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Characterization of Toxicological Properties of L-Lysine Polymers in CD-1 Mice

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We recently showed that polylysine, the polymer of lysines, retains anti-prion activity. Although the effectiveness of prion inhibition by polylysine was demonstrated with the regimen tolerated in mice, a determination of quantitative polylysine toxicity is necessary to precisely address the *in vivo* toxicity level of polylysine. In this communication, we report the results of body weight monitoring and hematologic tests performed in CD-1 mice that received two different tolerable dosages of polylysine for an either 5-day or 4-week period. We found that there was no significant alteration in overall serum chemistry, blood cell count, and body weight gain compared with controls. The only notable quantitative change with statistical significance was the decrease of platelet numbers in mice subchronically administered with polylysine. Our results suggest that polylysine is harmless in mice if administered for a short period, but administrations of polylysine in mice may require considerate attention for safety in future investigations as mice chronically receive tolerable doses of polylysine.

Keywords: Polylysine, toxicity, mice, body weight, hematology test, serum chemistry

Introduction

Prion diseases of humans and animals are fatal neurodegenerative disorders [16]. Despite numerous attempts to modulate the progress of disease in hosts, prion diseases remain incurable [17]. We recently showed that polylysines with various molecular weights commonly share the activity to inhibit prion propagation [18]. Polylysine suppressed propagation of the disease-causing isoform of prion protein (PrP^{Sc}), the only biochemical entity that comprises prions, in the *in vitro* prion amplification system. Polylysine also removed PrP^{Sc} from the neuroblastoma cell lines with chronic prion infection. Furthermore, polylysine exhibited a potent activity to delay the onset of disease in a prion-infected mouse model and decreased the level of PrP^{Sc} accumulation in the brain. Through these studies, we proposed a mechanism that polylysine inhibits prions by its interaction with plasminogen, a protein cofactor that assists in PrP^{Sc} propagation [10, 11], forming a nanoscale complex [7].

Polylysine is a chemically synthesized polymer of L-lysine. The biological activity of polylysine was addressed to control bacterial and viral pathogens, as well as cancer cells [1, 9, 13]. Moreover, it has been popularly used as a vehicle for drug delivery [2, 5, 15, 19, 24].

Although our recent study demonstrated that efficacious anti-prion activity of polylysine was achieved under the non-toxic concentrations in cultured cell lines and below the maximum tolerable doses in animal models of prion diseases [18], polylysine has been regarded as a toxic substance for years [3, 12, 21, 23, 25]. Animals that received polylysine *via* the intravenous route showed hemagglutination and erythrocyte destruction in the circulation [12, 23]. The level of *in vivo* toxicity differed as polylysine was given by the different delivery routes. The half maximal lethal dose (LD_{50}) was determined as 45 mg/kg/day, when polylysine

with the average molecular mass of 50 kDa was injected intraperitoneally once a day for 5 days in mice [1]. However, the LD_{50} was determined to be much lower (~ 12 mg/kg) when a single intravenous dose of polylysine with identical molecular masses was given to mice, [6, 21]. In addition, *in vitro* toxicity studies using polylysines with different molecular masses revealed that toxicity increases as the molecular mass of polymers increases, suggesting that polylysine toxicity is correlated with polymerization degrees, which indicate the number of lysine monomers within the molecule [18]. Much additional information on polylysine toxicity is available from previous studies. For instance, many different aspects of polylysine toxicity in a few animal species, including rats, guinea pigs, and rabbits, have been reported [3, 6].

Despite the abundant description from earlier studies, quantitative data of polylysine toxicity such as hematologic indeces in mice are unavailable from the literatures. Although our recent investigation briefly describes polylysine tolerance in mice when they were intravenously and intraperitoneally injected with sublethal doses [18], we inquired whether polylysine causes toxic consequences at any level. To address polylysine toxicity in mice, we investigated the alteration of toxicity parameters in a quantitative manner. Here, we report the outcomes of body weight change, blood cell count, and blood chemistry measurements.

Materials and Methods

Materials

Polylysine with an average molecular mass of 50 kDa was purchased from Sigma-Aldrich (USA). Stock solution at the concentration of 1 mg/ml was prepared in double-distilled water and saved at −20°C until it was used for dilution. Upon necessity, the polylysine stock was further diluted 1,000-fold with phosphate-buffered saline (PBS, pH 7.4; Invitrogen, USA). The diluted polylysine solution was sterilized by passing through a syringe filter device (0.2 μm pore size; EMD Millipore, USA) and used immediately for animal injection.

Animals

Four-week-old female outbred CD-1 mice were purchased from Harlan Laboratories Inc. (USA). The mice were kept in a standard "shoebox" cage with irradiated bedding (7090A Teklad Aspen Sani Chips, Harlan Laboratories Inc.). The mice were fed with irradiated 2018 Teklad Global 18% Protein Rodent Diet (Harlan Laboratories Inc.). Water was provided to mice *ad libitum* using a plastic water fountain (Lustar Productions Co., USA). The temperature and relative humidity were maintained between 18°C and 25°C and 40% and 60%. A consistent and uninterrupted light-dark cycle (14 h lights on/10 h lights off cycle) was maintained for the lighting condition. After a week in the animal facility, the now 5-week-old mice were randomly grouped and subjected to polylysine administration.

Study Design

In vivo toxicity test parameters were survival, weight loss, behavioral changes, alterations of cell number in the blood, and biochemical changes in the sera. For the acute toxicity test, mice were grouped into three (n = 6−8/group). Groups 1 and 2 received 4 and 8 mg/kg polylysine, respectively, for 5 days. Injection was made once every 24 h. The mice in Group 3 received PBS and served as a control. For the toxicity test followed by subchronic polylysine administration, mice were grouped into three (n = 6/group). Groups 4 and 5 received 4 mg/kg polylysine daily for 2 weeks and once every 48 h for 4 weeks, respectively. Then, each group was kept for an additional 4 or 2 weeks with no treatment until 6 weeks had passed after the initial injection. Group 6, the control groups for Groups 4 and 5, received vehicle only.

Animal Observation

During and after the administration of polylysine, mice were closely observed to see if there were any side effects by monitoring body weight and behavioral changes, such as drinking and eating patterns. Mice were weighed before and after the polylysine administration for short-term toxicity studies. When mice received polylysine for a subchronic period, weight measurement was performed weekly until the endpoint of the studies.

Administration of Polylysine

Polylysine was administered intraperitoneally into the abdominal cavity using a 1 ml syringe and a 26 gauge, 1/2 to 1 inch needle with a short bevel. The injection was made in the lateral aspect of the lower left quadrant. The volume injected into a mouse was 0.1−0.2 ml.

Hematology and Serum Chemistry

For sample preparation, whereas mice for the acute toxicity test were euthanatized 6 h after the last dosing, mice for the subchronic test were euthanatized on the last day of the 6-week period. At the time of euthanasia, sera and whole blood were immediately collected from the left ventricle of the heart using methods described elsewhere [4]. EDTA-treated blood (0.5 ml) for blood cell count and 0.2 ml of serum for chemical analysis were sent to The Division of Laboratory and Animal Resources at the University of Kentucky. The blood cell count was performed using a Heska CBC-Diff Veterinary Hematology System (Heska, USA). The serum chemistry test was conducted using a Hemagen Analyst chemistry analyzer system with a VET 16 Panels + rotor (Hemagen Diagnostics Inc., USA).

Statistical Analysis

Quantitative data were expressed as the mean ± standard deviation. The differences between the test and control groups

were compared using the one-way analysis of variance method. The statistically significant difference was set by *p*-values smaller than 0.05.

Results

Survival and Behavioral Changes

No mortalities occurred during the study. In both acute and subchronic toxicity tests, mice survived without showing clinical signs and impaired motor activities. However, it was noteworthy that mice that had received polylysines manifested temporal hypoactivity, which disappeared within 20–30 min. Other behavioral changes that reflect toxicity in organs were not demonstrated.

Body Weight Changes

The relative gain of body weights during acute and subchronic intraperitoneal administrations in mice is depicted in Fig. 1. When comparing body weights before and after administration, mice that received 4 or 8 mg/kg/day polylysines for 5 days showed body weights similar to the levels measured before administration, whereas mice that received vehicle (PBS) gained body weights which was statistically significant ($p = 0.029$) (Fig. 1A). More importantly, the relative changes of body weights between polylysine-treated and control groups were similar, and the difference among groups was not statistically meaningful ($p = 0.076$) (Fig. 1A). Dose responsiveness was not exhibited either. During subchronic administration, the average body weight of mice in each group increased ~ 5.6–8.6 g over the 6-week period. This increase was nearly 20–30% of the body weight measured prior to polylysine administration. The results of body weight changes in subchronic tests resembled the data described in acute tests. Mice gained body weight after polylysine administration, but the relative changes in body weight gain were not different among vehicle- and polylysine-treated groups ($p = 0.367$) (Fig. 1B). These results suggest that body weight gain in mice that received 4 mg/kg polylysine every 24 h for 2 weeks or every 48 h for 4 weeks is not different from that of the controls. The doses and durations employed for polylysine administration in mice were below the level that leads to body weight changes.

Blood Cell Counts

Overall, the hematology test for acute and subchronic polylysine toxicity showed certain differences in some criteria of complete blood count analysis. In acute tests, granulocyte differential was significantly increased ($p = 0.015$) (Table 1), although this was not consistently observed in subchronic tests (see Table 2). Owing to statistical insignificance, differences of the means in some criteria were not considered as indications of toxicity. In other criteria, the values measured from control and treated groups did not fall into the reference ranges. In subchronic tests, it is notable that the concentration of platelets decreased as the duration of polylysine administration lasted longer ($p < 0.001$) (Table 2). The differences of some values measured for each test criterion were statistically significant, but alterations varied within the normal reference ranges.

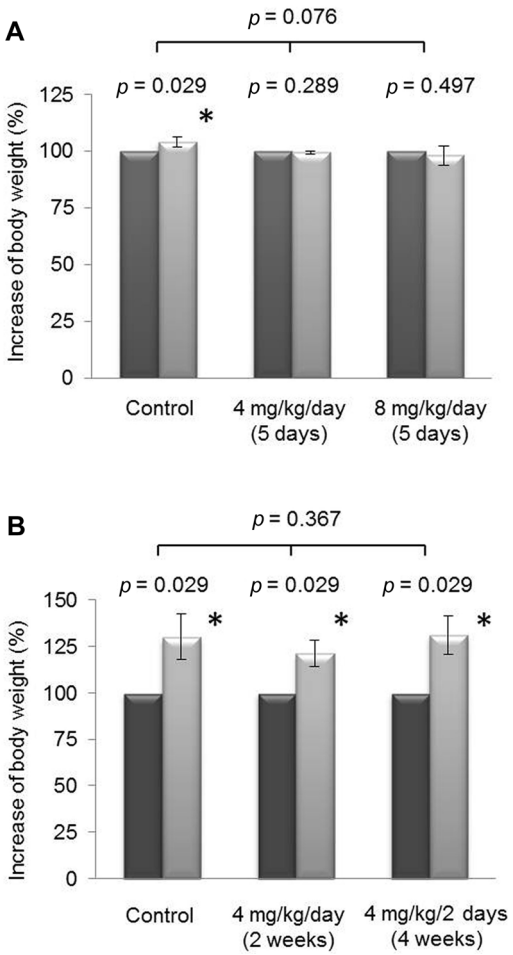

Fig. 1. Changes in body weights of mice.
Average increases of mouse body weight (n = 6–8) from the acute (**A**) and subchronic (**B**) tests were presented as percent changes compared with vehicle only. Control group: dark gray; polylysine-treate group: light gray. The *p*-values immediately above the bar were obtained from the analysis of comparison between vehicle-only and polylysine-treated groups. The *p*-values on the top of the bracket in each panel were obtained from the comparison among different groups that received different polylysine regimens. Asterisks denote the *p*-values smaller than 0.05.

Table 1. Complete blood cell count for acute toxicity of polylysine.

	Units	Control		4 mg/kg/day (5 days)		8 mg/kg/day (5 days)		*p*-Value[a]	Ref. value[b]
		Mean	SD	Mean	SD	Mean	SD		
WBC	10^3/μl	7.60	1.61	7.67	5.23	6.70	4.56	n.s.	1–9.1
LYMF	%	73.97	3.93	57.33	4.31	57.73	5.75	n.s.	50–86
GRAN	%	16.67	3.17	34.03	5.23	32.07	7.14	0.015	10–43
MONO	%	9.37	1.27	8.63	2.27	10.20	1.51	n.s.	0–8
HCT	%	48.17	8.53	44.27	3.27	44.03	7.68	n.s.	36–48
MCV	fL	58.83	6.62	54.53	5.70	57.83	6.39	n.s.	69–85
RBC	10^6/μl	8.34	2.31	8.15	0.75	7.66	1.38	n.s.	4.7–8.0
HGB	g/dl	13.93	3.00	13.53	1.10	12.70	1.90	n.s.	12.6–16.6
MCH	pg	16.90	1.10	16.63	0.49	16.67	0.64	n.s.	23–30
MCHC	g/dl	28.83	1.92	30.70	2.27	29.03	2.57	n.s.	31–39
RDW	%	16.97	1.69	17.50	0.78	16.07	1.80	n.s.	13 ± 0.9
MPV	fL	7.10	0.82	7.87	0.55	7.37	0.74	n.s.	6 ± 1.1
Platelets	10^3/μl	1,153.33	257.96	1,178.00	286.69	908.00	440.76	n.s.	1,000–1,400

[a]*p*-Values represent the values among different groups that received different polylysine regimens.

[b]RDW and MPV reference values are presented in a form of mean ± standard deviation and adopted from Smith and Jarecki (2011) *Atlas of Comparative Diagnostic and Experimental Hematology*, 2nd Ed., Blackwell Publishing. The rest are presented as upper and lower ranges and adopted from *Baseline Hematology and Clinical Chemistry Values* (1986) Technical Bulletin, Charles River.

Serum Biochemistry

In addition to complete blood counts, we assessed biochemical changes in the serum to measure the toxicity of polylysine. Following both acute and subchronic polylysine administrations, the biochemical measurements revealed that the values for some parameters remained unchanged, but others exhibited a small range of variations in each group (Tables 3 and 4). Most of these changed values did

Table 2. Complete blood cell count for subchronic toxicity of polylysine.

	Units	Control		4 mg/kg/day (2 weeks)		4 mg/kg/2 days (4 weeks)		*p*-Value[a]	Ref. value[b]
		Mean	SD	Mean	SD	Mean	SD		
WBC	10^3/μl	7.93	0.40	10.13	0.12	5.63	0.21	<0.001	1–9.1
LYMF	%	78.33	1.84	77.07	0.31	78.60	0.20	n.s.	50–86
GRAN	%	13.60	1.40	15.57	0.47	13.73	0.12	0.050	10–43
MONO	%	8.07	0.57	7.37	0.25	7.67	0.31	n.s.	0–8
HCT	%	47.00	0.26	47.17	0.61	43.87	0.29	<0.001	36–48
MCV	fL	50.53	0.06	56.83	0.15	49.40	0.17	<0.001	69–85
RBC	10^6/μl	9.30	0.05	8.29	0.12	8.87	0.06	<0.001	4.7–8.0
HGB	g/dl	15.20	0.10	13.80	0.17	13.97	0.06	<0.001	12.6–16.6
MCH	pg	16.33	0.06	16.63	0.06	15.77	0.12	0.004	23–30
MCHC	g/dl	32.37	0.21	29.33	0.06	31.93	0.23	<0.001	31–39
RDW	%	19.30	0.17	20.47	0.23	19.63	0.12	<0.001	13 ± 0.9
MPV	fL	6.47	0.06	6.50	0.10	6.77	0.06	0.005	6 ± 1.1
Platelets	10^3/μl	1,040.67	6.43	923.33	36.12	770.00	9.85	<0.001	1,000–1,400

[a]*p*-Values represent the values among different groups that received different polylysine regimens.

[b]RDW and MPV reference values are presented in a form of mean ± standard deviation and adopted from Smith and Jarecki (2011), *Atlas of Comparative Diagnostic and Experimental Hematology*, 2nd Ed., Blackwell Publishing. The rest are presented as upper and lower ranges and adopted from *Baseline Hematology and Clinical Chemistry Values* (1986), Technical Bulletin, Charles River.

Table 3. Serum biochemistry for acute toxicity of polylysine.

	Units	Control		4 mg/kg/day (5 days)		8 mg/kg/day (5 days)		*p*-Value[a]	Ref. value[b]
		Mean	SD	Mean	SD	Mean	SD		
Alkphos	U/L	n.d.		n.d.		n.d.			86–246
AST	U/L	74.5	20.5	134.0	80.6	124.0		n.s.	24–472
ALT	U/L	54.0	49.3	56.0	53.2	23.0		n.s.	28–190
Amylase	U/L	1,557.3	239.9	1,499.3	79.7	1,192.5	433.5	n.s.	
BUN	mg/dl	16.3	1.4	13.2	2.1	24.5	14.5	n.s.	21–43
Glucose	mg/dl	281.3	49.4	325.3	95.9	72.5	77.1	0.035	185–320
Phos	mg/dl	13.3	1.3	12.6	3.8	13.0		n.s.	7.4–18.6
Calcium	mg/dl	9.9		<5.0		<5.0			8.4–12.4
Albumin	g/dl	3.4	0.4	3.1	0.2	2.9	0.1	n.s.	1.8–3.7
Chol	mg/dl	122.7	17.4	96. 7	13.3	136.5	37.5	n.s.	31–91
Uric acid	mg/dl	5.7	0.2	8.0	2.7	4.7		n.s.	
CK	U/L	206.0	148.5	517.5	501.3	434.0		n.s.	
Creatinine	mg/dl	0.3	0.2	0.4	0.2	0.4	0.1	n.s.	0.2–0.8
Bilirubin	mg/dl	0.1	0.0	0.1	0.0	0.1		n.s.	0–0.5
T.P.	g/dl	5.4	1.0	5.2	0.3	6.4		n.s.	3.0–5.6
Globulin	g/dl	2.0	0.8	2.1	0.2	3.4		n.s.	1.2–2.2
BUN/Crea		57.6	28.8	24.9	21.4	58.4	15.6	n.s.	53.8–115
Alb/Glob		1.9	0.7	1.4	0.1	0.9		n.s.	1.0–1.9

Some mean values are shown without standard deviations owing to insufficient numbers of samples to perform statistics.

[a]*p*-Values represents the values among different groups that received different polylysine regimens.

[b]The reference values are presented as upper and lower ranges and adopted from *Baseline Hematology and Clinical Chemistry Values* (1986), Technical Bulletin, Charles River.

not correlate with the administration of polylysine, and these values were within the normal range. In acute toxicity tests, no drastic changes occurred among values of each test parameter, and the decrease of the glucose level was proportional to the increase of the polylysine dose (p = 0.035) (Table 3). However, this observation was not consistently witnessed in mice that were given polylysine for an extended period (Table 4). In subchronic tests, although the changes in some parameters, such as aspartate aminotransferase, alanine aminotransferase, blood urea nitrogen, phosphorous, albumin, cholesterol, and creatinine kinase, were statistically significant (p = 0.001–0.03), altered values were near or within the normal ranges (Table 4). These data indicate that polylysine administration for a subchronic period does not induce toxic biochemical events and, thus, is tolerable in mice.

Discussion

Translation of our preclinical studies dealing with the anti-prion activity of polylysine [18] toward practical *in vivo* application requires detailed investigations in toxicological perspectives of polylysines. Because systematic, quantitative descriptions on polylysine toxicity in mice have not been reported, we conducted a series of tests on several parameters to fill such a deficiency. In assessing polylysine toxicity, we monitored the survival, weight loss, and changes in blood system integrity and serum chemistry of wild-type CD-1 mice. The data reported in this communication will contribute as guidance for the future pharmacological and toxicological studies of polylysine in mice.

Our quantitative measurements on the various aspects of polylysine toxicity support that polylysine is tolerable in mice under the conditions used for the study. The doses of polylysine employed in mice were determined by extrapolation from *in vitro* efficacy and cytotoxicity data described previously [18]. Since the minimum effective dose of polylysine for the complete elimination of prions and the maximum non-toxic dose of polylysine in ScN2a cells overlapped in the range of 2–10 μg/ml, two extrapolated dosages (4 and 8 mg/kg) of polylysine that fall within the aforementioned range were intraperitoneally delivered for

Table 4. Serum biochemistry for subchronic toxicity of polylysine.

	Units	Control		4 mg/kg/day (2 weeks)		4 mg/kg/2 days (4 weeks)		*p*-Value[a]	Ref. value[b]
		Mean	SD	Mean	SD	Mean	SD		
Alkphos	U/L	106.7	4.0	107.7	3.5	109.3	2.3	n.s.	86–246
AST	U/L	109.3	0.6	113.0	2.0	111.7	0.6	0.03	24–472
ALT	U/L	31.0	0.0	28.3	0.6	36.3	0.6	0.004	28–190
Amylase	U/L	1,706.0	26.2	1,767.3	29.9	1673.0	81.6	n.s.	
BUN	mg/dl	20.1	0.5	21.5	0.5	23.5	1.6	0.018	21–43
Glucose	mg/dl	335.7	5.1	322.3	6.7	322.0	17.7	n.s.	185–320
Phos	mg/dl	13.2	0.2	14.5	0.4	15.7	1.0	0.007	7.4–18.6
Calcium	mg/dl	13.3	0.5	12.9	0.4	13.8	0.9	n.s.	8.4–12.4
Albumin	g/dl	3.5	0.1	3.7	0.1	3.4	0.1	0.025	1.8–3.7
Chol	mg/dl	129.0	3.6	153.7	3.1	159.0	10.2	0.003	31–91
Uric acid	mg/dl	5.6	1.1	4.5	0.6	5.5	0.4	n.s.	
CK	U/L	481.4	3.5	566.7	4.9	515.7	1.5	0.001	
Creatinine	mg/dl	0.2	0.1	0.3	0.1	0.3	0.1	n.s.	0.2–0.8
Bilirubin	mg/dl	n.d.		n.d.		n.d.			0–0.5
T.P.	g/dl	5.6	0.1	5.7	0.2	5.5	0.1	n.s.	3.0–5.6
Globulin	g/dl	2.1	0.1	2.0	0.2	2.1	0.2	n.s.	1.2–2.2
Bun/Crea		85.2	25.2	88.7	26.0	91.0	20.9	n.s.	53.8–115
Alb/Glob		1.7	0.1	1.9	0.2	1.6	0.2	n.s.	1.0–1.9

Some mean values are shown without standard deviations owing to insufficient numbers of samples to perform statistics.

[a]*p*-Values represents the values among different groups that received different polylysine regimens.

[b]The reference values are presented as upper and lower ranges and adopted from *Baseline Hematology and Clinical Chemistry Values* (1986), Technical Bulletin, Charles River.

a short (5 days) or a subchronic (2–4 weeks) period. Prior to quantitative testing, the dosages used for the test were regarded as safe and tolerable in mice because the LD_{50} of polylysine with molecular masses of 35 and 70 kDa were determined to be 30–60 mg/kg in previous studies [1]. Since intravenously administered polylysine is cleared rapidly from circulation and targets mostly the liver of animals [8, 14], we chose an alternative, intraperitoneal route to minimize liver targeting and deliver polylysine to the target organs, such as the brain and spleen, in which prions replicate and accumulate. The accumulation of intraperitoneally delivered polylysine in the brain and spleen was confirmed by *ex vivo* imaging assays (Ryou, unpublished data).

During the short and subchronic periods of polylysine administration, mice in both the treated and control groups survived without any signs of toxicity. The food and water consumption and body weight of mice remained within the normal range. Despite that temporal hypoactivity was observed after polylysine injection, mice returned to normal routine behaviors. It is possible that transient discomfort near the ventral injection sites of mice may be responsible for such a temporal behavior change.

Our quantitative measurements by the complete blood counts followed by statistical analysis demonstrated an increase of granulocyte differential in the acute test and a decrease of platelet numbers in the subchronic test. The increase of granulocyte differential is suggestive of an inflammatory process that may be associated with chemical poisoning and other toxic states [22], but the validity of this phenomenon is obscure because it was an isolated case resulting from the acute test and this was not repeated in the subchronic test. The decrease of platelet numbers in the subchronic test suggests that a prolonged exposure of polylysine may cause abnormal bone marrow processing to decrease platelet production and could result in a blood clotting problem in mice. However, a low platelet count can also be caused by increased platelet destruction due to certain chemotherapeutic drugs or trapping platelets in the spleen [20]. Because red blood cells, platelets, and white blood cells arise in bone marrow, no drastic alteration of red and white blood cell populations in both acute and

subchronic tests proposes a possibility that polylysine is not associated with abnormal bone marrow processing. The elucidation of the mechanism that facilitates the polylysine-induced platelet decrease requires further investigations in the future.

The serum biochemistry test revealed the different levels in the subset of toxicity parameters, such as glucose, aspartate aminotransferase, alanine aminotransferase, blood urea nitrogen, phosphorous, albumin, cholesterol, and creatinine kinase. However, it was insufficient to address that polylysine is toxic, because the altered values were not different from the normal reference values. Thus, our serum biochemistry data suggest that there is no evidence that polylysine causes harmful effects in mice. Separately, the values of certain parameters obtained from the control group were completely off from the reference values, although they should correspond to one another. We speculate that this discrepancy is due to the intrinsic characteristics of the outbred strain, CD-1 mice. Thus, it is possible that the same CD-1 strain bred in the facility of different suppliers could be not completely identical. The CD-1 mice used in this study were from Harlan Laboratories Inc., whereas the reference data were based on CD-1 mice from Charles Rivers.

In conclusion, no differences in survival, weight gain, and the values of toxicity parameters in blood system integrity and serum chemistry suggest that the polylysine treatments described in this study did not contribute to toxicity in mice. However, the decrease of platelet numbers upon subchronic polylysine administration suggests a potential risk of polylysine treatments for extended periods.

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Abbreviations

WBC, total white blood cell count; LYMF, lymphocyte differential; GRAN, granulocyte differential; MONO, monocyte differential; HCT, hematocrit; MCV, mean corpuscular volume; RBC, total red blood cell count; HGB, hemoglobin concentration; MCH, mean corpuscular hemoglobin; MCHC, mean cell hemoglobin concentration; RDW, red cell distribution width; MPV, mean platelet volume; Alkphos, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; BUN, blood urea nitrogen; Phos, phosphorous; Chol, cholesterol; CK, creatinine kinase; T.P., total protein; S.D., standard deviation; n.d., not determined; n.s., not significant.

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