# EFFECT OF MALARIA INFECTION AND DEXAMETHASONE ON SPLEEN MORPHOLOGY AND HISTOLOGY

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**Abstract.** The purpose of this study was to determine the histopathological changes of the spleen caused by parasite infection and steroid use to investigate pathological effects due to infection in ICR mice. The mice were divided into 5 groups: non-malaria infected mice served as controls, mice with parasite infection only, and the other three groups; mice that were injected with dexamethasone (Dex) only, mice injected with Dex prior to and mice injected with Dex after malaria inoculation. Differences in spleen color between the groups were found. Compared to controls, malaria infected mice, and those injected with Dex only were significantly different ($p < 0.05$) in spleen weights and sizes. Histological changes were also seen in these two groups. Fused white pulps were found in the spleens of mice infected with malaria only, clear zones of white and red pulp were observed in the spleens of mice treated only with Dex; fibrinoids were also found in this group. The histology of spleens appeared normal except for infiltration by numerous megakaryocytes in the spleens of mice given Dex before or after parasite inoculation. Infection with malaria and use of Dex leads to destruction of typical features of spleen morphology and histology. However, uptake of Dex after malaria infection seems to reverse the pathology of the spleen.

**Key words:** malaria, dexamethasone, spleen, histology

## INTRODUCTION

Malaria is a parasitic disease that is a major cause of death in the world. It is especially devastating to pregnant women, infants and children under age 5 years (Leeanne *et al*, 2008). The World Health Organization has reported there are 400-900 million new cases of malaria and approximately one to three million deaths annually (Greenwood *et al*, 2005). Malaria is usually found in tropical and subtropical countries, such as Thailand. It has been recognized as a public health problem. There has been an increase in the number of malaria cases in Thailand, especially along the borders with Myanmar, Cambodia and Malaysia (Renard-Singhanetra *et al*, 1986; Kamolratanakul *et al*, 1994; Thimasarn *et al*, 2006). A main problem of malaria is its resistance to drug treatment.

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This problem in Thailand has been due to the overuse of antimalarial drugs. The villagers self treat with drugs purchased from pharmacies for common colds, fatigue, and infectious diseases, such as malaria (Kamolratanakul *et al*, 1994). Some villagers use antimalarial drugs habitually since they believe it helps them to be able to work in the forest. Many of the drugs contain steroids, such as dexamethasone. Dexamethasone (Dex) is a member of glucocorticoid class of steroid hormones with antiinflammatory and immunosuppressive effects used to treat inflammatory and autoimmune symptoms. Dex has been used along with antimalarial therapy, to treat cerebral malaria for those with a coma for more than 24 hours, and appears to have a beneficial effect on survival (Kain and Keystone, 1998). Dex has also been used to treat histopathological changes, cerebral complications and death in malaria (Curfs, 1989). Dex at a dose of 0.5mg/kg combining with quinine has been used to reduce the severity of falciparum malaria infection (Hoffman *et al*, 1988). At present, the WHO recommends against the use of steroid in severe malaria, including cerebral malaria, since it has not been shown to improve overall outcomes and increases the risk of superimposed bacterial infection and gastrointestinal breeding. Dex may also decrease the efficacy of the immunological response. The spleen is a lymphoid organ that plays a role in human immunity, especially in the process of phagocytosis of the malaria pigment of the parasites (Chotivanich *et al*, 2002; Engwerda *et al*, 2005). A study of the use of steroid drugs is important in the setting of malaria infection to determine its effect on lymphoid organs and the pathology of the other organs. There is little information regarding the effect of steroid use on malaria and the pathology of the lymphoid organs. The purpose of this study was to investigate the morphological and histological changes in the spleen due to malaria, Dex and the use of Dex in malaria infection in a mouse malaria model.

## MATERIALS AND METHODS

Twenty-five 6 week old pathogen free female ICR mice weighing approximately 20 g each were obtained from the National Laboratory Animal Center, Mahidol University, Thailand and reared for at least one week on sterile, filtered water and a standard diet. The study was approved by the Ethics Committee on Animal Experimentation of the Faculty of Medicine Siriraj Hospital, Mahidol University. All mice were randomly divided into 5 groups; each group contained 5 mice. Group 1 was the untreated control group (C) in which the mice had been given only food and drinking water. Group 2 were mice inoculated with only *Plasmodium yoelii* infected erythrocytes (I). Group 3 were mice given 0.5 mg/kg of Dex for 20 days (Dex-only). Group 4 were mice given Dex for 20 days, then on Day 21, were inoculated with *P. yoelii* infected erythrocytes (Dex-BI). Group 5 were mice inoculated with *P. yoelii* infected erythrocytes first, then later injected with Dex (Dex-AI). *P. yoelii* infected erythrocytes were injected intraperitoneally at a dose of $1x10^6$ per mouse. The spleens of Group C and Dex-only mice were collected on Day 21, whereas the spleens of Groups I, Dex-BI and Dex-AI were collected when the percent parasitemia of each parasitized mouse reached 80%. Immediately after sacrificing, the colors of the spleens were observed and photographed and the weights, lengths and widths were recorded. The spleens were dissected after being fixed in 10% formaldehyde for 48 hours and subse-

Table 1
The average weight of spleens in grams. Each group, $n = 5$ and each value shows mean ± standard deviation (mean ± SD).

| Spleen | C | | I | | Dex-only | | Dex-BI | | Dex-AI | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Weight (grams) | 0.23 | 0.04 | 0.68[a] | 0.05 | 0.15[a] | 0.03 | 0.54 | 0.08 | 0.47 | 0.06 |

[a]Significant difference at $p < 0.05$

Table 2
The average length and width of spleens in millimeters. Each group, $n = 5$ and each value shows mean ± standard deviation (mean ± SD).

| Spleen | C | | I | | Dex-only | | Dex-BI | | Dex-AI | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Width | 4.4 | 0.06 | 9.8[a] | 0.08 | 2.4[a] | 0.06 | 6.0 | 0.07 | 5.6 | 0.05 |
| Length | 16.8 | 0.08 | 25.2[a] | 0.08 | 12.2[a] | 0.08 | 20.0 | 0.01 | 21.2 | 0.08 |

[a]Significant difference at $p < 0.05$

quently dehydrated in graded ethanol, cleared in xylene and later embedded in paraffin. The spleens were cut in serial sections 5 µm thick with a microtome and mounted on albuminized slides. The sections were then stained with hematoxylin-eosin. Finally, the slides were examined under light microscopy and photomicrographed. The weight, length and width of spleens were expressed as mean ± SD in grams and millimeters, respectively, and the differences of weights, lengths and widths among the samples were analyzed using the *t*-test. Differences were considered significant at $p < 0.05$.

## RESULTS

The colors of the spleens among the 5 groups appeared different. The Group C spleens appeared reddish-brown in color, Group I spleens appeared brownish-black, Group Dex-only spleens appeared pale-yellowish in color, and Groups Dex-BI and Dex-AI spleens appeared reddish-yellow with a slightly brownish color. Fig 1 shows the differences in spleen sizes. The mean spleen weights are shown in Table 1 and the mean lengths and widths are shown in Table 2. The spleen weights in Group I and Dex-only were different from Group C ($p < 0.05$), however Group Dex-BI and Dex-AI were not different from Group C. The spleen weights of Group I were significantly heavier than Group C spleens. The spleen weights of Group Dex-only were significantly lighter than Group C. Group I and Group Dex-only spleens were significantly different from Group C spleens ($p < 0.05$): Group I spleens were larger and Group Dex-only spleens were smaller than Group C spleens. However,

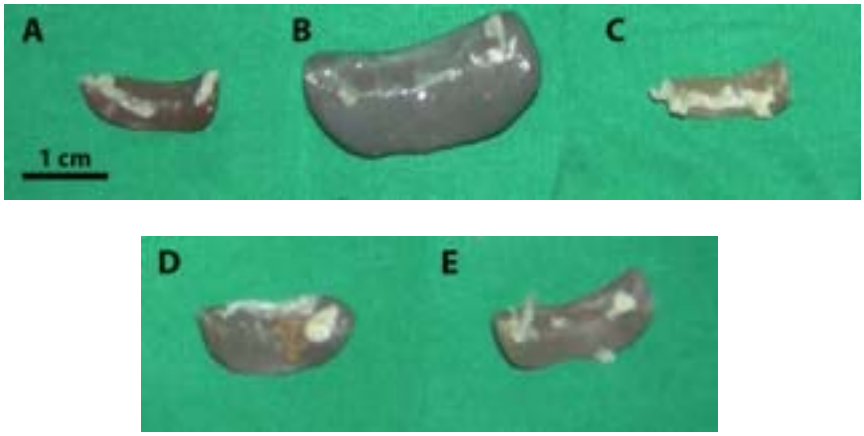


Fig 1–Spleen sizes. A is the representative spleen in Group C; B is the representative spleen in Group I; C is the representative spleen in Group Dex-only; D is the representative spleen in Group Dex-BI; E is the representative spleen in Group Dex-AI.

there were no significant differences on spleen size when comparing Dex-BI and Dex-AI with Group C spleens.

On histological examination (Fig 2), Group C spleens (Fig 2A, 2B) had normal spleen histology with clear white pulp (WP) zone and red pulp (RP) zone and a marginal zone (MZ) in between them. The sinusoids (S) arranged in cord-like structures had a normal appearance. In Group I (Fig 2C, 2D) the spleen zone were not clearly differentiated into WP, MZ and RP. No germinal centers were found in Group I sections. Degraded hemoglobin (malaria pigment or hemozoins) was found in the sinusoids of Group I, these were also distended. In Group Dex-only (Fig 2E, 2F), the WP, MZ and RP zone were organized, similar manner to C group, but on hematoxylin and eosin staining Group Dex-only was more reddish in color than in the Group C. Group Dex-only had pink fibrins or fibrinoids (FB) inside the sinusoids. Groups Dex-BI (Fig 2G, 2H) and Dex-AI (Fig 2I, 2J) had histological features common to both Groups C and Dex-only. Groups Dex-BI and Dex-AI had clear WP, MZ and RP zones and FB in the sinusoids. However, both Groups Dex-BI and Dex-AI had megakaryocytes (MK) present, especially in the RP zone, which is different from the other groups.

## DISCUSSION

The spleen is a secondary lymphoid organ, important for immunity and blood filtration and has been reported to decrease parasitemia (Alves *et al*, 1996). We observed differences in color among the spleens of the different groups of mice. Parasite infection and glucocorticosteroid use can cause changes in the physical appearance of the spleen. Dex used after infection, seems to cause a change in spleen appearance by reducing or treating spleen damage. Splenomegaly was seen only in Group I mice.

Histological changes were also seen under light microscopy. Hyperplasia of the RP and WP explains the increase in infected spleen size. Our results are con-

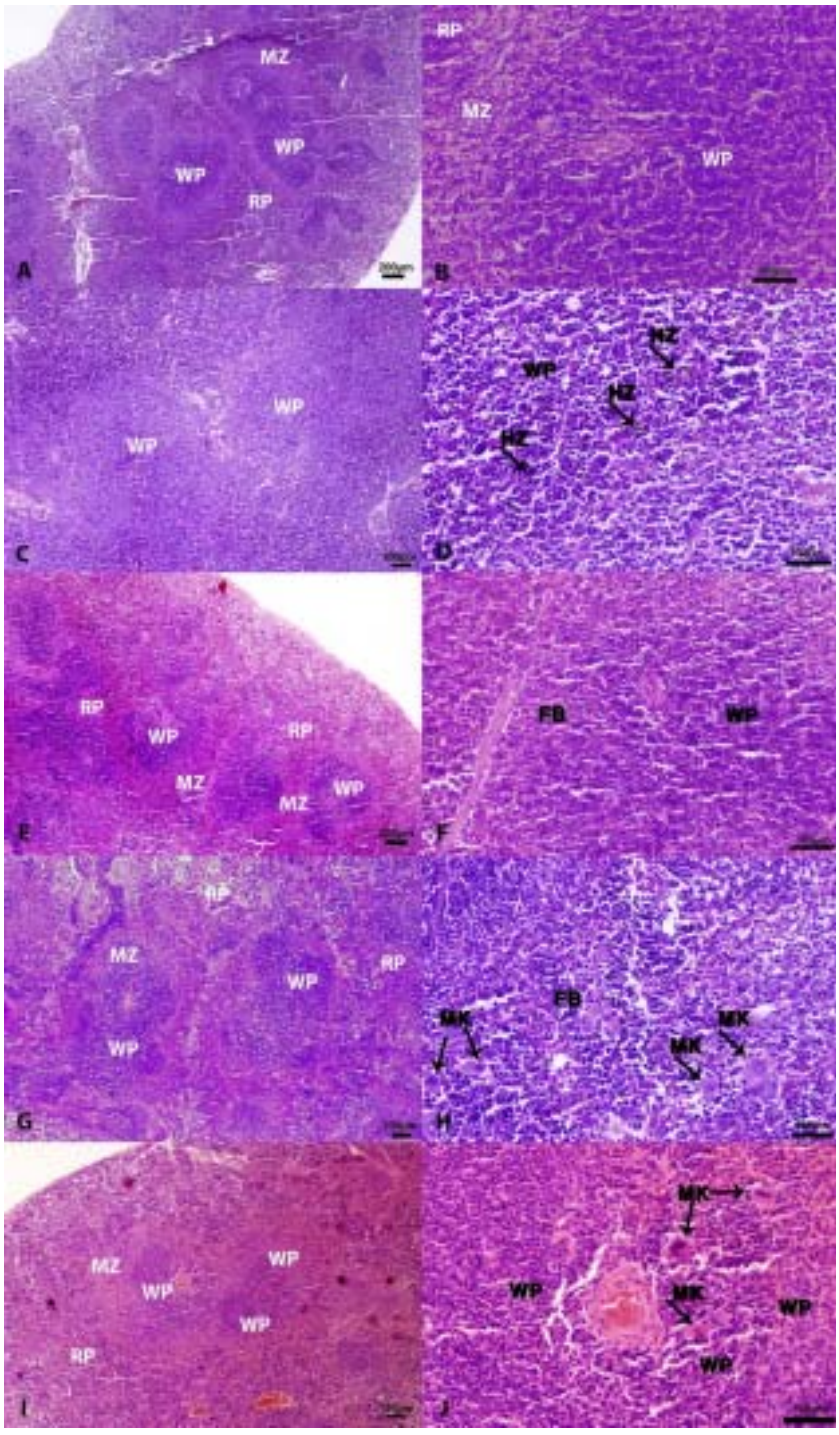


Fig 2–Histology of spleen sections. Spleen sections obtained from control mice (2A and 2B), Group I mice (2C and 2D), Group Dex–only mice (2E and 2F), Group Dex-BI mice (2G and 2H) and Group Dex-AI mice (2I and 2J) are shown. Each group is displayed at 4x and 20x magnification. WP, white pulp; RP, red pulp; MZ, marginal zone; HZ, hemozoin; FB, fibrinoid; MK, megakaryocyte.

sistent with previous reports (Carvalho *et al*, 2007; Dkhil 2009) showing splenomegaly in both *P. berghei* and *P. chabaudi* infected mice. They also reported a disappearance of the marginal zone, disorganized germinal centers and blurred red pulp. The smaller spleens seen in the Dex-only mice may have been the result of apoptosis of cells within the follicular. FB were observed in the sinusoids, suggesting accumulation of cells able to obstruct arterial supply, which could result in growth inhibition and spleen ischemia. No significant differences in weight and size between the Group Dex-BI and Group Dex-AI spleens were seen. Dex appears to act as a protective factor before or after parasite infection. This result may support a similar study (Goya *et al*, 2003) which reported glucocorticoids used to treat the spleen can reduce its weight by up to 31%. No germinal centers were found in any of investigated spleens. Before the experiment, we expected to find germinal centers in the infected spleens, since a germinal center is found because of antigen stimulation; in this case, malaria parasite antigens. However, no germinal centers were seen. This may be explained by the fact that parasite infected spleens may stop centroblast transformation. Infected spleens may be unhealthy and most centroblasts may be destroyed, therefore no germinal center is formed. These observations lead us to make the conclusion the B-cells in the spleens were defective. Long term use of Dex without infection also causes defective spleens due to B-cell defects. Smaller B-cells develop and transform into larger B-cells and then memory B-cells can function properly. Long term use of Dex could affect B-cell transformation by suppressing growth. The presented MK in Groups Dex-BI and Dex-AI spleens may the spleen is a site for hematopoiesis and malaria is associated with thrombocytopenia, thus stimulating the production of MK in the spleen. Dex may play a role in accelerating production of platelets. Our results show changes in morphology and histopathology of spleens infected with malaria and treated with Dex.

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