Research Square

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Metallothionein alone or in combination with Prussian blue attenuates acute thallium systemic toxicity in rats.

Laura Anaya-Ramos
Universidad Autonoma del Estado de Morelos Facultad de Farmacia
Araceli Díaz-Ruíz
Instituto Nacional de Neurologia y Neurocirugia Manuel Velasco Suarez
Camilo Ríos
Instituto Nacional de Neurologia y Neurocirugia Manuel Velasco Suarez
Sergio Montes
Instituto Nacional de Neurologia y Neurocirugia Manuel Velasco Suarez
Yoshajandith Aguirre-Vidal
Universidad Autonoma del Estado de Morelos Facultad de Farmacia
Sara García-Jiménez
Universidad Autonoma del Estado de Morelos Facultad de Farmacia
Veronica Baron-Flores
Universidad Autonoma Metropolitana - Xochimilco
Antonio Monroy-Noyola (amonroy83@hotmail.com)

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Abstract

Background: Acute Thallium (Tl) toxicosis is still a health problem, worldwide. Oral administration of Prussian blue (PB) is the antidotal treatment of election. On the other hand, metallothionein (MT) is a low-molecular-weight protein, with high content of cysteines (25–30%). MT is able to chelate metals as an efficient endogenous mechanism of detoxification. It is also a potent antioxidant.

Methods: In this study, we tested the ability of MT at two doses (100 and 600 μg/rat), administered alone or in combination with Prussian blue (PB) (50 mg/kg) to decrease thallium (Tl) toxicity. A sublethal dose of Tl (16mg/kg) was injected i.p. to male Wistar rats. Antidotes were administered twice-daily, starting 24h after Tl injection, for 4 days. Tl concentrations were analyzed in body organs and brain regions, 5 days after Tl injection.

Results: Results showed a diminution ($p<0.05$) of Tl concentrations in all organs by effect of PB alone or in combination with MT-100 and MT-600, whereas MT-100 only decreased Tl concentrations in testis, spleen, lung and liver. Likewise, Tl in brain regions was also diminished ($p<0.05$) by effect of PB and both MT-100 alone or in combination in most of the regions analyzed ($p<0.05$). The greatest diminution of Tl was achieved when the antidotes were combined. Plasma markers of renal damage, increased after Tl administration. Both PB and MT, either alone or in combination, prevented the raise of renal markers of Tl Toxicity.

Conclusions: Our findings demonstrate that the combined treatment of PB + MT is a good antidotal option against thallotoxicosis.

Background

Thallium salts are mainly found in iron, copper and sulfide ores of the earth's crust. Those compounds are only minor components, therefore, thallium (Tl) is found in a concentration range of 0.3–0.5 μg/g, rendering it as a trace element of the earth's crust [1]. In the early 1900s, thallium sulphate salts were used in the treatment of tuberculosis, dysentery and venereal diseases. It was also been used as a depilatory for human and veterinary use and especially for the manufacture of the rodenticide “Zelio”, containing 2% of thallium sulphate as active ingredient [2]. Due to the toxic effects of Zelio and other products in humans, its applications for human purposes were suspended, while the manufacture of the rodenticide banned. Nowadays, Thallium-201 is used in the medical field at very low doses for the diagnosis of myocardial activity. Environmental concern has been raised, as different salts and organic compounds of Tl are being disseminated to the environment, as a byproduct of cement plants and in the manufacturing of cosmetics and jewelry, as well as optical, electrical and electronic products, including semiconductors [3]. For this reason, cases of human poisoning are still reported, due to accidental causes in the working environments. The most severe cases of human thallotoxicosis leading to death are those reported in the scientific literature resulting from suicide or homicide acute intoxications [4–6]. Also, it has been reported that human chronic thallotoxicosis could occur by environmental exposure, due to the consumption of vegetables and fruits contaminated with Tl coming from adjacent farmlands located in the vicinity of cement plants, municipal incinerators and sulfuric acid factories. Those sources of pollution represent the highest health risk for Tl human intoxication, including contaminated food crops irrigated with wastewater [1, 7–9]. Thallium salts are soluble, odorless, tasteless and highly toxic, for this reason, it was widely used in rodenticides and insecticides [10]. Rat Tl LD_{50} is about 30–32 mg/Kg, while human LD_{50} of thallium has been estimated to be 10 mg/kg, lower than As, Hg, Pb and Cu.

Despite the fact that the world health organization banned the use of “zelio” since 1973, there are still cases of acute human poisoning around the world, because the rat poison was not collected from human communities for its destruction [11, 12]. Since 2012 to 2017 it has been reported acute Tl poisoning in China, Mexico and Japan with at least 25 patients involved [13–16]. Cases of intoxication manifest gastrointestinal signs and symptoms (diarrhea and vomiting), dermatologic alterations (alopecia, eruptions of the face, Mee´s stripes in nails, eczematous lesions, anhidrosis, palmar erythema, stomatitis, and painful glossitis), cardiac (tachycardia and hypotension) and neurological dysfunctions (Disorientation, lethargy, ataxia, convulsion, psychosis, insomnia and coma) [2, 14, 17]. In severe cases of intoxication, individuals died less than a week after thallium ingestion [12, 15, 18].

Once ingested, Tl is transported into the cells by means of the active mechanism of the Na^+/K^+-ATPase and passively through K^+ channels, due to the similarity of charge and atomic radius to this monovalent cation [19, 20]. It is distributed throughout the organism crossing the blood-brain barrier (BBB) and the placenta barrier [21, 22]. Pre-clinical studies with rats have supported that the half-life of Tl in blood is 72 hours and its highest concentration has been measured in the kidney, testicles and heart [23]. Even when the brain is the organ that reports the lower concentration of Tl, it is considered a target organ of the acute thallotoxicosis [2].

The mechanism of thallium toxicity is based upon the inhibition of enzymes specific of the glycolysis, Krebs cycle and oxidative phosphorylation. Those inhibitions produce a depletion of the levels of ATP in the cells [24]. Tl has a high affinity for the sulfhydryl groups of amino acids such as cysteine and methionine. The binding affinity of thallium for potassium sites has also been reported, and that binding has been implicated in the alterations of the cellular metabolic pathways dependent on this ion [25–27]. Tl^+ also increases reactive oxygen species (ROS) formation, which in turn play an important role in brain and liver tissue damage and organ dysfunctions by lipid peroxidation (LP) [28–30].

Potassium ferri-cyanoferrate II ($KFe[Fe(CN)_6]$), commonly known as Prussian blue (PB), is the antidote of choice against the human thallotoxicosis [31, 32]. This chelator agent is administered by oral route, decreasing the absorption of Tl to the enterohepatic circulation by 60–70% and therefore increasing elimination of Tl into feces [33]. Despite its effectiveness as antidote, in severe cases of human thallotoxicosis it´s administration is still inefficient, causing neurological and peripheral aftermath, including renal and hepatic lesions. In order to get a new therapy against Tl poisoning and to increase the efficacy of PB, other chelating agents have been administered alone or in combination with PB, such as; sodium diethyldithiocarbamate, dimercaprol (British Anti-Lewisite), D-penicillamine and the thiol amino acid L-cysteine, as antidotes. However, rat studies have shown that when those chelating agents are administered alone, they cause removal of the metal from the deposit tissues, such as bone, muscle and others, redistributing it to the brain, which aggravates the symptomatology of the intoxication [34–36]. On the other hand, the administration of an endogenous metalloprotein, such as metallothionein I (MT-I), has shown a chelating and antioxidant effect (35%) on the liver of rats administrated with 32 mg/kg of thallium acetate [30]. Those in vivo studies suggest the need for a better characterization of the effects on other tissues and peripheral organs, using different doses of MT. In the present study, we characterized the effect of MT-I + MT-II administration to rats treated with a sublethal dose of thallium acetate (16 mg/Kg), either alone or in combination with PB.

Methods

Animals

We used male Wistar rats weighing 200 to 250 g; they were obtained from National Institute of Neurology and Neurosurgery, México. Rats were housed four per acrylic cage and maintained under standard laboratory conditions (12:12 light–dark cycles, 23 ± 2 °C) and 40% relative humidity, had free access to food and water. All animals were treated following the guidelines established internationally and nationally by the Mexican Official Standard NOM-062-ZOO-1999, the guidelines for Care and Use of Laboratory Animals of the National Institutes of Health (USA) and in accordance to the ethical principles and regulations specified by the Animal Care and Use Committee of the National Institute of Neurology and Neurosurgery.

Antidotal treatments

Animals (n = 46) were administered with a single i.p. dose of 16 mg/kg of thallium acetate (Tl), according to reports by Rios and Monroy [35]. Twenty-four hours later, animals were randomly allocated into each of six groups, as follows: group 1: animals dosed only with Tl plus vehicle (C), group 2: rats administered with Tl plus Prussian blue by oral route (PB group) (50 mg/kg twice-daily, for four days) according to the report by Montes et al. [37] Group 3: rats dosed with Tl plus a single i.p. dose of metallothionein (100 μg/rat) (MT-100); group 4: animals with Tl plus MT-100 in combination with PB (PB + MT-100), group 5: rats dosed with Tl plus a single i.p. dose of metallothionein (600 μg/rat), according to report Kılıç and Kutlu [30] (MT-600); group 6: animals dosed with Tl and MT-600 in combination with PB (PB + MT-600). Likewise, we included a healthy control (HC) group (n = 5) to know the normal values, only for the studies of renal and hepatic functions. Finally, all animals were killed 5 days after Tl intoxication to obtain Tl concentration in body organs and brain regions. All animals were euthanized by decapitation. They were previously anesthetized with an overdose of pentobarbital (100 mg/Kg i.p.) as established by the guidelines of the official Mexican standard and the bioethics committee of the National Institute of Neurology and Neurosurgery.

Thallium analysis

The levels of Tl in kidney, testis, spleen, heart, lung, and liver (peripherals organs) and hippocampus, striatum, hypothalamus, midbrain, cerebellum and cortex (brain regions) were analyzed by atomic absorptions spectrophotometry using the analytical conditions reported previously by our research group [23]. Tissue samples were acid-digested in metal-free concentrated nitric acid (Suprapur Merck) and analyzed using an atomic absorption spectrophotometer (Perkin Elmer 3110) equipped with a graphite furnace (Perkin Elmer HGA-600) and auto sampler (AS-60). All of the material used (polypropylene tubs and tips) in Tl analysis was previously washed and immersed in a 3% nitric acid solution for 24 h and rinsed with deionized water to avoid external Tl contamination. The quantification of metal in biological tissues was performed using a calibration curve constructed with a thallium standard (Perkin Elmer). Results of Tl content in peripheral organs and brain regions are expressed as μg of thallium per gram of wet tissue.

Biochemical markers of liver and renal damage

To determine biochemical markers of renal and hepatic functioning, blood samples were withdrawn and stored in clean tubes. After centrifugation at 1500 g, for 10 min, serum was stored at -20° C until analysis. Creatinine, urea, alanine amino-transferase (ALT), aspartate amino- transferase (AST) and total proteins (TP) were measured using Cobas c 111 autoanalizer (Roche, USA), with commercially available kits (Roche diagnostics).

Statistics

An exploratory analysis of the data was performed to determine normal distribution (Kolmogorov-Smirnov's test) and homogeneity of variances (Levene's test). In order to obtain normal distribution of the data, a logarithmic transformation was applied and then, logarithmic values were analyzed using one-way ANOVA followed by Dunnett's

test. All analyses were performed using an SPSS 22.0 software (Chicago, Illinois, USA). Differences were considered statistically significant when $p < 0.05$

Results

Thallium levels in peripheral organs

Thallium levels in peripheral organs after antidotal treatment are shown in Fig. 1. In panel A, the mean ± one SEM of kidney thallium levels (Tl) are shown; values are expressed as μg of Tl per gram of wet tissue. As can be observed, kidney Tl in the control group (C) averaged 11.97 ± 2.21 (n = 11), while the group of Tl with PB were 5.44 ± 0.64 (n = 13), showing a 54% of diminution, as compared to group C average ($p < 0.05$). Likewise, the Tl plus MT-100 group averaged 10.05 ± 1.40 (n = 5), a diminution of 16% as compared to group C. Also, the mean of the group PB + MT-100 was 2.34 ± 0.25, in this group, the highest decrease was observed, 80% vs C group (n = 5) ($p < 0.05$). The group MT-600 averaged 22.25 ± 5.26 (n = 7), an increase 85% vs C group ($p < 0.05$). Finally, the group with PB + MT-600 averaged 10.60 ± 0.89 (n = 5).

Panel B, shows the results of Tl levels in testis. The mean value o group C was 10.80 ± 0.95, while for the PB group averaged 3.52 ± 0.27, showing a reduction of 67%. Likewise, the groups MT-100 and MT-600 showed diminutions of Tl of 32 and 17%, respectively. Likewise, the groups of the combined therapy PB + MT-100 and PB + MT-600, averaged 2.48 ± 0.27 and 3.36 ± 0.31, respectively, showing the highest Tl decrease in the PB + MT-100 group (77%). All groups were different from C ($p < 0.05$) except treatment with MT-600.

Panel C shows the results of the analysis of Tl in spleen. Tl concentration averaged 3.98 ± 0.43 in group C, while the mean of the PB group was 1.20 ± 0.09, 70% lower than group C. Meanwhile, the MT-100 group averaged 2.70 ± 0.26, a 32% lower than group C; the combination of antidotes at the two doses of MT (PB + MT-100 and PB + MT- 600) showed the greatest decrease of Tl, as compared to group C levels (71 and 73%, respectively). All groups were different from C ($p < 0.05$) except treatment with MT-600.

Tl levels measured in the heart are shown in panel D. The mean value of group C was 4.72 ± 0.40, while in the PB group the value was 1.73, 63% lower than group C. Likewise, in the group MT-100, the mean was 3.91 ± 0.37, a diminution of 17% vs group C. Finally, the combination of antidotes, groups PB + MT-100 and PB + MT-600, produced the greatest decrease of Tl levels, 74% (1.24 ± 0.15) and 68% (1.53 ± 0.11) vs group C, respectively. In this organ only PB antidotes combined with MT at 100 and 600 were different when compared with group C ($p < 0.05$).

Panel E shows the results of lung Tl. The mean of group C was 4.30 ± 0.55, while the PB group averaged 1.61 ± 0.13; a reduction of 63% vs group C. Likewise, in the MT-100 group we observed a mean of 2.41 ± 0.35, 44% lower than group C average. Again, the combination of antidotes showed the greatest decrease as compared to the control group average, showing diminutions of 81% (PB + MT-100) and 66% (PB + MT-600), respectively. All groups were different from C ($p < 0.05$) except treatment with MT-600.

Liver Tl concentrations are shown in panel F. The mean value of group C was 4.18 ± 0.050, while the PB group averaged 1.21 ± 0.09, a reduction of 71% as compared to group C. MT-100 and MT-600 groups showed mean values of 2.73 ± 0.09 and 2.96 ± 0.35, respectively, diminutions of 35 and 29% both vs group C, respectively. Again, the combination of antidotes had the greatest reduction of Tl content, which was 78% and 74%, respectively, as compared to the average of group C. All groups were different from C ($p < 0.05$).

Thallium levels in brain regions

Figure 2 shows the Tl levels in different brain regions. Panel A shows the means ± SEM of Tl in the hippocampus; the results are expressed as µg of Tl per gram of wet tissue. As observed, control group (C) Tl averaged 2.13 ± 0.20 (n = 11), while PB group the mean was 0.72 ± 0.05 (n = 13), 66% lower than group C ($p < 0.05$), meanwhile, the mean was 1.92 ± 0.034 (n = 5) in the group MT-100. Likewise, mean Tl of group PB + MT-100 was 0.49 ± 0.09; this group showed the lowest decrease of Tl observed, 77% (n = 5), when compared with group C ($p < 0.05$). Group MT-600 averaged 1.86 ± 0.020 (n = 7), finally in the group PB + MT-600, the average was 0.72 ± 0.05 (n = 5), showing a diminution of 66% vs group C ($p < 0.05$). Only PB antidotes combined with MT at 100 and 600 were different when compared with group C ($p < 0.05$).

Panel B shows the mean Tl levels in the striatum. Tl concentrations averaged 2.05 ± 0.17 in group C, while in the PB group the mean was 0.71 ± 0.04, 65% lower than that observed in group C ($p < 0.05$). Meanwhile, group MT-100 averaged 1.47 ± 0.05, a 28% reduction compared to group C mean ($p < 0.05$). The combination of antidotes PB + MT-100 and PB + MT-600 showed the highest decrease of Tl levels vs group C, with reductions of 75 and 66%, respectively ($p < 0.05$). All groups were different from C ($p < 0.05$) except treatment with MT-600.

Panel C shows the mean Tl concentrations in the hypothalamus. Tl in Group C averaged 2.19 ± 0.21, while the mean value of group PB was 0.70 ± 0.04; 68% lower than the mean of group C. On the other hand, groups MT-100 and MT-600 averaged 1.61 ± 0.21 and 1.80 ± 0.21, showing a diminution of 26 and 18%, respectively, when compared to group. Again, the combination of antidotes showed the greatest Tl reduction as compared to the mean of group C: 77% (PB + MT-100) and 67% (PB + MT-600). All groups were different from C ($p < 0.05$) except treatment with MT-600.

Tl levels in the midbrain are shown in panel D. Group C showed a mean of 1.71 ± 0.017, while PB group averaged 0.61 ± 0.04; 64% lower than group C. Likewise, the mean of MT-100 group was 1.18 ± 0.09; 31% lower than group C. Meanwhile, MT-600 group averaged 1.54 ± 0.016. Again, the combination of antidotes showed the greatest reductions, 74% (PB + MT-100) and 68% (PB + MT-600), respectively, when compared to group C. All groups were different from C ($p < 0.05$) except treatment with MT-600.

Panel E shows Tl levels measured in cerebellum. The mean value of group C was 1.56 ± 0. 13, while in the PB group the mean value was 0.52 ± 0.04; 66% lower than group C. Groups MT-100 and MT-600 showed mean values of 1.21 ± 0.09 and 1.23 ± 0.16, respectively, Tl diminutions of 22 and 21%, respectively, as compared to group C. Finally, the combination of antidotes showed the greatest reduction as compare to control group mean (0.40 ± 0.06 and 0.40 ± 0.03 respectively) or approximately 74% of diminution for both groups. All groups were different from C ($p < 0.05$).

Panel F shows Tl levels in the cortex. There is a Tl concentration of 1.63 ± 0.13 in group C, while in the PB group the average value was 0.50 ± 0.03, 69% lower than that observed in group C ($p < 0.05$). Groups MT-100 and MT-600 showed means of 1.27 ± 0.07 and 1.29 ± 0.15, 22 and 21% lower than group C, respectively ($p < 0.05$). Similarly, the combination of antidotes with the different doses of MT (PB + MT-100 and PB + MT-600) showed the highest reduction of the levels of Tl, as compared to group C. That reduction was 75 and 69%, respectively ($p < 0.05$). All groups were different from C ($p < 0.05$).

Renal and hepatic biochemical markers

The results of antidotal treatments on renal and hepatic thallium toxicity markers are shown in the Table 1. We observed a significant increase in serum creatinine and urea of 2.4 and 3.6-fold, respectively, by effect of Tl intoxication (Group C), as compared healthy control (HC) group. No alterations in those markers were found in all of the groups receiving treatment either with PB or MT antidotes alone or in combination, as compared to HC averages.

Table 1
Renal and hepatic biochemical biomarkers.

Group	Creatinine mg/dL	Urea mg/dL	ALT U/L	AST U/L	TP g/dL
HC	0.36 ± 0.01	42 ± 2.8	83 ± 8.7	310 ± 11.1	7.3 ± 0.07
C	0.86 ± 0.24*	150 ± 34.9*	59 ± 3.9	276 ± 26.2	7.5 ± 0.19
PB	0.41 ± 0.02	57 ± 8.8	72 ± 2.1	335 ± 28.2	7.6 ± 0.20
MT-600	0.42 ± 0.03	62 ± 11.7	58 ± 5.3	281 ± 30.2	7.5 ± 0.20
PB + MT-600	0.36 ± 0.01	44 ± 1.6	61 ± 7.2	311 ± 42.6	7.7 ± 0.21

Alanine amino-transferase (ALT), aspartate amino- transferase (AST) and total proteins (TP). HC, Healthy-control animals, with no treatment, C: animals with thallium (Tl) plus vehicle, PB: Tl plus Prussian blue, MT-600: Tl and metallothionein, PB + MT-600: animals Tl plus combination of treatments. One-way ANOVA followed by the Dunnett's test. * $p < 0.05$ vs healthy control (HC) group.

Discussion

The results presented here, are in agreement with what was previously published by Montes et al. [37]. PB is an effective antidote of election for thallotoxicosis. The mechanism of Tl decorporation by PB is well-known; during its passage through the intestine, PB exchange K^+ by Tl^+ ions on the surface of the lattice of K Fe (Fe $(CN)_6$) (PB). Once exchanged, PB forms a stable compound with thallium, that is in turn excreted into the feces, accelerating Tl decorporation [38]. MT, on the other hand, has been proposed as a chelating antidote for Tl in thallotoxicosis [30]. In our hands, MT was moderately effective as an antidotal treatment against acute thallium systemic poisoning, when given alone (100 or 600 µg) or in combination with PB. MT reduced the thallium levels in tissues of metabolic importance, such as lung and liver, and also in the two brain regions containing the highest concentrations of thallium, such as hypothalamus and cerebellum. The systemic effect of MT did not produce an undesirable redistribution of Tl to the central nervous system (target organ of thallotoxicosis), as it has been observed after the administration of other chelating agents, such as D-penicillamine or endogenous thiols such as L-cysteine [35, 36], as MT is unable to cross the blood-brain-barrier [39] then, it is possible that a putative Tl-MT complex may not be able to cross to the brain to produce a redistribution of the metal. Particularly, the administration of 600 µg of MT showed a systemic, rather than cerebral, increase of renal Tl concentrations produced by a redistribution of the metal to the kidney. Interestingly, this renal thallium accumulation did not induce significant kidney damage, as the levels of creatinine (a biomarker of kidney dysfunction) were similar to the HC group values. Tl decorporation is more evident in the kidney and hippocampus, when MT is administered simultaneously to PB (PB + MT-100 and 600 groups). This additive pharmacological effect of MT in combination with PB has been reported for others exogenous chelating agents studied experimentally in vivo [37]. Taken together, results of the present study suggest a chelating effect of MT on Tl [30, 40, 41]. Exogenous administration of MT has been reported to be protective against heavy metals' intoxication [42]. In this study, hepatic Tl accumulation decreased at both doses of MT (100 or 600 µg), as reported by Kılıç, and Kutlu [30] in a similar rat model of acute thallotoxicosis. It is important to remark that most of the effects of MT were not dose-dependent in body organs and brain regions, suggesting that the lower dose of MT (100 µg/rat) is achieving the maximal effect of the protein on Tl decorporation. In fact, the highest dose of MT employed (600 µg/rat), was the one that produced a redistribution effect on kidney Tl.

The use of MT as an antidote in thallotoxicosis protected against the damage to peripheral organs by decreasing organ concentrations of thallium without increasing the metal concentration of the brain. Because this effect of decorporation of Tl by MT was modest, it is possible that other MT-induced mechanisms are also participating to prevent Tl-induced damage. The antioxidant actions of MT are well-known and have been suggested as an advantage of the protein compared to other chelating agents [43]. As Tl is able to induce free radicals' overproduction with consequent lipid peroxidation and formation of reactive oxygen species [29], an antioxidant action of MT may be an additional protective mechanism to explain why the protein protected from the renal effects of acute thallium poisoning, in spite of the redistribution of Tl to the kidney. The increased renal Tl concentrations observed in the group of animals treated with MT-600 may also be the reflection of a higher excretion of the metal by this organ. This remains speculative until future experiments.

The present study contributes with relevant pre-clinical data to evaluate the pharmacological efficacy of MT alone or in combination with PB for the antidotal treatment of acute thallotoxicosis. For most of the organs and brain regions, both antidotes showed a summation of effects when combined, with no statistically significant interaction. That result indicates that PB and MT are acting by different, unrelated mechanisms. As MT is unable to cross the blood-brain barrier efficiently, thallium is been chelated only in the periphery, without redistribution to the brain. This represents an advantage of the protein over other chelating agents that aggravate thallium toxicity when administered alone [35, 39].

Conclusion

The results presented in this work reinforces the idea of a combined treatment using PB plus a chelator as an effective antidote schedule against acute thallium toxicity, as suggested by the U.S. Food and Drug Administration [31, 32]. Also, our results encourage the investigation on the participation of endogenous mechanisms of protection against acute thallotoxicosis, such as those exerted by MT.

Declarations

Ethics approval and consent to participate

The guidelines for animal care was approved by the biology committee, in accordance with the official Mexican standard, that established internationally and nationally by the Mexican Official Standard NOM-062-ZOO-1999 (which observes technical specifications for the production, care and use of laboratory animals) and the guidelines for Care and Use of Laboratory Animals of the National Institutes of Health (USA). Finally, the protocol was registered with the National Institute of Neurology and Neurosurgery with the number 75/12.

Consent for publication

Not applicable.

Availability of data and materials

The datasets during and/or analyzed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Conceptualization, A.D.-R. and A.M.-N.; Formal analysis, L.A.-R., A.D.-R. and C.R.; Investigation, L.A.-R., S.M., Y.A.-V. and S.G.-J.; Methodology, S.M.; Project administration, A.D.-R. and A.M.-N.; Resources, A.D.-R., V.B.-F and A.M.-N.; Supervision, A.D.-R. and A.M.-N.; Visualization, L.A.-R., Y.A.-V. and S.G.-J.; Writing – original draft, L.A.-R.; Writing – review & editing, C.R., V.B.-F. and A.M.-N.

All authors have read and approved the manuscript

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Author detail

[1]Laboratorio de Neuroprotección, Facultad de Farmacia. Universidad Autónoma del Estado de Morelos Cuernavaca, Morelos. México.

[2]Departamento de Neuroquímica, Instituto Nacional de Neurología y Neurocirugía, Ciudad de México. México.

[3]Laboratorio de Neurofarmacología Molecular, Departamento de Sistemas Biológicos, Universidad Autónoma Metropolitana-Xochimilco. Ciudad de México. México.

Abbreviations

Tl: Thallium; PB: Prussian blue; MT: Metallothionein; BBB: Blood-brain barrier; ROS: Reactive oxygen species; LP: Lipid peroxidation; ALT: Alanine amino-transferase; AST: Aspartate amino- transferase; TP: Total proteins.

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Figures

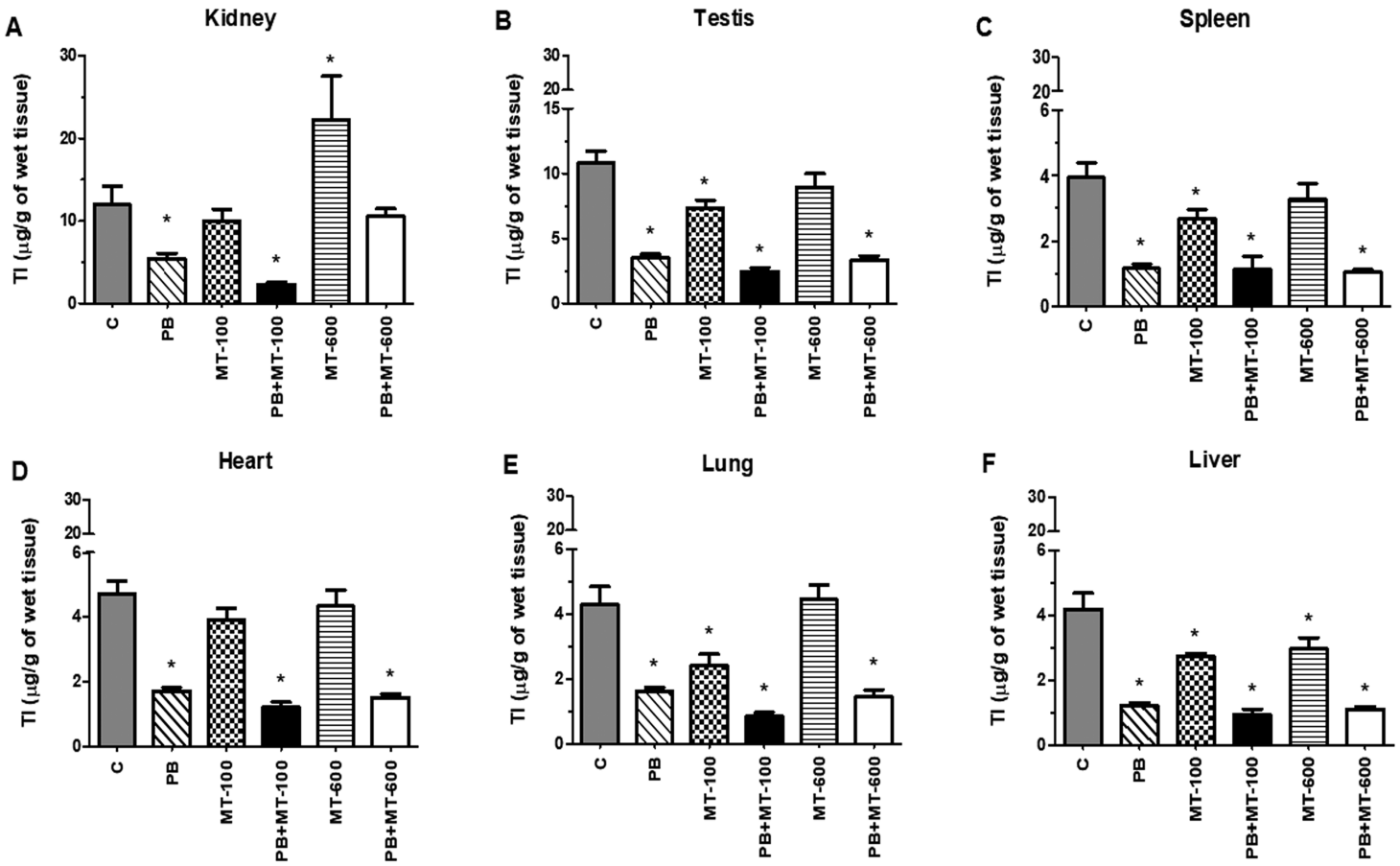


Figure 1

Effect of Prussia blue and Metallothionein administered alone or in combination upon thallium in organs. Levels in kidney (panel A), testis (panel B), spleen (panel C), heart (panel D), lung (panel E) and liver (panel F). The values are shown as mean ± SEM and are expressed in µg/g of tissue wet of thallium (Tl). Control group (C): Tl and vehicle, Prussia blue group (PB): Tl plus PB at 50 mg/kg four days, Metallotioneins groups (MT-100 and MT-600): Tl and MT 100 or 600 µg/ rat; PB + MT-100 and PB + MT-600 groups: Combined treatments of PB and MT to 100 and 600 µg/ rat respectively. One-way ANOVA followed by the Dunnett's test. * $p < 0.05$ vs C group.

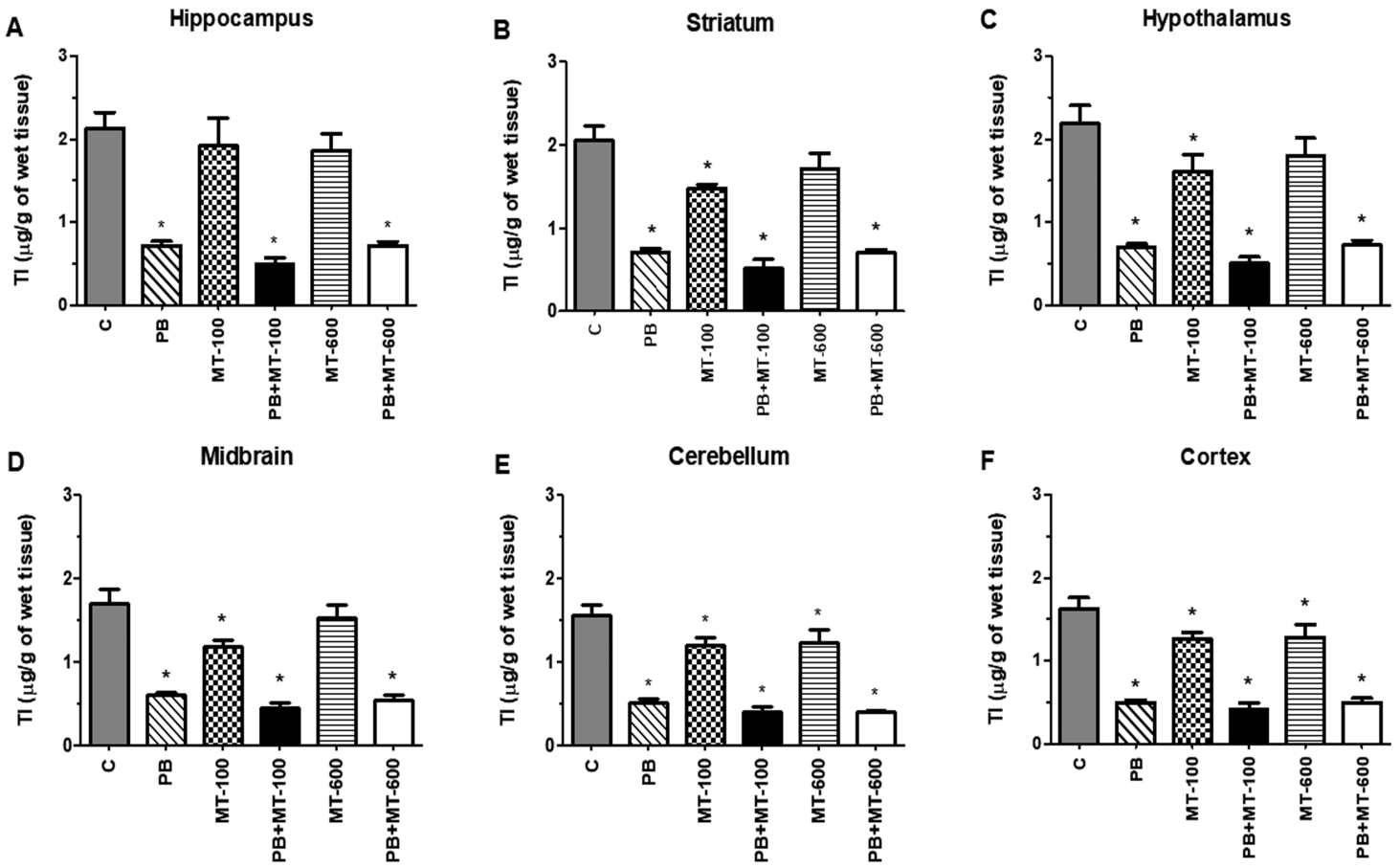


Figure 2

Effect of Prussia blue and Metallothionein administered alone or in combination upon thallium in brain. Levels in hippocampus (panel A), striatum (panel B), hypothalamus (panel C), midbrain (panel D), cerebellum (panel E) and cortex (panel F). The values are shown as mean ± SEM and are expressed in μg/g of tissue wet of thallium (Tl). Control group (C): Tl and vehicle, Prussia blue group (PB): Tl plus PB at 50 mg/kg four days, Metallotioneins groups (MT-100 and MT-600): Tl and MT 100 or 600 μg/ rat; PB + MT-100 and PB + MT-600 groups: Combined treatments of PB and MT to 100 and 600 μg/ rat respectively. One-way ANOVA followed by the Dunnett's test. * $p < 0.05$ vs C group.

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