Form No.5

Abstract of Thesis/Dissertation

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Title : Repositioning of orally administered compounds for the treatment of African trypanosomosis

(ドラッグ・リポジショニングによる新規トリパノソーマ病経口治療薬開発に関する研究)

African animal trypanosomosis (AAT) is a devastating disease of animals caused by *Trypanosoma congolense, T. brucei brucei, T. evansi*, and *T. vivax*. The disease is endemic in 36 African countries, negatively affecting the economy of these countries by reducing animal production. In the absence of vaccines, various trypanocidal drugs are used for the treatment of nagana. However, few available drugs were discovered decades ago and are either inaccessible to farmers in remote areas, or are associated with severe toxicity. Most importantly resistance has widely developed against their usage. Therefore, safe, effective and easily administrable drugs are urgently in need.

Due to the disadvantages of the currently available trypanocidal drugs, farmers have resorted to using fake drugs in increased numbers, which has resulted in the reluctance of the pharmaceutical industries regarding introduction of new drugs. Drug reposition has therefore proven advantageous, which is the adaptation of the already existing drugs and/or compounds effective for a certain disease to treat another disease. The already established compounds for one disease are screened for a different infection without having to go through the clinical screenings and various step associated with the new drugs to bring a faster relief to the infection.

As a result the e main aim of the current study was to determine efficacy of azithromycin (AZM), triclosan (TCS) and curcumin CUR on the AAT causative agents, namely, *T. congolense, T. b. brucei* and *T. evansi* as well as evaluate their cytotoxicity *in vitro* and toxicity *in vivo* using mouse models.

Chapter 1 tested the trypanocidal effect of AZM *in vitro* against *T. congolense, T. b. brucei* and *T. evansi* and the cytotoxicity on the host cells, MDBK and NIH 3T3 cells. Furthermore, the oral trypanocidal effect of AZM was determined on *T. congolense* and *T. b. brucei* – infected mice. The treatment terms differed from short (7 days) and long (28 days) term treatment. Additionally, in order to determine the possible mode of action of AZM on *T. congolense and T. b. brucei,* Transmission Electron Microscopy (TEM) was conducted from *in vitro* treated *Trypanosoma* parasites. The *in vitro* IC_{50} values of AZM on *T. congolense , T. b. brucei* and *T. evansi* was 0.19 ± 0.17; 3.69 ± 2.26 and 1.81 ± 1.82 µg/mL, respectively, while the cytotoxicity effects values were > 25 µg/mL in both the cells. AZM was more effective against *T. congolense* infected mice than *T. b. brucei*, *in vivo*. The short term treatment of AZM on *T. congolense* resulted in a relapse post treatment while the long term treatment resulted in trypanocidal activity at 300 and 400 mg/kg with observed survival rates of 80 and 100%, as compared 70 and 70% survival rate observed in the long term treatment of *T. b. brucei* infected mice. AZM oral efficacy on *Trypanosoma* infected mice is probably due to its ability to inhibit the protein synthesis of micro-organisms by reverse binding onto the 50 S ribosomal complex and thereby inhibiting the translation process of the amino acid to proteins.

Chapter 2 aimed to determine the trypanocidal effects of TCS on *T. congolense, T. b. brucei* and *T. evansi*, the cytotoxicity on the host cells, the toxicity in mice and finally the trypanocidal efficacy *in vivo* against *T. congolense* infected mice. The toxicity tests were conducted in order to determine the safe and tolerable doses for Balb/c mice. The *in vitro* efficacy of TCS was observed at 1.93 ± 0.86; 1.82 ± 0.21 and 1.06 ± 0.07 µg/mL for *T. congolense*, *T. b. brucei* and *T. evansi* respectively, with cytotoxicity of 15.15 ± 6.83 and 2.20 ± 1.01 µg/mL on MDBK and NIH 3T3 cells, respectively. A single tolerable dose (LD_{50}) of TCS was recoded at 1414.21 mg/kg, however, a repeated tolerable dose was 300 mg/kg body weight of the mice. Furthermore,

observed toxicity signs included hepatomegaly, elevated levels of liver enzymes, fluctuations of the haematological parameters and the presence of megakaryocytes and dose dependent hydropic degeneration. The oral treatment was more effective on *T. congolense* infected mice as compared to the intraperitoneal treatment which resulted in a significant suppression of parasitaemia and a prolonged survival rate of the mice even though the mice were not relieved from the infection. The suppression of parasitaemia after oral treatment with TCS was attributed to high bioavailability of orally administered compound. Chapter 3 aimed to demonstrate the effect of CUR and CUR nanoparticle *in vitro* on *T. congolense, T. b. brucei* and *T. evansi*, cytotoxicity effects on the MDBK and NIH 3T3/HFF cells. Additionally, the study aimed to determine the extent of diarrhoea caused in CUR treated healthy mice. Both CUR and CUR nanoparticle trypanocidal efficacy were demonstrated *in vivo* against *T. congolense* infected mice.

CUR nanoparticles were synthesized using three different methods, the antisolvent precipitation with a syringe pump (ASPS), evaporative precipitation of nanosuspension (EPN) and wet-milling (WM). All the CUR nanoparticles were 2 folds more effective on the *T. congolense* as compared to free CUR *in vitro,* nonetheless, the in *vivo* tests were conducted only on the ASPS produced nanoparticles. The *in vitro* efficacy of CUR on the trypanosomes was 1.36 ± 0.31; 2.82 ± 1.22 and 2.37 ± 3.07 µg/mL, while the ASPS prepared CUR nanoparticle efficacy was 0.58 ± 0.50; 10.43 ± 9.43 and 4.86 ± 0.13 µg/mL on *T. congolense* , *T. b. brucei* and *T. evansi*, respectively. Both CUR and CUR nanoparticles showed moderate efficacy orally, compared to the intraperitoneal treatment. Histopathology examinations revealed the possibility of diarrhoea induced by CUR administration, evident with the presence of a dose dependent atrophy of the villi and the thinning of mucosa propria. The solubility of CUR was improved by the preparation of nanoparticles. The efficacy of CUR and CUR nanoparticle were evidently affected by the solvent, the presence of food and the treatment period. None of the CUR treated mice were cleared of the infection, however, the survival rate of the orally treated mice was significantly prolonged as compared intraperitoneally treated mice. CUR nanoparticles, cured few mice while some of the mice relapsed from the infection probably due to the short half-life of CUR. CUR and CUR nanoparticles possess efficacy orally on the trypanosomes as compared to the intraperitoneal treatment. The applicability of oral treatment in the natural host of nagana still remains challenging

and therefore further and thorough studies are necessary in order to address this issue. Thus far, dogs and cats are easier to treat orally while ruminants could receive the drugs either through food or water. High concentrations were tested in this study in order to demonstrate the effect of the compounds on trypanosomes, even though monotherapy is no longer advisable for the treatment of trypanosomosis.

In conclusion, AZM is a promising compound for the treatment of *T. congolense* and *T. b. brucei*, however, there is still much research needed to ascertain the applicability in the natural host. The TCS significantly suppressed trypanosome parasitaemia in mice, but due to its short half-life and rapid elimination in the bloodstream, parasitaemia increased steadily post treatment, until all the mice died. The CUR nanoparticle improved the pharmacokinetics of CUR free compound and the efficacy, both *in vitro* and *in vivo*. This study has shown that AZM has trypanocidal effects particularly against *T. congolense* which means that it has potential to be used for treatment of *T. congolense* - Nagana. Furthermore, it has been observed in this study that TCS significantly suppressed parasitaemia during early treatment which means if it can be used in combination with other compounds or as a hybrid then it can contribute significantly in treatment of trypanosomosis. Moreover, the production of CUR nanoparticle improved the oral treatment against trypanosome infection, indicating an enhanced bioavailability and absorption of the nanoparticle compounds in mice.

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2. Abstract should be between 1,800 and 2,200 characters in Japanese, or be between 1,000 and 1,400 words in English.

3. Do not include figures and tables.

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