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Oral Administration versus Intra-peritoneal Injection of Pb Affects Its Concentration in Selected Rat Tissues

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Pb poisoning is a serious health problem that continues even after the banning of its primary source, lead-based paint, in 1978 and tetraethyl lead in gasoline. This is seen most dramatically in children living in low-income housing, where the houses contain lead-based paint. Other sources of Pb include water pipes found in older houses; soil and other water sources located near industrial factories. It can result in retarded growth, diminished IQ, and developmental abnormalities in children [1] and adults can be plagued by hypertension, anemia, hearing loss, kidney damage, peripheral neuropathies, infertility, diminished life span, and encephalopathy, listed in the order associated with increasing blood lead levels [2]. It is the most dramatic in children with one out of every ten above the CDC established limit of Pb level in blood of 10 µg/dL [3].

Our laboratory is focused on using ionophores in the treatment of Pb intoxication; specifically monensin [4], ionomycin [5], A23187 [6], and nigericin [7]. These and other polyether ionophores have been shown to transport Pb²⁺ ions across phospholipid bilayers in model membranes and form complexes with Pb²⁺ in solution [4-7]. Due to the nature of ionophores, higher concentrations of Pb are necessary to elicit better results, and different methods of administration might vary those conditions. We have extended our studies into a living system using the rat as our model. During separate studies, we provided Pb to the rats either through their drinking water or by intra-peritoneal (IP) injection in the form of Pb(acetate)₂. We have seen a trend in Pb accumulation such that its method of administration affects their levels in selected organs: rats that were given Pb by IP injection showed a higher concentration of the metal and smaller relative errors compared to their oral counterparts. This study examines these marked differences in Pb concentration between oral administration and IP injection.

During past studies, we have observed a marked difference in the concentration of Pb in selected rat tissues and felt that it would be of importance to further examine this trend. Using Pb loading data from past experiments [4,8, unpublished data], we wanted to gain a better understanding as to why the mode of Pb administration changes the Pb concentration in the body. Table 1 compares the tissue Pb levels after the 3 week Pb loading period by either oral or IP injection. Across all organs examined, the IP injections gave much higher concentration

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of Pb compared to its oral counterparts. Specifically, the order of Pb levels observed in the IP group was femur > kidney > liver > brain ~ muscle > heart, with the highest value exceeding the lowest one by approximately 1.5×10^3 . The order of Pb levels observed in the oral groups followed an almost identical pattern, or specifically femur > kidney > liver > brain > heart > muscle, with the highest value exceeding the lowest one by approximately 1.9×10^3 . These findings are similar to those reported by other groups [9-11]. Also in Table 1, we observed that the standard error was 3-5 fold lower in the organs that contained a large amount of Pb (kidney, liver, and femur) in the IP group compared to the oral groups. This suggests that each animal is receiving approximately the same amount of Pb by injection rather than through their water supply. These implications will be talked later on in this report.

To express the relative effectiveness of Pb loading in each group, we calculated the fraction of the Pb loading value of the IP group that remained after the 3 week treatment period and compared these values with the three oral group values. Figure 1 shows that the oral groups have dramatically (~60-98%) smaller amounts of Pb in all tissues, except brain, compared to their IP group counterpart and that these differences were also statistically significant. Rat brains tend to have smaller amounts of Pb (Table 1) compared to some of the other tissues and this may be why the data sets aren't statistically significant. The exception for brain is between the IP group and the Pb-loaded group (Figure 1) that was given Pb for 3 weeks and taken off for an additional 3 weeks (Oral #3). Over additional time after the treatment, the oral group lost Pb concentration in all tissues and therefore became significant compared to the IP group. Regarding the three oral groups, we compared their values together in the same format as used for Figure 1. Between the two Pb-loaded groups (Oral #1 and Oral #2) and the last oral group (Oral #3), we observed dramatic differences ($\geq 180\%$) in Pb tissue levels in liver, kidney, and muscle and these differences were also statistically significant. There was a large difference between the muscle values although this organ had amongst the lowest concentration of Pb compared to the other tissues (Table 1). Between Oral #1 and Oral #2 groups, there were no marked differences and thus none of them were statistically significant. The third oral group lost additional Pb concentration over the 3 weeks as this oral group was taken off the Pb whereas the first two groups were sacrificed earlier in the experiment.

This paper has shown that by giving rats IP injections of Pb, they have much higher levels of Pb in specific tissues and have smaller relative errors compared to their oral counterparts. The best explanation for this trend is that by administering Pb by IP injection, this allows Pb to get into the blood system faster where it can be distributed to the organs quicker than by oral administration. The rats that received the Pb orally *via* their drinking water had some of the Pb tied up in their feed where it passed through the gastrointestinal (GI) tract and was excreted by the kidneys and out of the body. This is seen most dramatically when comparing the IP injection group with that of the third oral group that was loaded with Pb for 3 weeks and then taken off the regimen for an additional 3 weeks before sacrificing the rats.

The purpose of Table 1 was to illustrate the fact that IP injection of Pb showed higher concentrations compared to the normal groups and the oral groups. As was seen, the tissues that had the greatest concentration of Pb were the femur, the kidney, and the liver. This is not surprising since the femur acts as a central pool for Pb and that the kidney and liver are involved in detoxification of Pb in the body. It should also be seen that the relative errors for the IP groups are all lower compared to the other groups. This is most evident in the femur where the average relative percent error was around 15% for the three oral groups and only about 3% for the IP group. This means that between individual rats within a group, the IP injections have higher precisions of Pb concentration compared to the oral groups. Again, this can be attributed to the fact that rats provided with Pb orally take it in at different rates depending upon the individual rats drinking patterns compared to the known concentrations during IP administration.

To understand the differences in Pb concentration for the two methods, we looked at the relative percent changes in the three oral groups compared to the IP group (Figure 1). Brain doesn't contain a high concentration of Pb (Table 1) and therefore we wouldn't expect to see much change between the test groups. In brain, Pb effects manifest at low overall levels and include cognitive impairment and reduced IQ [12-14]. However, muscle and heart were statistically different in the oral groups compared to the IP groups although they had low concentrations of Pb (Table 1). In heart, Pb accumulation generates a set of effects that are analogous to those produced by various forms of human cardiac disease [14]. The differences between the oral groups in Pb concentration doesn't vary greatly (Figure 2) and only shows a significance when the two oral groups are compared to the third oral group.

Comparing the six tissues, we can see that the femur had the highest concentration of Pb in the normal group (no Pb), IP injection group and all three oral groups. Bone Pb is problematic because this pool is large and is the main source of Pb that reestablishes an elevated blood concentration after treatment by existing methods (e.g. ethylenediaminetetraacetic acid or EDTA) [15-19]. Past experiments have shown [8, unpublished data] that known Pb ionophores that have a high affinity for Pb and that they maybe more effective at higher levels of Pb intoxication. Since IP injection shows a tendency of higher Pb levels in the tissues, this technique can be implemented to see how much more effective particular Pb ionophores have on removing Pb from the interested tissues. This possibility can be tested experimentally and is being considered for future studies.

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Comparison	Tissue (relative percent change)					
	Muscle	Heart	Brain	Liver	Kidney	Femur
Oral #1 vs. IP	-93.8*	-63.4*	-11.5	-86.7*	-71.2*	-84.3*
Oral #2 vs. IP	-94.5*	-60.2*	-19.4	-88.3*	-65.9*	-83.8*
Oral #3 vs. IP	-98.0*	-61.1*	-38.5*	-98.2*	-92.5*	-87.0*

Figure 1.

Comparison	Tissue (relative percent change)					
	Muscle	Heart	Brain	Liver	Kidney	Femur
Oral #1 vs. Oral #3	+214.3*	-5.7	+44.0	+629.7*	+282.7*	+20.6
Oral #2 vs. Oral #3	+180.1*	+22.9	+31.1	+545.6*	+352.5*	+24.8
Oral #1 vs. Oral #2	+12.2	-8.1	+9.9	+13.0	-15.4	-3.4

Figure 2.

Figure 3.

Table 1

Tissue Pb concentrations (average $\pm$ relative percent error.)

Methods	Muscle	Heart	Brain	Liver	Kidney	Femur
Normal	0.037 ± 97.4	-0.010 ± 33.6	0.006 ± 150.1	-0.003 ± 100.5	0.056 ± 59.9	0.254 ± 14.8
IP injection	1.19 ± 23.0	0.445 ± 10.8	1.33 ± 5.1	43.4 ± 5.4	103.4 ± 7.2	668.3 ± 2.8
Oral #1	0.073 ± 20.1	0.163 ± 18.3	1.18 ± 19.9	5.77 ± 25.3	29.8 ± 25.9	104.6 ± 15.3
Oral #2	0.065 ± 20.9	0.177 ± 20.4	1.07 ± 23.4	5.10 ± 19.6	35.2 ± 28.9	108.3 ± 15.6
Oral #3	0.023 ± 21.1	0.173 ± 15.2	0.817 ± 25.7	0.789 ± 26.8	7.79 ± 18.2	86.8 ± 12.7