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Short Communication

Effect of Combination Songga-Wood-Stem (*Strychnos Ligustrina* Blume) And Antimalaria-Act on Il-10 Production of Malaria

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Abstract
Malaria is one of the deadliest infectious diseases in the world. Artemisinin-Based-Combination-Therapy (ACT) an antimalaria recommended by WHO, is starting to experience resistance in Asia. Songga-wood-stem having an immunoprotective effect is traditionally used as antimalaria in a certain community. IL-10 produced during malaria, gives an immunopathological-protection but interfere the ability to control *Plasmodium*-infection. Whether IL-10-production affected by both Songga-wood-stem and ACT together had not been studied. Objective of this study was to determine the effect of combination of ethanolic-extracted-Songga-wood-stem (EESWS) and ACT on IL-10, a protective-cytokine against malaria-immunopathology. This experimental-study used *post-test-only-randomized-controlled-group-design*. The thirty-*Swiss-webster*-mice were grouped into 5 groups. The one-group of healthy-mice (K1), and the four-groups infected with *Plasmodium berghei* ANKA (PbA) which were untreated-K2-group, ACT-treated-K3-group, EESWS-treated-P1-group, and EESWS-ACT-combination-treated-P2-group. IL-10-level of stimulated-splenocytes-culture was examined by ELISA-method. Data-analysis-used was *One-Way-Anova-Welch-test* and *Post-hoc Games-Howell.* P1 and P2-groups had higher IL-10-levels than K1 (p=0.038). Groups of P1 and P2 showed lower IL-10-levels than K3 (p=0.001). IL-10-level of P2-group was not different than P1 (p=0.135). The conclusion is the EEWS or EESWS-ACT-combination-therapy restrains the increase-splenic-IL-10-production above normal value in the healing phase of malaria infection.

Keywords: *ACT; IL-10; malaria; Plasmodium, Strychnos lucida*
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INTRODUCTION

Malaria, one of the deadliest infectious diseases in the world, is until now still a major problem on international and national scale.[1,2] WHO recommends *Artemisinin-Based-Combination-Therapy* (ACT) as the treatment of uncomplicated malaria caused by *P. falciparum*. The ACT-effectiveness, however, is starting to experience resistance in Asia.[3] One solution for antimalarial-treatment is to combine of natural-extract-ingredients and standard-antimalarial-drugs. This can potentially slow the occurrence of parasite-resistance to standard-antimalarial-drugs.[4] The ethanolic-extract of Songga-wood-stems (EESWS) in mice infected with *P.berghei* ANKA (PbA) showed strong antimalarial activity with an IC50 of 8,478 mg/Kg weight. This is reinforced by GCMS-analysis which proves that EESWS contains strychnine alkaloid compounds which are very active as antimalarials.[5]

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Songga-wood-plants in addition to having antimalarial activity, also have immunomodulatory-effect during malaria in mice-model. EEWS-adjuvant-administration has protective immunomodulatory-activity against severe-PbA-infections by an increase-splenic-immunoprotective-chemokines-CXCL12-production, without inhibiting the infection-control.[6] The various-immunomodulatory-benefits shown by Songga-wood-plants is expected to be immunoprotective in tackling various-malaria-immunopathological-responses. The immunoprotective-response is successful in controlling the infection without triggering the immunopathologies often associated with malaria, including severe-anemia and cerebral-malaria (CM).[7,8] The occurrence of malaria cases is often associated with an imbalance between the overproduction of pro-inflammatory cytokines and the response of anti-inflammatory cytokines.[7] IL-10 is an anti-inflammatory and an important-immune-regulatory-cytokine in the host.[6,9] It is expected that the EESWS-ACT-combination plays a protective role by inducing sufficient spleen-IL-10-production of PbA-infected-Swiss-mice.

MATERIALS AND METHODS

A *true-experimental*-study with *post-test-only-randomized-controlled-group-design* was approved by Health Research Ethical Committee Faculty of Medicine Universitas Diponegoro (Ethical Clearance No. 43/EC/FK-UNDIP/IV/2021). The research was carried out at the Experimental-Animal-Laboratory of Sultan-Agung-University (Unissula), the Integrated-Biomedical-Laboratory-Unissula and the GAKY-laboratory of the Faculty of Medicine, Diponegoro-University. This research used thirty-female-Swiss-mice, and detailed research-intervention-protocols were mentioned elsewhere.[6] Control-groups were K1, K2 and K3-groups; treatment-groups were P1 and P2-groups. The K1-group consisted of healthy-mice, and those of K2, K3, P1 and P2-groups were inoculated with PbA intraperitoneally. K2-group was without any treatment, and K3-group was given ACT. P1 and P2-groups were given EESWS, obtained from Maluku Province, and extracted using ethanol in UNISSULA-biomedical-laboratory, and ACT-EESWS-combination, respectively. The culture-supernatant of lipopolysaccharide (LPS)-stimulated splenic-cells was collected and measured for the IL-10-levels using IL-10-*Enzym-Linked-Immuno-Assay* (ELISA)-kit (Legend Max™, Biolegend Inc, USA).[10] Data analysis was used statistical software on a computer. Each data was tested for normality using *Saphiro-Wilk-test*. The *One-Way-Annova-test* was carried out to see a different mean between the five-research-groups. The magnitude of the difference in the mean between two-groups was further analyzed using the *Games-Howell Post Hoc Test* for IL-10. The significant difference was indicated by $p < 0.05$.

RESULTS

The normality-test showed that the splenic-IL-10-production-data in each group was normally distributed ($p>0.05$). *Levene's-homogeneity-test* showed that the five-groups had different-data-variations ($p<0.001$), and *one-way-ANOVA-Welch-Test* showed a significant different among those-groups ($p<0.001$). The different-mean between two-experimental-animal-groups was then analyzed using the *Post-Hoc-Games-Howell-test* (Table). The splenic-IL-10-production of P2 and P1-groups was not different ($p=0.135$). P1 and P2-groups showed higher-splenic-IL-10-production than K1 and K2-groups, although significant-differences were only found between the treatment-groups (P1 and P2) and K1 ($p=0.001$ and $p=0.038$). P1 and P2-groups showed a significantly-lower-splenic-IL-10-production than K3-group ($p=0.001$ and $p=0.026$). IL-10-production in K3-group was significantly higher than K1 and K2 ($p=0.001$), while there was no difference between K1 and K2 ($p=0.619$).

Table. Gamess-Howell post-hoc-test of spleen-IL-10-production

Group	mean±SD (pg/ml)	P Value			
		K2	K3	P1	P2
K1	29.48 ± 4.89	0.619	0.001*	0.001*	0.038*
K2	55.43 ± 38.99		0.001*	0.173	0.054
K3	550.00 ± 82.00			0.001*	0.026*
P1	106.37 ± 3.51				0.135
P2	282.54 ± 143.76				

*significant, $p<0.05$

DISCUSSION

The splenic-IL-10-production in the EESWS-treated-P1 and EESWS-ACT-treated-P2-groups was significantly higher than the healthy-control-K1-group (Table). The culture-supernatants of this study were obtained from a previous-study showed the no difference of parasitemia-levels among groups of mice treated with EESWS-treated-P1, EESWS-ACT-treated-P2 and ACT-treated-K3-groups, and these three-groups showed significantly-lower-parasitemia-levels than the PbA-infection-control-K2-group which did not receive any therapy.[6] These together indicate that the EESWS and the EESWS-ACT-combination are associated with an increase-IL-10-production above normal during the PbA-infection-recovery-phase. The EESWS and the EESWS-ACT-combination are associated with normal-spleen-production of CXCL12, a chemokine that increases IL-10-production, in the PbA-infection-recovery-phase.[6] Other mediators, therefore may involve in the increase in IL-10-production. It worthy of note was that the highest IL-10-production among the groups was observed in the PbA-infected-ACT-treated-K3-group, and the differences were significant (Table). A significantly lower IL-10-production in the P1 and P2-groups than K3-group indeed was noticed. This indicates that either EESWS or EESWS-ACT-combination-treatment associates with a restricted-IL-10-elevation in the malaria-recovery-phase. The PbA-infection prevents the increase-anti-inflammatory-cytokine-production. The

PbA-infected-control-K2-group and the healthy-control-K1-group showed no different IL-10-production (p = 0.619; Table). This was in accordance with the finding that the spleen-CXCL12-production in the K2 and K1-groups was not different.[6] These indicate that the day7-PbA-infection associates with inhibition of a significant increase in spleen-IL-10 and CXCL12-production.

ACT was associated with the increase-splenic-IL-10-production in the recovery-phase of PbA-infected-Swiss-mice (Table). The inhibition-IL-10-production of ACT on PbA-infection is thus not proven. ACT reduces the proportion of IL-10-producing-Th2-cells in autoimmune-experimental-animals.[11] ACT therefore might have different-effect on the different-diseases. Interestingly, IL-10 protects the severity of the immunopathology of *Plasmodium*-infection, but IL-10 inhibits the control of *Plasmodium*-infection and the recurrence of parasitemia. Research is needed to prove whether the recurrence of parasitemia can be protected by EESWS treatment or the EESWS-ACT-combination.

CONCLUSION

The EESWS-ACT-combination or EESWS alone might constrain the increase-splenic-IL-10-production above normal in Swiss mice in the recovery phase of PbA-infection.

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