United States
Environmental Protection
Agency

Health Effects Research
Laboratory
Research Triangle Park NC 27711

Research and Development

EPA-600/S1-81-015 Aug 1981

EPA

Project Summary

Sensitive Biochemical and Behavioral Indicators of Trace Substance Exposure: Part II. Platinum

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The overall objective of this project was to characterize Pt [$Pt(SO_4)_2$, NA_2PtCl_6] intoxication in a model mammalian system (the mouse) employing acute (single) and serially repeated (multiple) dose exposure paradigms. Platinum lethality parameters were defined in both sexes of the adult mouse and Pt tissue/organ distribution was monitored as a function of time and dose. In coordination with the tissue distribution study, investigations of the effects of Pt on selected parameters of open field, exploratory and social behavior, passive avoidance learning and the acquisition of two-way active avoidance learning were undertaken. Correlations among tissue levels of Pt and open field behavioral parameters (ambulations and rearings) were probed employing statistical methodology.

Since numerous xenobiotics are known to cross the placenta and/or accumulate in maternal milk, studies of Pt tissue/organ distribution in the gravid female and the embryo/fetus/offspring were undertaken. Platinum levels were monitored as a function of time. Coordinate studies examined developmental and behavioral parameters of the offspring (as neonates, pups and adults) of exposed mothers. In addition, maternal behavior was examined as a function of pup retrieval and neonate and pup activity levels were assessed. Ambulations and rearings in the open field, passive avoidance learning and rotarod performance were assessed in adult offspring of exposed mothers.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Results

Presently, we have no data to explain the disproportionately high tissue concentrations of Pt in animals receiving $Pt(SO_4)_2$ intragastrically (IG) at the LD_{25} level as compared to the LD_5 level. Conceivably, the higher Pt dose may perturb the GI tract in some manner, resulting in facilitated Pt absorption. The high Pt concentrations found in the blood of such animals may damage the kidneys (which normally eliminate Pt quite efficiently), thereby decreasing Pt excretion and increasing tissue uptake. In addition, it is well known that some metals, such as cadmium and lead, are able to induce the synthesis of their own

binding proteins. Platinum may have a similar ability.

The analysis of variance (ANOVA) of the open field behavioral measures revealed that the higher Pt dose depressed ambulations significantly and rearings marginally, relative to other treatments, from 4 hours through 7 days post administration. A correlation analysis revealed highly significant negative correlations between behavioral measures and tissue levels of Pt in all tissues except brain and muscle. Since brain Pt levels were not related to behavior, it is not clear how non-CNS tissue Pt levels affect behavior. In earlier work with alveolar macrophages in tissue culture, a 50% loss of viability (ability to exclude trypan blue) after a 20 hour exposure to 300 mM $PtCl_4$ was reported. Protein and RNA synthesis were inhibited by 50% by 60 mM Pt and DNA synthesis was inhibited by 50% by 10 mM Pt. The maximum tissue Pt concentration observed in our study was 50 ppm in the kidney, 4 hours post administration. Fifty ppm is approximately equivalent to 250 mM Pt. Therefore, cellular processes may have been disrupted to some extent in the LD_{25} animals which, in turn, may produce a general malaise and depression of the activity component of certain behaviors.

$Pt(SO_4)_2$-Single (Acute) Dose Effects on Exploratory Behavior

The high rate of explorations of the 4 hour low pH control group, for which no satisfactory explanation is presently available, may have sufficiently accentuated the difference between the 4 hour time point and all other times of observation to account for the finding of a significant main effect of time as well as the significant time by treatment interaction.

At 1, 3 and 7 days post IG administration, the mean number of explorations for the 2 Pt groups (LD_5, LD_{25}) and the low pH control group was lower than that of the saline control group, but not significantly. Possibly, this trend was due to some nonspecific effect of the low pH or sulfate content of the solutions. In any case, it is apparently not a specific Pt effect. Thus, in this study, Pt exposure *per se*, does not appear to have effected changes in exploratory behavior.

$Pt(SO_4)_2$-Single (Acute) Dose Effects on Activity Wheel Performance

Platinum at either the LD_5 or LD_{25} levels appeared not to affect wheel running scores. All groups exhibited the lowest scores during the first 24 hours post administration. This may have resulted from trauma induced by handling the dose administration. The relative depression of scores observed on the last 2 days of testing may have been due to the effect of time (habituation). Trend analysis demonstrated that the performance curves of all treatment groups followed the same trend, independent of treatment. Thus, there were no overt effects of Pt exposure on activity wheel performance.

$Pt(SO_4)_2$-Single (Acute) Dose Effects on Passive Avoidance Learning

Intragastric administration of $Pt(SO_4)_2$ at the LD_5 or LD_{25} levels had no observable effect on passive avoidance learning by the adult mouse.

$Pt(SO_4)_2$-Single (Acute) Dose Effects on Active Avoidance Learning

Platinum sulfate at the LD_5 or LD_{25} levels had no effect either on the acquisitions of two-way active avoidance learning or activity in the apparatus.

$Pt(SO_4)_2$-Single (Acute) Dose Effects on Social Behavior

At the IG LD_5 or LD_{25} levels, $Pt(SO_4)_2$ had no effect on the measure of murine social behavior investigated.

Na_2PtCl_6-Single (Acute) Dose Tissue Distribution and Open Field and Exploratory Behavioral Studies

Relative to the dose administered, the fairly low tissue/organ Pt levels observed suggest that Na_2PtCl_6 is only poorly absorbed from the gut. With the exception of the spleen, the biological half-life of Pt administered as Na_2PtCl_6 appears to be short.

Na_2PtCl_6 depressed ambulations and rearings in the open field. However, the effect was transitory and clear-cut effects were found only at 4 hours post administration. At 1 and 3 days post administration, the LD_5 exposed groups had depressed rearings relative to the saline controls groups but not the low pH control groups.

Na_2PtCl_6 had no observable effect on exploratory behavior at 1, 3 and 7 days post administration. Unfortunately exploratory behavior was not examined 4 hours post administration when the strongest depression of open field behavior had been observed.

$Pt(SO_4)_2$-Multiple (Serially Repeated) Dose Effects on Tissue Distribution and Open Field Behavior

This study was undertaken to gain insight into the effects of subchronic exposure to Pt on certain behaviors of the mouse. Subchronic exposure was simulated via the use of a limited repeated dose experimental design (1 to 10 doses). Adult male mice were subjected to repeated IG administration (every 72 hours) of relatively high dose of $Pt(SO_4)_2$ (each dose equaled the 7 da LD_1, which is approximately 40% of the 7 day LD_{50}). This design resulted only in marginal adverse effects on the general activity and exploratory behavior of the adult animal. Tissue/organ Pt levels while highly variable, generally increased with the number of dose except for the brain, in which no Pt was detected in any animal. The absence of Pt in the brain is not surprising since the highly charged Pt cation should be excluded by the blood brain barrier. Of the three behavioral measures studied only rearings in the open field showed significant correlation with tissue Pt levels. Since this correlation involved number of tissues and not the brain, general systemic effect, rather than neurotoxic effect, seems to be indicated. Inorganic Pt compounds have been shown to inhibit DNA, RNA and protein synthesis in tissue culture. Thus, it may be that Pt has a depressive effect on cellular metabolic processes and exposure to Pt above a threshold level results in general malaise which is reflected in the slight depression in behavior observed in this study.

$Pt(SO_4)_2$ Developmental Studies

The predominant effect of maternal administration of $Pt(SO_4)_2$ on days 7 and 12 of gestation was in offspring weight. The *in utero* Pt exposed offspring were lighter than low pH exposed offspring. This effect was powerful, beginning on day 0 and continuing through day 4

post partum. As indicated by the significant multivariate effect for postnatal exposure, type of foster mother exposure also has a significant effect on offspring weight. Thus, on day 45 *post partum*, pups (exposed to Pt or low pH *in utero*) reared by Pt exposed foster mothers weighed less than Pt or low pH gestationally exposed pups reared by low pH exposed mothers. The reason for the weight differences is unknown. However, it may be that reduced weight could have important consequences, such as reduced viability.

Administration of $Pt(SO_4)_2$ to lactating mothers resulted in symptoms of toxicity in offspring. Offspring assigned to mothers administered Pt during lactation exhibited depressed neonatal activity ($p < 0.006$) and lower adult weights ($p < 0.015$) than controls. Pt may have affected these offspring directly through the maternal milk supply and/or indirectly by affecting some aspect of maternal behavior.

Na_2 $PtCl_6$ Developmental Tissue Distribution and Behavioral Studies

Maternal blood, kidney, and whole embryo Pt levels were highest 1 day post administration of Na_2PtCl_6 on day 7 of gestation. No Pt was detected in the maternal brain. By day 5 post administration, Pt was undetectable in fetal tissues, but the placental level was 2.5 fold greater than that of maternal blood.

Except for the maternal spleen, the Pt levels of maternal, fetal, and placental tissues peaked on day 1 post administration of Na_2PtCl_6 on day 12 of gestation. A low level of Pt (0.43 ± 0.01 ppm) was detected in maternal brain tissue. Placental Pt levels were greater than blood levels throughout the course of the experiment, reaching concentration factors, compared to blood, of 1.3 and 4 2 on days 1 and 5 post administration, respectively.

Whole body Pt levels in the suckling offspring of dams receiving Pt on day 2 *post partum* were highest on day 1 post administration. By day 12 post administration, except for the digestive tract and its contents, no Pt was detectable in the tissues of the sucklings.

Effects of maternally administered Na_2PtC1_6 on neonatal and adult offspring behavior were limited to day 12 gestational exposure. The neonatal effects were expressed as lower day 8 *post partum* activity scores for *in utero* Pt exposure compared to PBS exposure. For the adult offspring, there was a prenatal x postnatal exposure interaction indicating that Pt exposed mothers reduced the performance of offspring on those tasks with a large activity component (i.e., ambulations, passive avoidance trial 1 latency and passive avoidance difference scores). In this case, gestational exposure to Pt appears to have persistent effects on the mother that are transmitted postnatally to offspring. This may occur directly through residual Pt in the milk or indirectly through maternal behavioral alteration resulting in neglect of the offspring.

Conclusions

In summary, $Pt(SO_4)_2$ administered via the IG route appears to be poorly absorbed. However, with the exception of brain tissue, the absorbed Pt achieves general systematic distribution. With repeated exposure, tissue concentrations tend to increase with dose; again, with the exception of brain which did not accumulate Pt. While Pt did affect behavior under conditions of single and multiple dose exposure, these effects were weak except when very high (LD_{25}) individual doses were employed In the latter case, the behavioral effects were more pronounced and lasted for up to 7 days post administration (end of observation). The consistent pattern of relationship between tissue levels of Pt and behavior is suggestive of interference with cellular processes induced by Pt at concentrations greater than some threshold value. This interference is manifested as behavioral malaise.

It is difficult to predict the potential danger of increased anthropogenic redistribution of Pt in the environment. Toxicity has been observed in humans exposed to high Pt levels in the workplace or during antitumor chemotherapy. However, such dose levels are many times those that would ever by expected to occur in the general environment. Nevertheless, since exposure to Pt appears to alter certain behavior of the mouse, the possibility of subtle adverse biological and behavioral effects resulting from long-term low level (environmental) exposure of humans cannot be disregarded especially in the light of the fact that Pt can be methylated in a manner analagous to that of mercury. By altering its physicochemical properties, methylation may, as in the case of mercury, greatly enhance the toxicity of Pt and its bioaccumulatability

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The complete report, entitled "Sensitive Biochemical and Behavioral Indicators of Trace Substance Exposure. Part II. Platinum," (Order No. PB 81-160 897; Cost: $8 00, subject to change) will be available only from:

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