, since previous studies with platinum anti-cancer agents have shown that ip and iv administration results in similar plasma AUCs (Los et al., 1989), biodistribution to most organs (Los et al., 1989), and acute toxicity (LD50) (Mathe et al., 1985), it appears unlikely that this will affect the interpretation of these data. Biodistribution (Gregg et al., 1992) and morphometric changes (Tomiwa et al., 1986; Muller et al., 1990; Cavaletti et al., 1991, 1993; van der Hoop et al., 1994) suggest that the dorsal root ganglia appear to be the major site of neurotoxicity in both humans by iv administration and Wistar rats by ip administration. Furthermore, the primary electrophysiological effect of platinum treatment is a decrease in sensory nerve conduction velocity in both humans (Mollman

### TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>Cisplatin (2 mg/kg)</th>
<th>Ormaplatin (2 mg/kg)</th>
<th>Oxaliplatin (4 mg/kg)</th>
<th>Ormaplatin (2 mg/kg)</th>
<th>Oxaliplatin (4 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Kidney Pt/g Tissue</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cisplatin</strong></td>
<td>8.39 ± 0.65</td>
<td>5.75 ± 0.41</td>
<td>7.82 ± 1.23</td>
<td>1.43 ± 0.05</td>
<td>1.24 ± 0.27</td>
</tr>
<tr>
<td><strong>Ormaplatin</strong></td>
<td>9.59 ± 0.69</td>
<td>5.47 ± 0.54</td>
<td>14.1 ± 3.8</td>
<td>0.41 ± 0.13</td>
<td>1.25 ± 0.22</td>
</tr>
<tr>
<td><strong>Oxaliplatin</strong></td>
<td>2.10 ± 0.33</td>
<td>0.52 ± 0.07</td>
<td>1.14 ± 0.07</td>
<td>0.02 ± 0.02</td>
<td>0.52 ± 0.05</td>
</tr>
</tbody>
</table>

* Determined by atomic absorption as described under Materials and Methods. The treatment protocol is described in the legend to Fig. 1. In experiment 2 the treatment phase was followed by an 8-week recovery period. Statistical analysis of the data was performed as described under Materials and Methods.

** Significantly different from control, p ≤ 0.01.

### TABLE 2

<table>
<thead>
<tr>
<th></th>
<th>Cisplatin (2 mg/kg)</th>
<th>Ormaplatin (2 mg/kg)</th>
<th>Oxaliplatin (4 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell area* (μm²)</td>
<td>127 ± 4</td>
<td>124 ± 2</td>
<td>107 ± 4**</td>
</tr>
<tr>
<td>Nuclear area* (μm²)</td>
<td>9.0 ± 1.4</td>
<td>18.5 ± 0.7**</td>
<td>18.2 ± 2.4**</td>
</tr>
<tr>
<td>% Cells with multiple nucleoli*</td>
<td>48.6 ± 3.0</td>
<td>55.2 ± 4.8</td>
<td>64.3 ± 6.2</td>
</tr>
<tr>
<td>% Cells with eccentric nucleoli*</td>
<td>90.0 ± 14</td>
<td>155 ± 22</td>
<td>142 ± 15</td>
</tr>
<tr>
<td># Cells evaluated per animal*</td>
<td>140 ± 16</td>
<td>154 ± 16</td>
<td>154 ± 16</td>
</tr>
</tbody>
</table>

* Significantly different from control, p ≤ 0.01.

### TABLE 3

<table>
<thead>
<tr>
<th></th>
<th>Cisplatin (2 mg/kg)</th>
<th>Ormaplatin (2 mg/kg)</th>
<th>Oxaliplatin (4 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1: Immediately following drug treatment</td>
<td>2.56 ± 0.37*</td>
<td>1.14 ± 0.07*</td>
<td>1.24 ± 0.27*</td>
</tr>
<tr>
<td>Experiment 2: Immediately following drug treatment</td>
<td>2.10 ± 0.33*</td>
<td>0.41 ± 0.13*</td>
<td>1.25 ± 0.22*</td>
</tr>
<tr>
<td>Experiment 2: Following an 8-week recovery period</td>
<td>0.02 ± 0.08*</td>
<td>-0.02 ± 0.02*</td>
<td>0.52 ± 0.05*</td>
</tr>
</tbody>
</table>

* Determined by atomic absorption as described under Materials and Methods. The treatment protocol is described in the legend to Fig. 1. In experiment 2 the treatment phase was followed by an 8-week recovery period. Statistical analysis of the data was performed as described under Materials and Methods.

** Significantly different from control, p ≤ 0.01.

---

DISCUSSION

The analysis of the data was performed as described under Materials and Methods. The treatment protocol is described in the legend to Fig. 1. Statistical analysis of the data was performed as described under Materials and Methods.

** Significantly different from control, p ≤ 0.01.

** Significantly different from control, p ≤ 0.01.
is the major platinum complex in the circulation following
platin. The exact mechanism of this unique neurotoxicity is not
known at present. However, in a pharmacokinetic and biotrans-
differences in the accumulation of platinum in the DRGs. It
was of particular interest to note that while ormaplatin caused
was compared with morphometric alterations of DRG. Thus, it was
possible to assess whether the observed differences in neuro-
differences in their biodistribution. In contrast, the greater
were simply due to differences in their biotransformations rather than
differences in their initial neurotoxicity of ormaplatin than oxaliplatin.
Thus, the observed differences in the neurotoxicity of ormaplatin and
immediately following drug treatment appear to be related to differences in their biodistribution rather than
to the DRG, since it was not observed for either ormaplatin or oxaliplatin in the kidney, and was not observed
for ormaplatin in the liver.

A scaling factor of 5.2 has been suggested for converting
doses in mg/kg for rats to mg/m² for humans (Whitefield and
Applying this scaling factor to the total cumulative
doses used in this study would correspond to 94 mg/m² for
cisplatin and ormaplatin and 187 mg/m² for oxaliplatin. This is
approximately two- to four-fold less than the cumulative doses
associated with the onset of neurotoxicity in clinical studies
(Mollman, 1990; Mollman et al., 1988; Schilder et al., 1991;
O’Rourke et al., 1994; Extra et al., 1990; Misset et al., 1991),
which suggests that the Wistar rat model is a relatively sensi-
tive model for evaluating neurotoxicity.

Scrceni et al. (1997) have measured the neurotoxicity of
ormaplatin, oxaliplatin, and Pt(dach)Cl₂ by the nerve conduc-
tion velocity assay in Wistar rats. However, those studies
focused primarily on the relative neurotoxicity of the different sterioisomeric conformations of the dach carrier ligand and on the
time required for onset of neurotoxicity rather than the

### TABLE 4

<table>
<thead>
<tr>
<th>Treatment</th>
<th>μg Pt/g tissue for</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatin (2 mg/kg)</td>
<td>Ormaplatin (2 mg/kg)</td>
</tr>
<tr>
<td>Experiment 1: Immediately following drug treatment²</td>
<td>0.814 ± 0.119**</td>
</tr>
<tr>
<td>Experiment 2: Immediately following drug treatment²</td>
<td>1.03 ± 0.08**</td>
</tr>
<tr>
<td>Experiment 2: Following an 8-week recovery period²</td>
<td>0.317 ± 0.036**</td>
</tr>
</tbody>
</table>

* Determined by ICP-MS as described under Materials and Methods. The
treatment protocol is described in the legend to Fig. 1. In experiment 2 the
treatment phase was followed by an 8-week recovery period. Statistical anal-
sis of the data was performed as described under Materials and Methods.

* Means ± SEM, n = 6/group except for ormaplatin and oxaliplatin
following the 8-week recovery where n = 3.

* Significantly different from control, p ≤ 0.05.

** Significantly different from control, p ≤ 0.01.

et al., 1990; Hamers et al., 1991) and Wistar rats (van der Hoop
et al, 1988, 1990; Hamers et al., 1991, 1993; McKeage et al.,
1994). Finally, our data show that immediately following drug
treatment in the Wistar rat model oxaplatin has significantly
more neurotoxicity than either cisplatin or oxaliplatin, while
cisplatin and oxaliplatin have fairly comparable neurotoxicity.
These data in the Wistar rat model system are consistent with
clinical reports that neurotoxicity is particularly severe for
ormaplatin (Schilder et al., 1994; O’Rourke et al., 1994), while
cisplatin and oxaliplatin appear to have relatively comparable
neurotoxicity that is manageable at therapeutic doses (Machover et al., 1996; de Gramont et al., 1997). Thus, both our data
and the previous studies described above suggest that Wistar rats
provide a reasonable model system for evaluating the
relative neurotoxicity of platinum compounds.

In these studies platinum accumulation in the DRG was
compared with morphometric alterations of DRG. Thus, it was
possible to assess whether the observed differences in neuro-
toxicity represented intrinsic differences in the neurotoxic poten-
tial of the platinum compounds, or were simply due to
differences in the accumulation of platinum in the DRGs. It
was of particular interest to note that while ormaplatin caused
the greatest neurotoxicity immediately following drug treat-
ment, it resulted in the least platinum accumulation in the
DRG. These data suggest that ormaplatin possesses an unique
mechanism of neurotoxicity compared to cisplatin and oxali-
platin. The exact mechanism of this unique neurotoxicity is not
known at present. However, in a pharmacokinetic and biotrans-
formation study in Wistar rats, we have shown that Pt(dach)Cl₂
is the major platinum complex in the circulation following
ormaplatin infusion (present at a 10-fold higher concentration
than ormaplatin) and only a minor biotransformation product
following oxaliplatin infusion (Luo et al., manuscript in prepa-
ratin and oxaliplatin infusions (Luo et al., manuscript in prepara-
in addition, Pt(dach)Cl₂ was 3.8-fold more neurotoxic
than oxaliplatin in a rat DRG neurite outgrowth assay (Luo et
al., manuscript in preparation). These data suggest that plasma
levels of Pt(dach)Cl₂ may be responsible for the greater initial
neurotoxicity of ormaplatin than of oxaliplatin. Thus, the ob-
served differences in the neurotoxicity of ormaplatin and ox-
aliplatin immediately following drug treatment appear to be
related to differences in their biodistributions rather than
differences in their initial neurotoxicity.

### TABLE 5

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Mortality²</th>
<th>Bloating³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td>Cisplatin (2 mg/kg)</td>
<td>3/10</td>
<td>1/10</td>
</tr>
<tr>
<td>Ormaplatin (2 mg/kg)</td>
<td>6/10</td>
<td>10/10</td>
</tr>
<tr>
<td>Oxaliplatin (4 mg/kg)</td>
<td>6/10</td>
<td>10/10</td>
</tr>
<tr>
<td>Lactose</td>
<td>0/6</td>
<td>0/6</td>
</tr>
</tbody>
</table>

* Toxicity which developed during the 8-week recovery period. The treat-
ment protocol is described in the legend to Fig. 1.
² Most of the deaths in the cisplatin group occurred during the first 3 weeks
of recovery. Most of the deaths in the ormaplatin and oxaliplatin groups
occurred during the last 2 weeks of recovery.
³ Severe bloating of the peritoneal cavity.
extent of neurotoxicity. In contrast, our studies focused on the clinical formulations of oxaliplatin and ormaplatin and on the extent of neurotoxicity achieved after the administration of a large cumulative dose of each drug. The data of Screnci et al. (1997) suggested that the trans-R,R isomer of the 1,2-diaminocyclohexane ligand was more neurotoxic than the trans-S,S and cis-R,S isomers for ormaplatin, oxaliplatin, and Pt-(dach)Cl₂. Since oxaliplatin is formulated with the trans-R,R dach isomer and ormaplatin is formulated with a mixture of the trans-R,R and trans-S,S dach isomers, our data do not provide any direct evaluation of the relative neurotoxicity of the dach isomers.

During the recovery period, the majority of the animals treated with ormaplatin and oxaliplatin developed severe bloating of the peritoneal cavity associated with inflammation and/or fibrosis of the peritoneal surfaces. These symptoms may be akin to the mucositis that has been reported in patients receiving iv infusions of oxaliplatin (Levi et al., 1997). However, the severity of the peritoneal inflammation in the Wistar rat model may also represent localized irritation caused by the ip injections of ormaplatin and oxaliplatin. Since oxaliplatin is administered primarily by the intravenous route in humans, the severe peritoneal inflammation seen in this study is probably not clinically relevant. In addition to the evident peritoneal toxicity, a majority of the animals treated with ormaplatin and oxaliplatin died during the recovery period. These deaths may have resulted from peritoneal inflammation, neurotoxicity, or another unidentified toxicity associated with ormaplatin and
oxaliplatin in the Wistar rat model. These data suggest that future studies designed primarily to characterize the delayed-onset neurotoxicity associated with ormaplatin and oxaliplatin should most likely be conducted at lower drug doses.

Following an 8-week recovery period, the pattern of neurotoxicity was significantly different than the pattern observed immediately following drug treatment. At this time ormaplatin and oxaliplatin appear to have roughly comparable neurotoxicity which is significantly greater than for cisplatin. This pattern of neurotoxicity appears to be associated with greater retention of Pt in the DRG for ormaplatin and oxaliplatin than for cisplatin. While these data should be interpreted with caution due to the small number of ormaplatin-and oxaliplatin-treated animals that survived the recovery period, these data suggest that particular attention should be focused on delayed-onset neurotoxicity in clinical evaluations of oxaliplatin. In addition, these data suggest that different strategies may be optimal for reducing the neurotoxicity associated with cisplatin and oxaliplatin. Since Pt is cleared from DRG fairly rapidly following cisplatin treatment, ACTH analogs or nerve growth factors which stimulate regeneration of damaged neurons (Gao et al., 1988, 1990) may be particularly useful for reducing cisplatin neurotoxicity. On the other hand, since the accumulated Pt in the DRG is cleared relatively slowly for oxaliplatin, agents such as glutathione (Cascini et al., 1995; Tredici et al., 1994; Hamers et al., 1993) which may be able to prevent the initial accumulation of Pt adducts in DRG might be more successful at reducing oxaliplatin neurotoxicity.

ACKNOWLEDGMENTS

We thank Dr. Martine Bayssas (Debiopharm S. A., Lausanne, Switzerland) for kindly providing us with the oxaliplatin used in these experiments. We also thank Dr. James Raymer and Dr. Margaret Goldberg (Analytical and Chemical Sciences, Research Triangle Institute, Research Triangle Park, NC) for advice in the use of ICP-MS and for use of the ICP-MS facility at the Research Triangle Institute. We thank Dr. Cindy Lawler (Department of Psychiatry, University of North Carolina) for advice in the design of the statistical analysis. Finally, we thank Dr. Feng Luo and Maria Varchenko for their technical assistance. Preparation, sectioning, and staining of the ganglia were performed by Victoria Madden and Dr. C. Robert Bagnell of the Microscopy Services Laboratory, Department of Pathology and Laboratory Medicine, University of North Carolina. The ICP-MS determinations were performed by Dr. Hong Ding in the Analytical and Chemical Services Laboratory, Research Triangle Institute, Research Triangle Park, NC.

REFERENCES


TABLE 6

Morphometric Analysis of Rat Dorsal Root Ganglia L4-L6 Following an 8-Week Recovery Period

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>Cisplatin</th>
<th>Ormaplatin</th>
<th>Lactose</th>
<th>Oxaliplatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell area (μm²)</td>
<td>886 ± 24</td>
<td>772 ± 33*</td>
<td>621 ± 72*</td>
<td>840 ± 45</td>
<td>635 ± 11**</td>
</tr>
<tr>
<td>Nuclear area (μm²)</td>
<td>157 ± 4</td>
<td>145 ± 5</td>
<td>108 ± 15*</td>
<td>154 ± 6</td>
<td>111 ± 2**</td>
</tr>
<tr>
<td>% Cells with multiple nucleoli</td>
<td>8.0 ± 0.7</td>
<td>9.7 ± 1.4</td>
<td>7.2 ± 2.8</td>
<td>7.9 ± 1.4</td>
<td>8.2 ± 2.2</td>
</tr>
<tr>
<td>% Cells with eccentric nucleoli</td>
<td>52.6 ± 3.6</td>
<td>56.1 ± 3.1</td>
<td>44.9 ± 8.8</td>
<td>53.8 ± 4.1</td>
<td>51.4 ± 3.4</td>
</tr>
<tr>
<td># Cells evaluated per animal</td>
<td>165 ± 14</td>
<td>166 ± 8</td>
<td>166 ± 23</td>
<td>139 ± 8</td>
<td>178 ± 10</td>
</tr>
</tbody>
</table>

* Significantly different from control, p < 0.05.
** Significantly different from control, p < 0.01.

*p Means ± SEM (n = 6 for saline, lactose, and cisplatin and n = 3 for ormaplatin and oxaliplatin).
NEUROTOXICITY OF PLATINUM DRUGS

351


