Studies on the Evaluation of the Toxicity of Various Salts of Lead, Manganese, Platinum, and Palladium

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Preliminary studies have been conducted on various parameters in order to assess the possible and relative toxicities of a number of metallic salts. Upon oral administration in lethal-dose experiments, two soluble Pt⁴⁺ salts were more toxic than the other salts tested. Following intraperitoneal injection in lethal-dose experiments, PbCl₂ was less toxic than several of the soluble or partially soluble salts of Pt⁺⁺, Pd⁺⁺, and Mn⁺⁺. An intake of a total of approximately 250 mg of Pt⁺⁺ per rat in the drinking fluid over a 30-day interval did not affect the activities of aniline hydroxylase and aminopyrine demethylase in rat liver microsomes. In rats receiving soluble Pt⁺⁺ salts in the drinking fluid, the highest concentration of Pt was found in the kidney and an appreciable concentration was found in the liver.

Introduction

Preliminary studies have been conducted on various parameters in order to assess the possible and relative toxicities of a number of metallic salts. The chloride, sulfate, and oxide salts of lead, manganese, platinum, and palladium were studied, since it is considered that some of these salts may be included in automotive emission products.

Materials and Methods

All experimental studies were conducted with male Sprague-Dawley rats. The animals were received at 3–3.5 weeks of age and were maintained for 1–1.5 weeks before use. The mean body weights were usually 100–110 g when the rats were used for the lethal-dose experiments or started on the diets.

In the lethal dose experiments, the salts were administered orally (via stomach tube) or intraperitoneally. The rats were observed through a 14-day observation period. In the completed experiments, the LD₅₀ values were calculated by the method of Litchfield and Wilcoxon (1).
In the diet experiments, four rats were maintained per cage. The metallic salt under study was dissolved in the drinking fluid. Animals consumed feed and drinking fluid ad libitum. Analyses for metals were performed on samples from three lots of feed (Purina Laboratory Chow). The feed contained (mean ± standard deviation): 56 ± 5 mg Mn/kg feed and 0.99 ± 0.07 mg Pb/kg feed; the analyses of the three lots for platinum were 0.09, <0.02, and <0.02 mg Pt/kg feed. Measurements were made of the body weights of individual rats and feed and fluid consumption per cage of four rats at 7-day intervals during the course of each diet experiment.

At the termination of the dietary experiments, samples of liver were used for the isolation of microsomes. Aniline hydroxylase was measured by the method of Imai et al. (2), modified by the addition of HgCl2 (3). Aminopyrine demethylase was measured by the formation of formaldehyde (Nash reaction) (4).

The analyses of the rat tissues for platinum, lead, and manganese were carried out by Yoakum, Stewart, and Sterrett (5) of Stewart Laboratories, Inc. by an emission spectrochemical method.

**Results and Discussion**

In Table 1 are presented, for various salts, the preliminary data on the LD50 values, the doses lethal to 50% of the rats following oral administration or intraperitoneal injection. Upon oral administration, the toxicities of the salts are in the following decreasing order (expressed on a molar basis): PtCl6, Pt(SO4)2·4H2O > PdCl2·2H2O, RuCl3 > MnCl2·4H2O, PdSO4, PbCl2, PtCl4 > PtO2 > MnO2, PdO. Thus, upon oral administration, the two soluble Pt4+ salts are the most toxic of the compounds studied. As one might anticipate, the highly insoluble salts are least toxic and include PtCl2 (Pt2+), and oxides of platinum, manganese, and palladium. The relative order of toxicities may be modified somewhat by the period of observation. Although the soluble Pt4+ salts kill early, usually within the first 1–2 days following oral administration, the palladium salts and PbCl2 often kill 4–10 days after administration.

The LD50 doses following intraperitoneal injection are also presented in Table 1. The relative order of the LD50 values are, on a molar basis: PdCl2·2H2O > Pt(SO4)2·4H2O, MnCl2·4H2O, PdSO4, PtCl4 > PbCl2, PtCl2. The most toxic salt was PdCl2·2H2O. PbCl2 was less toxic following intraperitoneal injection than the soluble Pt4+ salts and MnCl2·4H2O.

In the process of preparing rats for subsequent biochemical experiments, measurements were made at 7-day intervals of the weight gain by individual animals and the feed and drinking fluid consumption per cage of four rats. Usually each control rat gained 50–60 g per week during each of the first 4 weeks on the diets. The weight gain by rats which consumed 8.3 mM MnCl2·4H2O (one week) or 3.7 mM PbCl2 (four weeks) did not differ from the weight gain by control rats (Fig. 1A). Furthermore, when the drinking fluid was a saturated solution of PtCl2 or of PdCl2·2H2O, no statistically different weight changes were observed, although the PdCl2·2H2O appeared...
to cause some decrease (Fig. 1B). The saturated solution of PtCl$_2$ contained only a trace of Pt (14 μg Pt/l.); the saturated solution of PdCl$_2$·4H$_2$O has not been analyzed yet for Pd.

A soluble salt of Pt$^{4+}$, when added to the drinking fluid as 0.54mM PtCl$_4$, did not affect the weight gains for the four weekly or the total interval (Fig. 1C). When the Pt$^{4+}$ concentration was increased 3-fold with either 1.63mM PtCl$_4$ or Pt(SO$_4$)$_2$·4H$_2$O, the weight gains of the metal-treated rats was significantly less than the gains of the control rats during the first week on the diet. However, 1.63mM PtCl$_4$ did not decrease the weight gains during the second, third or fourth weeks. The decreases of 20% in weight gain during the first week were parallel to the decreases in feed consumption and fluid consumption which were also decreased by 20%.

The organ weights, expressed as the percentage of the body weight, of control and metal-treated rats are given in Table 2. PbCl$_2$ (3.67mM) did not significantly alter the organ weights through a 30–31 day diet; the total lead intake was approximately 750 mg of lead per rat for the entire interval. When essen-
Table 2. Effect of various salts in the drinking fluid on tissue weights. *

| Drinking fluid | Concen, mM | Dur. days | No. of rats | Body weight, g | Liver | Kidney | Spleen | Heart | Testes |
|----------------|------------|-----------|-------------|----------------|-------|--------|--------|-------|--------|
| PbCl₂          | Controls   | 3.67      | 29–31       | 8              | 309   | 3.41   | 0.92   | 0.28  | 0.32   | 1.04   |
|                | Controls   | 3.60      | 90–91       | 4              | 508   | 2.64   | 0.68   | 0.19  | 0.25   | 0.75   |
|                | Controls   | 0.54      | 29–30       | 8              | 297   | 3.52   | 0.86   | 0.43  | 0.31   | 1.06   |
|                | Controls   | 1.63      | 8           | 12             | 152   | 3.72   | 1.07   | 0.58  | 0.38   | 1.08   |
|                | Controls   | 1.63      | 8           | 12             | 145   | 3.72   | 1.13   | 0.57  | 0.39   | 1.11   |
| PtCl₄          | Controls   | 1.63      | 8–9         | 8              | 173   | 3.90   | 1.01   | 0.56  | 0.39   | 1.11   |
|                | Controls   | 1.63      | 8–9         | 8              | 149   | 3.69   | 1.05   | 0.54  | 0.37   | 1.21   |

* Since livers were used for isolation of microsomes, rats were fasted overnight (12–15.5 hr before collection of tissues; drinking fluid provided during fasting.

b Where mean of tissues from metal-treated rats is outside of the range of 92.0–108.0% of the mean of control rats, the percentage is given in parentheses.

† Statistical analysis (Student's t-test): *<0.05; 0.05< P <0.10; no marking is indicated where P >0.10.

Initially the same diet was continued for approximately 90 days, the kidneys were enlarged. These data are consistent with prior reports (6) and with our preliminary data which showed kidney enlargement in rats which received one-half this concentration over a 90-day interval. When PbCl₂·2H₂O was extracted with water to dissolve the readily soluble material and that solution used as the drinking fluid, no significant changes were observed in the organ weights over an 8-day interval.

The dietary administration of PtCl₄ did not affect any of the five organ weights when the PtCl₄ was included in the drinking fluid for approximately 30 days at 0.5mM or for 8 days at 1.6mM. Likewise, if 1.6mM Pt(SO₄)₂·4H₂O was given as drinking fluid for 8–9 days, there were no significant changes in organ weights. The total intake of platinum per rat was approximately 60 mg for each of these three Pt⁴⁺ diets. In contrast, if 1.6mM PtCl₄ is administered for approximately 30 days (total intake of approximately 250 mg of Pt per rat), the kidney weight was increased 6–10% in each of the three experiments.

At the termination of the dietary experiments, samples of liver were used for the isolation of microsomes. Measurements were made of the weight of microsomal protein isolated per gram of liver and the activities of two microsomal enzymes: aniline hydroxylase and aminopyrine demethylase.

None of the dietary treatments consistently affected the level of microsomal protein isolated per gram of liver (Table 3). The hepatic activities of aniline hydroxylase or aminopyrine demethylase are not significantly depressed when rats were maintained for only 30 days on 3.7mM PbCl₂ in the drinking water. The use of a saturated aqueous solution of PbCl₂·2H₂O as the drinking fluid appears to decrease the activities of both aniline hydroxylase and aminopyrine demethylase. Under various concentration and duration schedules, the addition of the soluble chloride (or sulfate) salt of Pt⁺ to the drinking fluid did not alter the activity of either microsomal enzyme.
Table 3. Effect of dietary salts on the activities of microsomal activities of rat liver.

| Drinking fluid | Conc, mM | Dura- | (Treated/control)×100* |
|----------------|---------|-------|------------------------|
| PbCl₂          | 3.67    | 31    | 107                    |
|                |         | 30    | 101                    | 79† 87 |
| PbCl₂          | 3.60    | 91    | 75                     |
|                |         | 71    |                        | 86   |
| PdCl₂·2H₂O     | Satd.   | 8     | 96                     |
|                |         | 67*   | 66**                   |      |
| PtCl₂          | 0.54    | 29    | 98                     |
|                |         | 98    | 106                    | 109  |
| PtCl₂          | 1.63    | 8     | 105                    |
|                |         | 107   | 104                    |      |
| PtCl₂          | 1.63    | 8     | 99                     |
|                |         | 121   | 125                    |      |
| PbCl₃          | 3.67    | 30    | 107                    |
|                |         | 93    | 99                     |      |

* Statistical analysis (t-test: ***, P < 0.01; *, P < 0.05; †0.05 < P < 0.10; no marking if P > 0.10. Each value is a comparison of the mean of four control values and values from four metal-treated rats.

- In control rats, approximately 43 mg of microsomal protein was obtained per gram of liver.
- Aniline hydroxylase: control values were approximately 18 mumole of p-aminophenol produced/mg protein/20 min incubation.
- Aminopyrine demethylase: control values were approximately 70 mumole of formaldehyde produced/mg protein/10 min incubation.

Analyses for lead, manganese, and platinum were conducted by Yoakum, Stewart, and Sterrett (5). In a series of rats treated for 90–91 days, the control rats ingested approximately 0.15 g of manganese (from the solid feed). The tissue concentration of Mn was 1.4 and 1.0 μg Mn/g wet tissue in the liver and kidney, respectively. In Mn-treated rats, which received 8.3mM MnCl₂·4H₂O as the drinking fluid and ingested approximately 2.3 g of Mn per rat during the 90–91 day interval, the concentration of Mn was somewhat increased, namely 2.8 and 1.6 μg Mn/g of wet tissue in the liver and kidney, respectively. The Mn concentration in spleen, heart, testes and blood was not increased in the tissues of Mn-treated rats.

A second group of rats received 3.6mM PbCl₂ in the drinking water for 90–91 days and ingested approximately 3 g of lead per rat during the interval; control rats ingested < 0.01 g of Pb in the solid feed during the same interval. Kidney showed a marked accumulation of Pb (to 11.1 μg Pb/g of wet tissue) in the lead-treated rats; in the same rats the concentration in liver was 1.2 μg Pb/g of wet tissue. The corresponding levels in the control rats were approximately 0.3 μg Pb/g of wet tissue in both kidney and liver. The other tissues (spleen, heart, testes, and blood) did not exhibit appreciably higher levels of Pb in the Pb-treated rats.

Soluble Pt⁺⁺ salts were included in the drinking fluid of rats for 8–9 days. The approximate total Pt intake (mg Pt per rat) and data on the tissue concentration of Pt in various tissues are presented in Table 4. Although the Pt concentrations in tissues of untreated control rats often attain levels measurable by the technique used by Stewart Laboratories, Inc., the levels are low and are generally less than 0.1 μg Pt/g of wet tissue. For the higher levels of Pt⁺⁺ intake in the Pt-treated rats, the highest tissue concentrations of Pt occurred in the kidney and ranged from 4.5 to 5 μg Pt/g of wet tissue. High levels, ranging from 0.7 to 2.5 μg Pt/g, also occurred in the liver. In contrast, brain showed only a very low level of Pt which may reflect a contribution from the blood. Separate experiments were conducted on the tissue concentrations of Pt in rats which received a saturated solution of PtCl₂ as the drinking fluid for 30–31 days. In the PtCl₂-treated rats, the mean Pt concentration for liver, kidney, and spleen was < 0.08 μg Pt/g of wet tissue.

In Table 5 are presented the Pt concentrations of tissues removed from rats which had survived for the 14-day observation period in lethal-dose experiments. The doses of Pt(SO₄)₂·4H₂O administered by both the oral and intraperitoneal routes were approximately 90% of the LD₅₀ values by the respective routes. During the two-week observation period, the rats gained weight at a rate from one-third to three-fourths the rate of the control rats. In the orally treated rats, April 1975
Table 4. Pt content of tissues of rats maintained on drinking fluid containing Pt salts.

|                     | Control | Pt(SO₄)₂·4H₂O | PtCl₄ |
|---------------------|---------|---------------|-------|
| Pt salt concn, mg Pt/l. | —       | 106           | 319   |
| Duration of diet, days | —       | 8             | 9     |
| Total Pt intake, mg Pt/rat | <0.01  | 26            | 80    |
| Tissue concentration of Pt, µg Pt/g wet tissue * |                     |     |       |
| Liver               | 0.07    | 0.85          | 2.2   |
| Kidney              | <0.02±0.02 | 0.04–0.09     | 0.73–0.97 | 2.0–2.5 |
| Spleen              | <0.23±0.45 | 0.26±0.05     | 4.6   |
| Heart               | <0.08±0.08 | (0.01–0.02)   | —     |
| Testes              | <0.014±0.010 | 0.04±0.05     | 0.25  |
| Brain               | —       | 0.015±0.002   | —     |
| Blood               | 0.05    | 0.22          | 0.23  |
|                     | 0.10±0.13 | (0.09–0.36)   | (0.19–0.27) |

*Values for control rats are those from diet experiments after approximately 8 or 30 days; 5–7 values for blood, spleen, and heart, 13–16 values for liver, kidney, and testes; standard deviation (±) is given for means with at least four values; ranges are indicated in parentheses for means of two values.

Table 5. Pt concentration in rat tissues following the administration of single high doses of Pt (SO₄)₂·4H₂O.

| Dose of Pt, mg Pt/kg | Controls (oral) | Pt(SO₄)₂·4H₂O |
|---------------------|-----------------|---------------|
| Tissue Concentration of Pt, µg Pt/g wet weight of tissue | Oral | Intraperitoneal |
| Liver               | <0.01           | 2.3           | 34 |
|                     | (0.004–0.006)   | (1.2–3.5)     | (30–38) |
| Kidney              | <0.008          | 16            | 37 |
|                     | (0.004–0.004)   | (13–19)       | (28–46) |
| Spleen              | <0.013          | 3.3           | 16 |
|                     | (0.007–0.011)   | (2.3–4.2)     | (12–20) |
| Heart               | 0.02            | 0.8           | 3.0 |
|                     | 0.013           | 0.5           | 1.2 |
| Testes              | 0.011           | 0.5           | 0.6 |
|                     | (0.009–0.013)   | (0.4–0.6)     | (0.9–1.5) |
| Brain               | 0.01            | 0.10          | 1.0 |
|                     | (0.07–0.14)     | (0.07–1.1)    |       |
| Blood               | <0.008          | 3.3           | 1.0 |

* Range of four values for control liver, kidney, and spleen, and range of 2–3 values of all other tissues are given in parentheses. Control values are the mean values of Pt concentration in two rats which received orally NaCl.

the highest concentration of Pt occurred in the kidney (approximately 16 µg Pt/g), and appreciable levels of Pt also occurred in liver and spleen (range, 1–4 µg Pt/g of wet tissue). In the intraperitoneally dosed rats, the kidney, liver, and spleen showed very high levels of Pt in the range of 10–40 µg Pt/g of wet tissue.

In a comparable lethal dose experiment, rats were treated orally with a dose of MnCl₂·4H₂O equivalent to 100% of the oral LD₅₀ value, and the tissues were analyzed in surviving rats at the end of the 14-day observation period. In contrast to the finding with the Pt salt, the oral administration of a single, large but nonlethal dose of MnCl₂·
Table 6. Mn concentration in rat tissues following the oral administration of a single large dose of MnCl₂-4H₂O.

| Tissue                | Controls | MnCl₂-4H₂O |
|-----------------------|----------|------------|
| Liver                 | 1.60±0.87| 1.9        |
| Kidney                | 0.75±0.50| (1.3-2.5)  |
| Spleen                | 1.46±1.99| (1.0-1.5)  |
| Heart                 | 0.55±0.35| 0.7        |
| Testes                | 0.44±0.35| 0.5        |
| Brain                 | 0.3       | 0.03       |
| Blood                 | 0.86±0.44| 0.4        |

* Control values are from rats treated orally with NaCl, and rats on diet experiments for approximately 8 or 30 days. Means ± standard deviations are given for 6-7 samples of spleen, heart and blood and for 13-18 samples of liver, kidney and testes from control rats; ranges are given in parentheses where two values are available from Mn-treated rats.

4H₂O to rats did not result in the retention after 14 days of excess concentrations of Mn in any of the tissues analyzed (Table 6). Due to low levels of absorption and/or a high capacity for excretion of the Mn, the tissue Mn levels of the experimental rats were approximately equal to the levels found in control rats.

These studies show that in rats treated with soluble Pt⁺⁺ salts, appreciable levels of the metal can be found in the kidney, liver, and spleen. Further studies will be necessary to determine the effects of the Pt and other metals on various biochemical reactions.

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