

TRICHOSTATIN A, A POTENTIAL DRUG FOR TREATMENT OF ANIMAL *BABESIA* INFECTIONS

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ABSTRACT

In the present study, we evaluated the inhibitory effect of trichostatin A on the asexual growth of bovine, equine, and canine Babesia parasites in vitro as well as on the in vivo growth of Babesia microti (B.microti) in mice. The growth of Babesia bovis (B.bovis), Babesia bigemina (B.bigemina), Babesia caballi (B.caballi), Theileria equi (T.equi), and Babesia gibsoni (B.gibsoni) species was significantly inhibited ($P < 0.05$) by very low concentrations of trichostatin A (IC_{50} values = 2.6, 2.4, 2.3, 2.4, and 2.3 nM, respectively). Furthermore, in B.microti-infected mice, trichostatin A caused significant higher ($P < 0.05$) inhibition of the growth of B.microti at the dose of 2 mg/kg body weight than that in the control group. These results indicated the trichostatin A might be a chemotherapeutic agent for treatment of babesiosis.

KEY WORDS: Chemotherapy; *in vitro*; *in vivo*; parasites.

INTRODUCTION

Babesia is an intraerythrocytic protozoan parasite that is transmitted to animals by ticks and babesiosis is also important with regard to zoonotic diseases. Recent our studies have shown that the use of histone deacetylase inhibitor, apicidin, can inhibit enzyme activity of recombinant *B.bovis* histone deacetylase protein (1) and the growth-inhibitory effect on *Babesia*

parasites both *in vitro* and *in vivo*. (2). Trichostatin A is one of inhibitors of histone deacetylase and has a broad spectrum of epigenetic activities. Therefore, the present study focuses on evaluation of the efficacy of Trichostatin A against *B.bovis* and *B.bigemina* for cattle, *B.caballi* and *T.equi* for horses, *B.gibsoni* for dogs *in vitro*, and *B. microti* for mice and humans *in vivo*.

MATERIALS AND METHODS

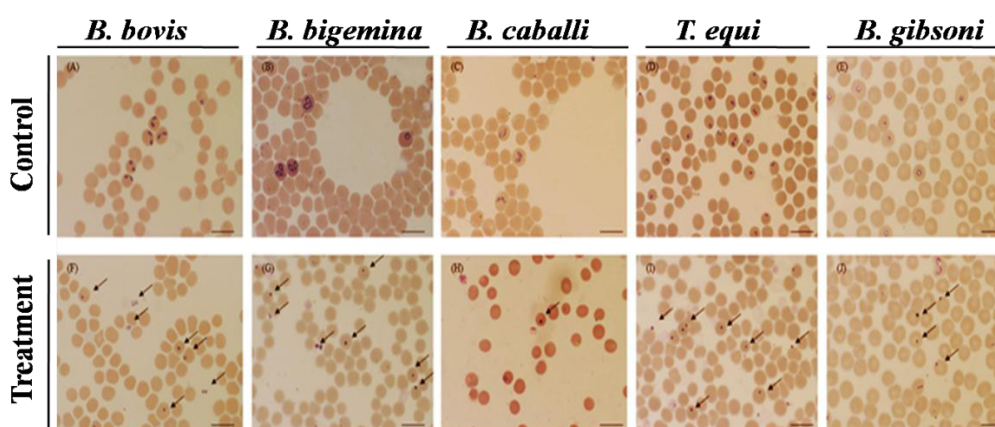
Inhibitory effects of Trichostatin A upon the *B.bovis*, *B.bigemina*, *B.gibsoni*, *B.caballi* and *T.equi* growth were examined as previously described (2 and 3). The *in vivo* growth inhibition assay for

Trichostatin A (2 mg/kg) was performed in BALB/c mice as previously described (4).

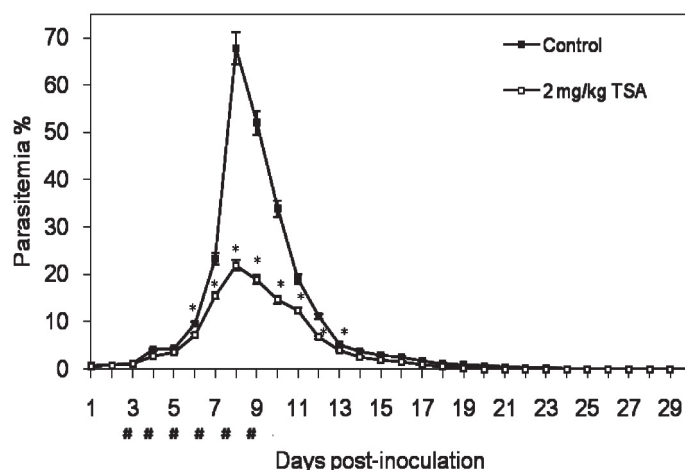
RESULTS

The growth of *B.bovis*, *B.bigemina*, *B.caballi*, *T.equi*, and *B.gibsoni* was significantly inhibited ($P < 0.05$) by Trichostatin A at concentration of 5 nM. In the presence of 25 nM of Trichostatin A, the growth of all parasites was completely suppressed on day 4. Subsequent viability tests showed that there was no re-growth of the five species of parasites at concentration of 5 nM for Trichostatin A. Moreover, Trichostatin A affected the morphology of *B.bovis*, *B.bigemina*, *B.caballi*, *T.equi*, and *B.gibsoni* parasites in the treated cultures.

To examine the effects of Trichostatin A on *B.microti*, infected mice were treated with Trichostatin A. In the treated group, levels of parasitemia were significantly lower than the control group ($P < 0.05$). Peak parasitemia reached an average of 21.9 % in treatment with the dose of 2 mg/kg, while in the control group it was 67.8 % (Dimethyl sylfoxid) at 8 days after inoculation. There were significant differences ($P < 0.05$) between the control and treated groups observed at days 6-13 post-infection.



(Figure1. Morphological changes of trichostatin A-treated *Babesia* parasites. Severe morphological changes in 5 nM trichostatin A-treated *B.bovis* (F), *B.bigemina* (G), *B.caballi* (H), *T.equi* (I), and *B.gibsoni* (J) after 3-day cultivation in comparison to the controls ((A) for *B.bovis*, (B) for *B.bigemina*, (C) for *B.caballi*, (D) for *T.equi*, and (E) *B.gibsoni*) are shown by arrows. Bar, 10 µm.)



(Figure 2. Inhibitory effects of trichostatin A given intraperitoneally at the dose of 2 mg/kg on the *in vivo* growth of *B.microti* for observations of 5 mice per experimental group. Each value represents the mean ± standard deviation for 2 experiments. Asterisks indicate a significant difference (Student's *t* test; $P < 0.01$) from days 3 to 13 post-inoculation between the trichostatin A-treated and dimethyl sylfoxid, control group.)

DISCUSSION

In this study, we conducted the first investigation of the *in vitro* and *in vivo* growth inhibition activity of histone deacetylase inhibitor, Trichostatin A, against bovine, equine, canine, and murine *Babesia* parasites. Exposure to higher concentrations of Trichostatin A completely suppressed the growth of bovine, equine, and canine *Babesia* parasites in *in vitro* cultures. Because treatment only with Dimethyl sulfoxid had no effect on parasitic growth, this growth inhibition of parasites was certainly due to effects of Trichostatin A.

Since Trichostatin A inhibited the growth of *in vitro*-cultured *Babesia* parasites, we evaluated its inhibitory effect in an *in vivo* model of *B. microti* in BALB/c mice. No sign of toxicity was observed on

treated mice. *B. microti* was suppressed in mice treated with Trichostatin A for 6 days at the dose of 2 mg/kg, with 78.1 % inhibition on day 8 p.i. This result is in agreement with our previous study that apicidin inhibited the growth of murine *Babesia* parasite *in vivo* (2). Therefore, inhibitors of histone deacetylase such as Trichostatin A and apicidin at low doses are not toxic to mice and might be used for the safe treatment of babesiosis. In conclusion, our present findings showed that the efficacy of Trichostatin A on the growth of *Babesia* parasites *in vitro* and *in vivo* with the absence of any toxic side effects for the first time. Therefore, Trichostatin A might be an excellent target for the development of novel anti-babesial agents.

REFERENCES

1. T. Munkhjargal, M. Aboulaila, A. Ueno, T. Sivakumar, Y. Nakano, M. Yokoyama, N. Yokoyama, and I. Igarashi. Cloning and characterization of histone deacetylase from *Babesia bovis*. *Veterinary Parasitology*. 190(3-4), 423-433. Dec, 2012a.
2. T. Munkhjargal, M. Aboulaila, T. Sivakumar, N. Yokoyama, and I. Igarashi. Inhibitory effect of apicidin on *in vitro* and *in vivo* growth of *Babesia* parasites. *The Journal of Protozoology Research*. 19, 42-49. Jan, 2009.
3. T. Munkhjargal, T. Sivakumar, B. Badgar, T. Nyamjargal, B. Purevtseren, D. Bayarsaikhan, Aboulaila, M. A. Terkawi, B. Byambaa, N. Yokoyama, and I. Igarashi. Prevalence and genetic diversity of equine piroplasms in Tov province, Mongolia. *Infection, Genetics and Evolutions*. 14, 178-185. Feb, 2013.
4. T. Munkhjargal, M. Aboulaila, M. A. Terkawi, T. Sivakumar, I. Madoka, B. Davaasuren, T. Nyamjargal, N. Yokoyama, and I. Igarashi. Inhibitory effect of pepstatin A and mefloquine on the growth of *Babesia* parasites. *American Journal of Tropical Medicine and Hygiene*. 87(4), 681-688. Oct, 2012b.