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NOTES

Dose dependency of prednisolone on the establishment of *Echinococcus multilocularis* infection in an alternative definitive host, Mongolian gerbil.

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Abstract

This study revealed the dose dependency of prednisolone tertiary-butylacetate (PTBA) treatment on the establishment of *Echinococcus multilocularis* in the small intestine of Mongolian gerbil *(Meriones unguiculatus)* and that some of the physiological parameters of host were correlated with the doses of PTBA and establishment of the worm. Twenty Mongolian gerbils were divided into 5 groups, according to the doses of PTBA; 0 mg, 0.5 mg, 2 mg, 5 mg and 10 mg per head. All animals were injected intraperitoneally with PTBA every other day from 6 days before to 6 days after infection. Doses of PTBA and the number of worms recovered at 7 days post-infection showed a positive correlation ($r=0.929$, $P<0.0001$). The increase of total protein (TP) and the decrease of the percentage of lymphocytes in the peripheral leukocytes were dependent on doses of PTBA (TP : $r=0.811$, $P<0.0001$, percentage of lymphocyte: $r=-0.92$, $P<0.0001$). The TP and the percentage of lymphocyte also correlated with the number of worms recovered (TP: $r=0.617$, $P=0.0049$; percentage of lymphocyte: $r=-0.800$, $P<0.0001$).

Key word: *Echinococcus multilocularis*, Mongolian gerbil, prednisolone tertiary-butylacetate

The natural definitive host of *Echinococcus multilocularis* is canine carnivores and many species of rodents play a role as the intermediate host. *E. multilocularis* is one of the most important zoonotic parasites in Japan and experimental infection of *Echinococcus* species in dogs accompanies a potential high risk for laboratory workers and requires special facilities for biohazard which are not easily available. Kamiya and Sato[6,7] succeeded in normal strobilation and egg production of E. multilocularis in golden hamsters and Mongolian gerbils *(Meriones unguiculatus)* treated with prednisolone tertiary-butylacetate (PTBA). It enables us to perform experimental infections of the tapeworm stage with minimum facilities for biosafety. The alternative definitive host models have been established for *Taenia crassiceps*

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using golden hamsters[14] and *T. solium* using chinchillas[10]. All of the models used glucocorticoids for requisite treatment, but the mechanism of the glucocorticoids in the establishment of the parasite has not yet elucidated, although the usefulness of such models has been supported by some researches[11,13]. The immunological and physiological change in rodents by PTBA treatment have not been known. In our previous study using this model for *E. multilocularis,* the number of worms recovered showed high variation among the host individuals. The variation may be due to the difference of susceptibility in Mongolian gerbils to PTBA treatment. Various side effects of PTBA has been known besides the anti-inflammatory effect[2]. On the peripheral blood, the reduction of the percentage of lymphocyte and the change of protein metabolism have been reported[2,5]. In the present study, the correlations were analyzed between the doses of PTBA treatment, the establishment of *E. multilocularis* and the selected physiological parameters, hosts total protein (TP) and percentage of lymphocytes in the peripheral leukocytes using Mongolian gerbils.

Twenty females, 14-19 weeks old Mongolian gerbils were raised in our laboratory. The animals were divided into 5 groups according to doses of PTBA (Codelcortone-T. B. A. ®, Banyu, Japan; 20 mg PTBA/ 1 ml solvent contained benzyl alcohol 9.2 mg, polysorbate 80 1.0 mg and 70 % D-sorbitol 0.5 ml); 0 mg (solvent only; 4 animals), 0.5 mg (4 animals), 2 mg (4 animals), 5 mg (4 animals) and 10 mg per head (4 animals). The solution was adjusted into 0.5 ml with the solvent and injected intraperitoneally to each animal every other day from 6 days before to 6 days after infection. The administration schedule of PTBA was based on Kamiya and Sato[6]. The animals were fed and supplied with water *ad libitum.* Protoscoleces of *E. multilocularis* used were originally isolated from a red backed vole *(Clethrionomys rufocanus bedfordiae)* captured in Tobetsu, Hokkaido, Japan in 1992 and kept in our laboratory by intraperitoneal passage in Mongolian gerbils. All animals were orally inoculated with 10,000 protoscoleces per head. The autopsy was performed under anesthesia with diethylether at 7 days post-infection. Peripheral blood samples were collected during autopsy from all animals. Sera were separated from blood and the TP was measured by protein refractometer (Erma, Japan). The percentage of lymphocytes in peripheral leukocytes was calculated in the Giemsa-stained blood smears. The small intestines were removed from the carcasses, opened and depressed using Petri dishes. The number of worms in the small intestine was counted under stereo-microscope.

The number of worms recovered at 7 days post-infection were 0 in untreated group, 0 - 4 (average ± S.D.; 1.5 ± 1.66) in 0.5 mg treated group, 4 - 120 (40.3 ± 47.56) in 2 mg treated group, 21 - 328 (199.5 ± 249.35) in 5 mg treated group and 52 - 1,111 (641.0 ± 379.39) in 10 mg treated group (Figure 1). The doses of PTBA and the number of worms recovered showed a positive correlation ($r = 0.747$, $P = 0.0002$). Both physiological parameters of peripheral blood, TP and the percentage of lymphocyte also correlated with the dose of PTBA (Fig. 2, 3; TP, $r=0.811$, $P<0.0001$, percentage of lymphocyte, $r=-0.920$, $P<0.0001$).

The effects of synthetic glucocorticoids are not only immunosuppression, but are wide and complex. The effects of glucocorticoid include the alteration of the metabolism of proteins, carbohydrates, lipids and minerals in various tissues[9,12]. The TP in the present study may not indicate only the protein content in serum, because the increase of lipids is also caused by glucocorticoid treatment and the refractive index of serum may have changed [2,12]. This TP

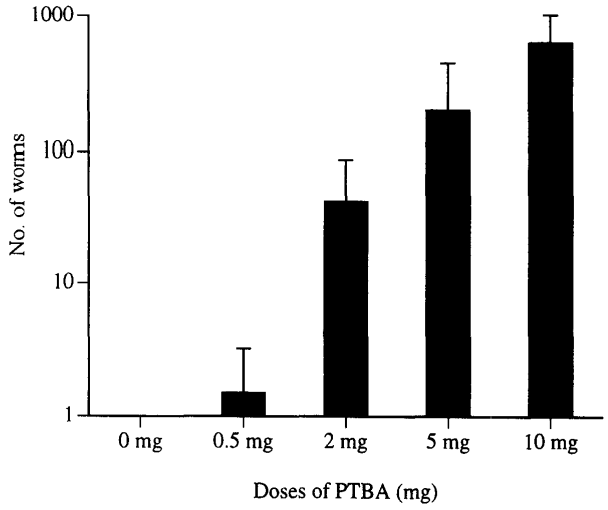

Fig 1. The number of worms recovered at 7 days post-infection from the small intestine of Mongolian gerbils orally inoculated with 10,000 protoscoleces of *Echinococcus multilocularis* and treated with various doses of PTBA . All data represent average ± S.D. for 4 animals.

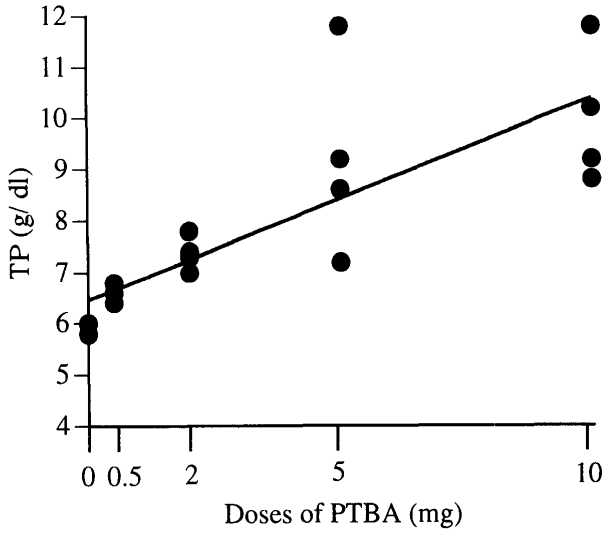

Fig 2. The correlation of PTBA doses and total protein (g/ dl) in sera from Mongolian gerbils orally inoculated with 10,000 protoscoleces of *Echinococcus multilocularis* and necropsied at 7 days post-infection. $Y = 0.395X + 6.454$, $r = 0.811$.

index may be related with the various changes as a result of PTBA treatment. Lymphocytes have many glucocorticoid receptors and show sensitive reaction to glucocorticoid treatment[2].

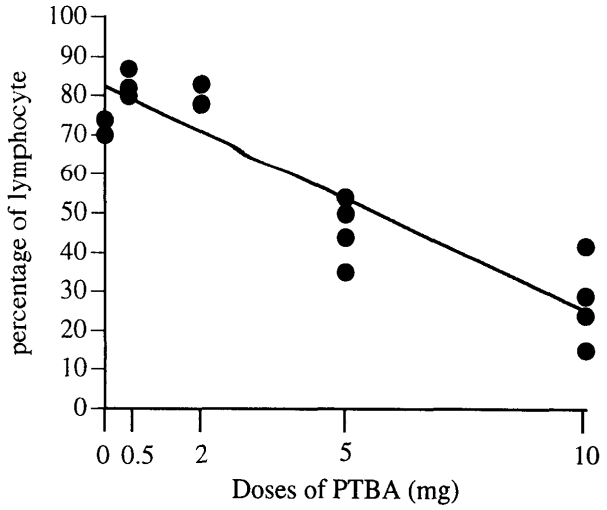

Fig 3. The correlation of PTBA doses and the percentage of lymphocyte in peripheral leukocytes from Mongolian gerbils orally inoculated with 10,000 protoscoleces of *Echinococcus multilocularis* and necropsied at 7 days post-infection. $Y = -5.712X + 82.38$, $r = -0.920$.

Intraperitoneal and subcutaneous injection of high dose of cortisol (5 mg/ 100 g of body weight) to adult rats resulted in a reduction in total protein content of the small intestinal mucosa and in maltase and alkaline phosphatase of the small intestine[9]. Administration of glucocorticoid inhibited the active transport of calcium in the small intestine of rat[8]. In addition, the low doses caused alteration in the morphology of the small intestine, which included reduction of villous height and crypt depth, decrement in cell production rates and the crypt population[15,16]. These effects of glucocorticoids are known to be dose-dependent[9], and those dose-dependent changes of the small intestine may have influenced the attachment and development of the worm. The influence of the present biological parameters to the establishment of worms remains to be understood. In present study, the correlation was recognized in the number of worms recovered with the increase of TP and the decrease of percentage of lymphocyte (Fig. 4, 5; TP, $r=$

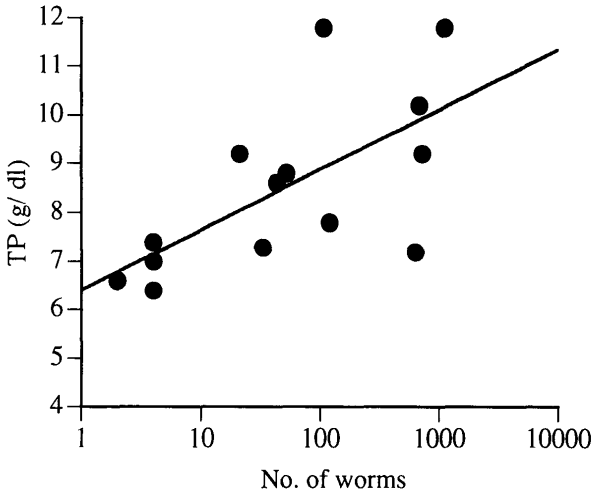

Fig 4. The correlation of the number of worms recovered and total protein (g/ dl) in sera from Mongolian gerbils orally inoculated with 10,000 protoscoleces of *Echinococcus multilocularis*, treated with various doses of PTBA and necropsied at 7 days post-infection. Y =0.003X+7.272, r=0.617.

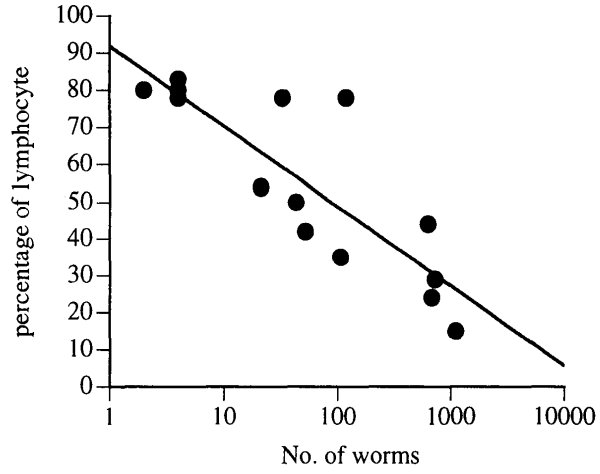

Fig 5. The correlation of the number of worms recovered and the percentage of lymphocyte in peripheral leukocytes from Mongolian gerbils orally inoculated with 10,000 protoscoleces of *Echinococcus multilocularis*, treated with various doses of PTBA and necropsied at 7 days post-infection. Y = −0.056X+71.077, r=−0.800.

0.617, P=0.0049, lymphocyte, r=−0.800, P < 0.0001). It means that the number of worms could be estimated using those parameters and individuals which are suitable for the alternative definitive host can be selected.

The Mongolian gerbil had been used for alternative hosts of various parasites, however, the biological and physiological characteristics of this animal remains to be clarified. Epilepsy seizure-prone and seizure-resistant strains of Mongolian gerbil have been established[3]. The recovery rate of worms in the present model showed high individual variations in Mongolian gerbils. This similar phenomenon have also been observed in dogs infected with *E. granulosus*[4]. Beagle dogs were more resistant to *E. granulosus* infection than Border collies[1]. Conversely, it suggests that the suitable strain of gerbil could be established for *Echinococcus* infection. The physiological parameters in the present study will be useful indexes to avoid individual variation in the alternative definitive host model for *E. multilocularis*.

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