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Liposomal glutathione as a promising candidate for immunological rheumatoid arthritis therapy

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ABSTRACT

Nano-medicine can passively accumulate in chronic inflammatory tissues via the enhanced permeability and retention phenomenon, or by being conjugated with a ligand that can bind to receptors over expressed by cells inside chronic inflammatory tissues, contributing to reduced systemic side-effects and increased efficacy. This article highlights the utilization of nanomedicine for potential treatment of rheumatoid arthritis. Rheumatoid arthritis was induced in rat model via 2 weeks intradermal injection of pristane at the base of the tail in a daily dose of 150 μl. Susceptible rat strains developed severe arthritis with a sudden onset 3 weeks post pristane injection. Three weeks post pristane administration; rats were treated intravenously with glutathione or liposomal-glutathione in a dose of 5 mg/kg daily for 30 days. Concomitant supplementation with the aforementioned antioxidants effect on proinflammatory marker C-reactive protein (CRP) was assessed. On the other hand, oxidative stress biomarker malondialdehyde (MDA) and rheumatoid factor (RF) compared with pristane treated group was also investigated. The results elucidated that glutathione and liposomal -glutathione significantly reduced rheumatoid factor, malondialdehyde and C-reactive protein levels with the superiority of liposomal -glutathione in this side reflecting its pronounced effect as anti-rheumatoid agent.

1. Introduction

A widespread understanding in the pathophysiology of chronic inflammatory autoimmune diseases, such as RA, affirms that the diseased tissue has high prevalence of macrophages, inflammatory mediators and necrotic monocytes lacking clearance via lymphatic system, which can become an inflammatory trigger in itself contributing to adaptive immune response [1, 2]. This can lead to boosting the delivery of nano-medicine. Nano-medicine can passively accumulate into chronic inflammatory tissues via the augmented permeability and retention phenomenon, leading to decreased systemic adverse-effects [1].

RA affects women three-times more than men and affecting 10% of the population in developing countries [3]. RA is characterized by synovial inflammation, which can lead to deformation, bone erosion in addition to loss of joint function. Other clinical manifestations include elevation in rheumatoid factor (RF), inflammatory markers (CRP, TNF-α and IL-6) in the blood, muscle soreness and joint tenderness [4].

Vast majority of inflammatory autoimmune diseases can be controlled, however not completely cured; NSAIDs and corticosteroids are the current available therapies for RA [5, 6]. NSAIDs are accompained with some side-effects as fluid retention, gastrointestinal bleeding and increased risk of heart disease. Corticosteroids in high-dose can cause gastrointestinal complications, glucose intolerance, liver toxicity and adrenal suppression [7, 8].

Nano-medicines are designed nowadays to target certain receptors, prevent the degradation of therapeutic agent, extend the time of retention in blood circulation and to be tailored for macrophage [9, 10].

The use of nanoparticles such as liposomal -glutathione increase site-specific drug targeting to inflamed tissues, by utilizing the disease state including, enhanced permeability or changes in pH of inflamed tissues.; thereby, allowing for maximum drug action with less adverse effect than traditional drug therapy [11, 12].

The focus of this study lies on the elucidation of the impact of liposomal -glutathione in comparison with glutathione on rheumatoid arthritis induced in rat model.

2. Material and methods

2.1. Chemicals

Pristane, glutathione and liposomal -glutathione were purchased from Sigma-Aldrich Co (St. Louis, MO, USA). ELISA kits for C-reactive

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protein and rheumatoid factor determination were provided from R & D systems (MN, USA). Kits used for the determination of malondialdehyde was obtained from Randox Company (UK). All other chemicals were of the highest analytical grade.

2.2. Animals

Forty male Wister albino rats, weighing 190–200 gm, obtained from the animal house of National Research Center were used in this study. Animals were housed in cages kept at standardized conditions (22 ± 5 °C, 55 ± 5% humidity, and 12 h light/dark cycle). They were allowed free access to water and pelleted standard chow diet.

All procedures relating to animal care and treatments strictly adhered to the ethical procedures and policies approved by Animal Care and Use Committee of National Research Center (AR110204), and complied with the Guide for Care and Use of Laboratory published by the US National Institute of Health.

2.3. Experimental design

After 1 week of acclimatization, animals were randomly divided into four groups (10 rats each) and were divided according to the following schedule:

Group 1: Animals were treated by saline (control group).

Group 2: Animals treated with pristane via 2 weeks intradermal injection at the base of the tail in a daily dose of 150 μl (0.04028 gm) in a total dose of 0.56gm which represent (2.8 gm/kg BW) and served as rheumatoid arthritis model [13].

3 weeks post pristane administration; rats were treated with glutathione or liposomal -glutathione [14, 15].

Group 3: pristane - treated animals were treated intravenously with glutathione in a dose of 5 mg/kg daily for 30 days [14].

Group 4: pristane - treated animals were treated intravenously with liposomal-glutathione in a dose of 5 mg/kg daily for 30 days [15].

2.4. Blood sampling

Animals were followed for any sign of sickness. At the end of the experimental period, rats were weighed, slightly anesthetized by ether and blood samples were collected from the sublingual vein. Sera were separated by centrifugation at 2555g for 10 min and were kept at − 80 °C for subsequent estimation of biochemical parameters. Animals were then sacrificed by cervical dislocation.

2.5. Measured parameters

2.5.1. Serum malondialdehyde (MDA) level

MDA expressed as thiobarbituric acid reactive substances was measured using diagnostic kits provided from Randox Company as previously described by [16].

2.5.2. Serum C-reactive protein and rheumatoid factor activities

The activities of C-reactive protein and rheumatoid factor were assayed using ELISA kits (R &D systems MN, USA) according to the manufacturer's instructions. The assays estimated the quantitative sandwich enzyme immunoassay technique. Specific antibodies were precoated onto the microplate. The standards, and samples were pipetted into the wells and C-reactive protein was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked secondary antibody specific for C-reactive protein and rheumatoid factor were added to the wells. Following color development, the assay was stopped, and the absorbance was read at 450 nm [17].

2.5.3. Statistical analysis

Data were expressed as means ±S.E.M. Statistical analysis was performed using Instat-3 computer program (Graph pad software Inc, San Diego, CA, USA). One way analysis of variance (ANOVA) by SPSS 12 program followed by Post hoc test was conducted. The level of significance was set at $p < 0.05$ using Tukey ¯s test.

Table 1
Effect of glutathione and liposomal glutathione on body weight following pristane administration.

Groups				
Parameter	Control	Pristane	Glutathione	Liposomal glutathione
Body weight	197.5 ± 2.5[a]	182.3 ± 1.8[b]	189.9 ± 1.7[c]	192.5 ± 1.65[d]

Data are expressed as means ±S.E.M (n = 10). P-value <0.05 is considered significant. Groups having the same letter are not significantly different, while those having different letters are significantly different from each other.

3. Results

3.1. Body weight coefficient

Body weight revealed a significant decrease post pristane treatment which was significantly increased post glutathione and liposomal glutathione treatment as represented in (Table 1).

3.2. Evaluation of clinical arthritis

Inflamed toes were observed every day; each inflamed one was given 1 point and up to 5 points for the affected 5 toes or the ankle and data was recorded in (Table 2).

3.3. Modulation of rheumatoid factor biomarker

A significant elevation in rheumatoid factor level was elucidated post pristane treatment by a percentage of 400% as compared with the normal value indicating a state of rheumatoid arthiritis (Fig. 1). Treatment with glutathione and nano-glutathione evidenced a significant reduction in RF level with a percentage of 200 and 140% respectively in comparison to animals treated with pristane with liposomal -glutathione showing the most significant effect.

3.4. Modulation of oxidative stress biomarker

Significant elevation in malondialdehyde level was elucidated post pristane treatment by a percentage of 180% as compared with the normal value indicating a state of severe oxidative stress (Fig. 2). Treatment with glutathione and nano-glutathione evidenced a significant reduction in MDA level with a percentage of 160 and 95% respectively in comparison to animals treated with pristane with liposomal -glutathione showing the most significant effect.

3.5. Modulation of inflammatory biomarker

Fig. 1 elucidated that, pristane treatment produced a significant

Table 2
Effect of glutathione and liposomal glutathione on clinical investigations following pristane administration.

Groups				
Parameter	Control	Pristane	Glutathione	Liposomal glutathione
Score of toe deformation	0[a]	56.4 ± 2.1[b]	29.2 ± 1.6[c]	20.3 ± 1.3[d]

Data are expressed as means ±S.E.M (n = 10). P-value <0.05 is considered significant. Groups having the same letter are not significantly different, while those having different letters are significantly different from each other.

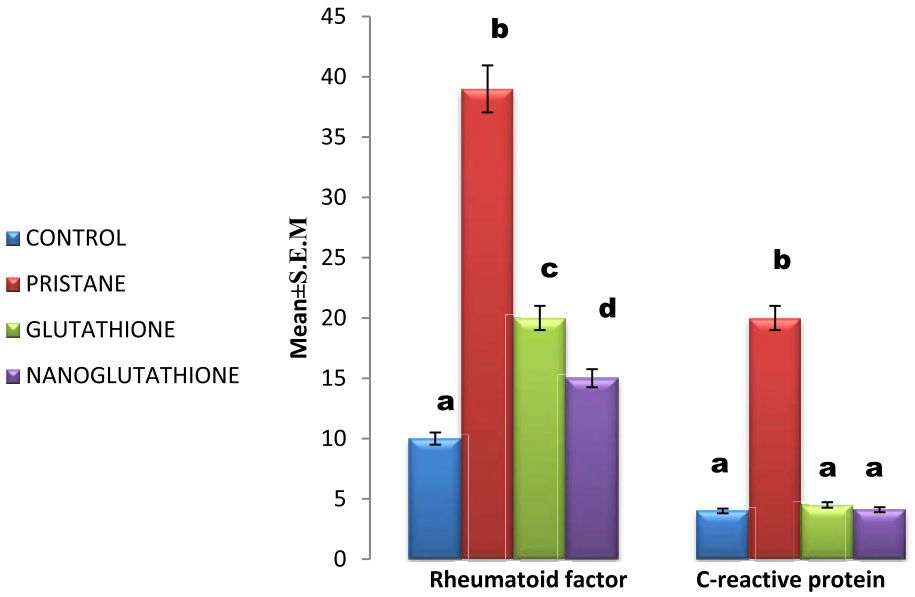

Fig. 1. Effect of glutathione and liposomal glutathione on serum rheumatoid factor and C-reactive protein following pristane administration. Data are expressed as means ±S.E.M (n = 10). P-value <0.05 is considered significant. Groups having the same letter are not significantly different, while those having different letters are significantly different from each other.

elevation in serum C-reactive protein level by a value of 500% as compared with the control value. On the other hand, there was a significant reduction in its level post glutathione and liposomal -glutathione treatment with a percentage of 112.5 and 102.5% respectively with liposomal -glutathione showing the most significant effect as compared with pristane treated group.

4. Discussion

A growing body of evidence contributes to nano-medicine application in the field of diagnosis, treatment or prevention of disease. Nano-medicines may include drug-loaded liposomes, nano-capsules and nanoparticles [9]. Nano-medicines were performed to: prevent the degradation of therapeutic agents, increase retention time and to be tailored for macrophage uptake or target certain receptors [18].

Over expression of inflammatory mediators in RA, contributed to increased tissue permeability. Moreover, inflamed tissues have more activated macrophages or other monocytes and changes in pH that can be utilized as targets for site-specific drug delivery systems as nano-carriers [19, 20].

In the current study, oxidative stress biomarker malondialdehyde (MDA) and RA biomarker rheumatoid factor (RF) were significantly elevated post pristane intoxication as compared with the control value. The results elucidated that liposomal-glutathione and glutathione significantly reduced rheumatoid factor and malondialdehyde levels with liposomal -glutathione reflecting the most pronounced effect.

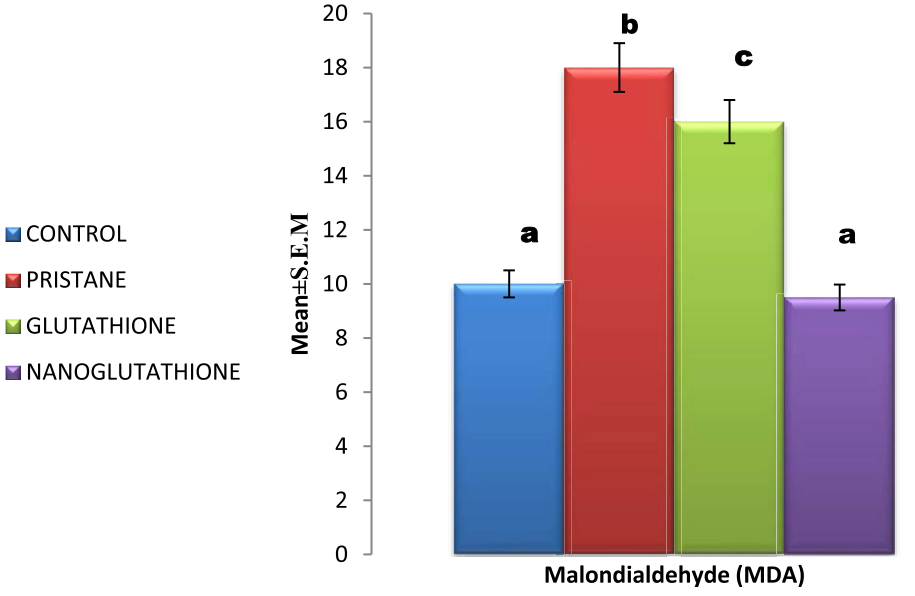

Fig. 2. Effect of glutathione and liposomal glutathione on serum malondialdehyde following pristane administration. Data are expressed as means ±S.E.M (n = 10). P-value <0.05 is considered significant. Groups having the same letter are not significantly different, while those having different letters are significantly differentfrom each other.

Exposure to pristane for 12 or 24 weeks promoted macrophage activation syndrome, characterized by hemophagocytosis in spleen and peripheral blood, as well as hypercytokinemia of IFN-γ, TNF-α, IL-4, and IL-6. In addition to a profound elevation in MDA and reduction in SOD, GSH, and catalase activities were also observed [21, 22].

In harmony, GSH is a good supplement for RA which can regulate immune cells preventing them from attacking the normal body cells. GSH can neutralize free radicals which can destroy tissues and muscles of the joints. It increases body energy and owns anti-inflammatory effect [23, 24].

Liposomal GSH is capable to bypass de novo glutathione synthesis contributing to elevating glutathione level, improving redox homeostasis and decreasing the effect of TGF-β [24, 25].

A significant elevation in C-reactive protein activity was elucidated in the present study meanwhile treatment with glutathione and liposomal glutathione was observed with liposomal glutathione elucidating the most significant effect.

Liposomal glucocorticoids elucidate local delivery and mounting in inflammatory sites, contributing to increased therapeutic efficiency and decreased systemic side-effects. Hofkens *et al* [26] showed that liposomal prednisolone phosphate reduced the proinflammatory macrophages activation *in vivo* [26, 27]. After macrophage uptake, significant reductions were seen in the expression of proinflammatory cytokines including CRP, TNF-α and IL-8 [26]. Selective biodistribution in inflamed tissues due to enhanced permeability and the resultant lower effective dose and longer duration of drug action leading to decreased dose frequency are a recurring theme with nano-medicines in RA models with less systemic side-effects [28].

5. Conclusion

Nano-glutathione may be a promising supplement for rheumatoid arthritis therapy and can improve oxidative stress as well as inflammatory markers. Further studies may be required and further investigations.

Declarations

Author contribution statement

Mai O Kadry: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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