Journal of Scientific and Innovative Research 2014; 3(5): 475-477
Available online at: www.jsirjournal.com

Short Communication

ISSN 2320-4818
JSIR 2014; 3(5): 475-477

Received: 29-07-2014
Accepted: 09-09-2014

Neeraj Khatri
Department of Bioscience and Biotechnology, Banasthali University, Rajasthan, India-304022 & CSIR-Institute of Microbial Technology, Chandigarh, India-160036

Nagesh Peddada
CSIR-Institute of Microbial Technology, Chandigarh, India-160036

Bhupinder Singh Chopra
CSIR-Institute of Microbial Technology, Chandigarh, India-160036

Ashok Kumar Gupta
CSIR-Institute of Microbial Technology, Chandigarh, India-160036

Renu Garg
CSIR-Institute of Microbial Technology, Chandigarh, India-160036

Ashish
CSIR-Institute of Microbial Technology, Chandigarh, India-160036

Veena Garg
Department of Bioscience and Biotechnology, Banasthali University, Rajasthan, India-304022

Correspondence:
Dr. Veena Garg
Professor, Department of Bioscience and Biotechnology, Banasthali University, Rajasthan, India-304022
E-mail:
veenagargin2014@gmail.com

Plasma gelsolin levels in healthy mice as a function of age and gender

*Neeraj Khatri, Nagesh Peddada, Bhupinder Singh Chopra, Ashok Kumar Gupta, Renu Garg, Ashish, Veena Garg**

Abstract

Plasma gelsolin (pGSN) has been reported to decline in many diseases of humans as well as animals. However, it is prerequisite to know normal pGSN levels in healthy animals so as to determine the dose of pGSN need to be given exogenously for repletion of gelsolin by gelsolin replacement therapy. Therefore, the purpose of this study was to quantify levels of injury healing protein, pGSN in different age (1, 3, 6 & 12 months) and sex (Male and female) groups of C57BL/6N mice by Enzyme Linked Immunosorbent Assay (ELISA). Our results showed that average pGSN levels at one month of age were 150.46±6.91 µg/mL in male and 123.14±18.11 µg/mL in female mice. However, these levels increased with the advancement of age and were in the range of 169.16±19.52 µg/mL and 214.15±34.88 µg/mL in male and female mice respectively from 3-12 months of age. There was no significant difference in the pGSN values with respect to age as well as gender of mice. These precise values of pGSN can be used as referral values while planning for gelsolin replacement therapy in clinical cases in mice. Since, gelsolin has been hypothesized to be the prognostic marker of health; therefore, it is proposed to monitor pGSN levels during routine health checkups.

Keywords: Plasma gelsolin (pGSN), Prognostic marker, ELISA.

Introduction

Plasma gelsolin (pGSN) is one of the most abundant plasma protein in human plasma and is primarily involved in the clearance of actin filaments released into circulation upon cell necrosis.[1, 2] Different studies have shown that pGSN levels decrease by 20-50% in a wide variety of disease conditions, while a minimum threshold level of pGSN (≥25%) is essential for maintaining the normal physiology of the body.[3, 4] pGSN levels at time of hospitalization below the critical threshold has been associated with poor prognosis. A decline in the level of pGSN in an individual reflects an onset of cellular injury resulting either from a trauma, inflammation or infection, which has led us to the hypothesis that pGSN level could be a "novel general prognostic marker of human health and disease conditions".[4] However, in order to qualify to be used as prognostic marker of health, determination of pGSN levels in healthy animals at different ages and genders etc. is pre-requisite. The information about the precise numbers of pGSN in healthy subjects would be effective in determining the dosage of exogenous recombinant gelsolin to be administered to diseased animals. Therefore, in the present study, we mapped pGSN levels in healthy mice keeping in mind about the variation of age and gender.

Materials and Methods

Animals

Blood samples were collected from inbred C57BL/6N mice of different gender and age groups (1, 3, 6 and 12 months of age) at the same time for estimation of pGSN levels. In all groups,

6 mice were taken. The mice were housed under normal conditions in the Animal Facility of the IMTECH, Chandigarh. Animals had free access to a standard diet and water. The experiments on animals were done after approval of Ethics Committee of the Institute as per guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Plasma was separated from the blood samples to be used in ELISA for quantification of pGSN.

Generation of polyclonal anti-gelsolin antibodies

Recombinant human pGSN antibodies used in the present study were raised in rabbit and has been reported previously.[5]

Quantification of pGSN levels using ELISA

A standard protocol of indirect ELISA was employed to determine the pGSN levels in mice and rats of different gender and age groups (at 1, 3, 6 and 12 months of age). Polystyrene 96-well plates (BD- falcon) were coated overnight with plasma samples diluted in coating buffer (carbonate-bicarbonate pH 9.6) at 4°C. The plates were then blocked with BSA for blocking the non-specific binding sites. The plates were washed with PBST and were incubated again for overnight with 1:3000 of rabbit anti-gelsolin polyclonal antibody at 4°C. After incubation, plates were again washed five times with PBST and 1:5000 dilution HRP-conjugated anti rabbit IgG (Sigma) was added to each well. After 1hr of incubation at room temperature, plates were again washed and Tetra Methyl Benzidine (TMB, Thermo Scientific) was added in each well. Subsequently, reactions were stopped with 2M H_2SO_4. Absorbance in each well was measured at 450nm with a microplate ELISA reader (Lab Tech).

Statistical analysis

The results are expressed as Mean ± S.D. All statistical analyses were done using Student unpaired t-test. A value of $p<0.05$ was considered statistically significant.

Results and Discussion

Our results showed that average pGSN levels at one month of age were 150.46±6.91 µg/mL in male and 123.14±18.11 µg/mL in female mice. However, these levels increased with the advancement of age and were in the range of 169.16±19.52 µg/mL and 214.15±34.88 µg/mL in male and female mice respectively from 3-12 months of age (Figure 1). It was observed that pGSN levels at one month of age were comparatively lower than the levels at 3, 6 and 12 months of age in both male and female mice. However, this difference in pGSN values was not statistically significant with respect to age as well as gender.

pGSN regulate the activity of actin and is present abundantly in plasma, with concentration of ~200±50 µg/mL.[6] Several clinical studies have reported a decline of 20-50% in pGSN levels patients affected with acute injury and the outcome of the injury is influenced by the rate at which pGSN values come to the normal levels. The pGSN appropriation at local site of injury for binding and capping of circulating actin is the main reason of diminished pGSN values in diverse acute inflammatory conditions following tissue damage.[7-9] Gelsolin replacement therapy has been tried in mice and rats model of burn and sepsis and it has been shown that administration of recombinant human gelsolin can save the animal by almost 90% in comparison to control mice.[10]

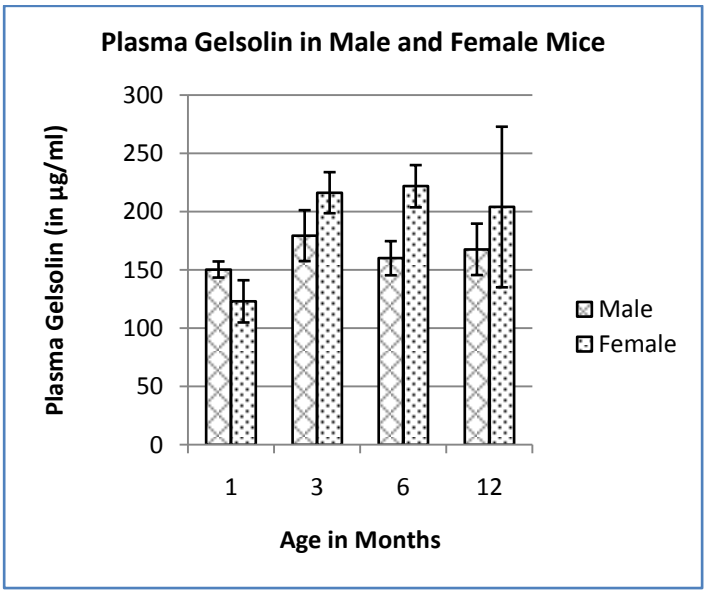

Figure 1: ELISA-based estimated values (Mean ± S.D) of pGSN in C57BL/6 male and female mice at different age group of mice (1, 3, 6 and 12 months of age of mice).

Pededda *et al.*, (2012) proposed a hypothesis describing gelsolin as prognostic marker of health as low levels of gelsolin have been reported in several pathological conditions. However, unless we know the precise levels of gelsolin in normal healthy animals of different age and sex group, it cannot be used as marker of health. Therefore, in this study we mapped pGSN levels in normal healthy animals. pGSN levels were initially low in both male and female mice at one month of age; however, with the increase in the age of mice, there was increase in the pGSN levels also. However, pGSN levels were little bit on higher side in case of female mice as compared to male mice at 3-12 months of age.

The awareness of pGSN levels in healthy animals will be valuable in deciding the dosage of gelsolin to be given exogenously for treatment. Based on pGSN levels of the diseased animals at the time of presentation for treatment, we can correlate the amount of gelsolin to be administered to bring gelsolin levels back to normal condition.

Conclusion

Our data showed that there is no variation in the pGSN levels at different age and gender of mice and normal gelsolin levels in healthy mice are in the range of ~ 170-215 µg/mL.

Acknowledgements

Authors acknowledge consistent support from faculty and staff of Department of Bioscience and Biotechnology, Banasthali University, Rajasthan and CSIR-IMTECH, Chandigarh.

References

1. Lee, W.M. and R.M. Galbraith, The extracellular actin-scavenger system and actin toxicity. N Engl J Med, 1992. 326(20): p. 1335-41.

2. Haddad, J.G., *et al.*, Angiopathic consequences of saturating the plasma scavenger system for actin. Proc Natl Acad Sci U S A, 1990. 87(4): p. 1381-5.

3. Lee, P.S., *et al.*, Relationship of plasma gelsolin levels to outcomes in critically ill surgical patients. Ann Surg, 2006. 243(3): p. 399-403.

4. Peddada, N., *et al*., Plasma gelsolin: a general prognostic marker of health. Med Hypotheses, 2012. 78(2): p. 203-10.

5. Peddada, N., *et al.*, Global shapes of F-actin depolymerization-competent minimal gelsolins: insight into the role of g2-g3 linker in pH/Ca^{2+} insensitivity of the first half. The Journal of biological chemistry, 2013. 288(39): p. 28266-82.

6. Yin, H.L. and T.P. Stossel, Control of cytoplasmic actin gel-sol transformation by gelsolin, a calcium-dependent regulatory protein. Nature, 1979. 281(5732): p. 583-6.

7. Thorstensson, R., *et al.*, Distribution of actin, myosin, actin-binding protein and gelsolin in cultured lymphoid cells. Experimental cell research, 1982. 140(2): p. 395-400.

8. Lind, S.E., *et al.*, Depression of gelsolin levels and detection of gelsolin-actin complexes in plasma of patients with acute lung injury. The American review of respiratory disease, 1988. 138(2): p. 429-34.

9. Rothenbach, P.A., *et al.*, Recombinant plasma gelsolin infusion attenuates burn-induced pulmonary microvascular dysfunction. Journal of applied physiology, 2004. 96(1): p. 25-31.

10. Lee, P.S., *et al*., Plasma gelsolin depletion and circulating actin in sepsis: a pilot study. PLoS One, 2008. 3(11): p. e3712.