Antifilarial actions of green tea extract and a synthetic heterocyclic thiazolidine derivative, Im8 compound in experimental mouse model

Sneha Hande¹, Kalyan Goswami¹*, Namdev Togre¹, Amisha Mandvikar² & Maryada Venkata Rami Reddy¹

¹Department of Biochemistry, Mahatma Gandhi Institute of Medical Sciences, Sevagram-442 102, Maharashtra, India.
²Institute of Pharmaceutical Education and Research, Borgaon (M), Wardha-442 001, Maharashtra, India.

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In spite of the advances in drug development and research against human lymphatic filariasis following the WHO mandate to address the disease-associated socioeconomic burden, diethylcarbamazine (DEC, N, N-diethyl-4-methyl-1-piperazine carboxamide) is the only available antifilarial drug to date. The major obstacle for further development of antifilarial drugs is the lack of validation of candidate drugs in the experimental animal models. Both, green tea extract and a synthetic heterocyclic thiazolidine derivative (Im8; 2-chloro-N-(4-phenylthiazol-2-yl), showed efficacy of antifilarial action in our earlier in vitro study and hence, they were screened in the present study for their antifilarial potential in the BALB/c mouse filariasis model. Mice were treated with 25 mg/kg dose of either Im8 or green tea extract or DEC or only with their respective vehicles. The untreated mice served as controls. Following insertion of the micropore chamber laden with microfilariae (Mf) of Brugia malayi, the drug or vehicle was administered s.c. in mice at 12 h intervals as 4 doses. After 12 h of administration of the last dose, the micropore chambers were removed to determine the action of the treatments as the loss of Mf motility. The green tea extract showed a significant antifilarial action and Im8 showed relatively less but significant antifilarial action as compared to the respective vehicle controls. Both the green tea extract and Im8 showed higher activity than that was exerted by DEC. These results revealed a greater efficacy of green tea and thiazolidine derivative, Im8 as the novel antifilarial agents in the experimental mouse model of filariasis.

Keywords: Brugia malayi, Elephantiasis, Lymphatic filariasis, Microfilariae, Nematodes, Roundworms

The tissue-dwelling filarial parasite, Brugia malayi, infects ~1.39 billion people in 73 countries worldwide. Considering the severity of the disease, life-long patient care, and socioeconomic burden of the lymphatic filariasis (LF), it is one of the major neglected tropical diseases (NTD) targeted for elimination¹. However, treatment options against this disease still rest on the only available drug, diethylcarbamazine (DEC; N, N-diethyl-4-methyl-1-piperazine carboxamide) (Fig. 1A), which although effectively targets the microfilarial form of B. malayi parasite indirectly in vivo but remain ineffective in vitro cell free system, reportedly due its dependence on host immunocytes to mediate its pharmacological action(s)². Due to long patency of filarial infections, DEC needs to be administered periodically for sustenance of the drug action which may lead to possible drug resistance³. The present therapeutic strategy under Mass Drug Administration programme (MDA) using DEC as mainstay is facing challenges to cover the entire endemic population for controlling the disease and to interrupt further parasite transmission⁴. Additionally, chances of developing side effects with limited option of drug molecules available in drug repository against the B. malayi parasite pose additional challenges to filariasis elimination or control⁵.

In this context, the World Health Organization (WHO) has campaigned for drug discovery research to recognize novel therapeutic agents (both synthetic compounds as well as herbal agents) which can effectively target the parasite while being safe to the humans⁶. However, in present situation, due to practical limitation of availability of the gerbil host, Jirds (Meriones unguiculatus) which is a relatively scarce animal, important data on the pre-clinical screening of the drug molecules from in vitro testing prior to the clinical trials is seriously lacking. Although, there is plethora of in vitro results suggesting various candidate agents (drugs), to date only a few potential new antifilarial drugs that have been tested in the pre-clinical animal filariasis models for further human trials.

*Correspondence:
Phone: +91 7152 284341. Ext. 262; Fax: +91 7152 284038
E-mail: goswamikln@gmail.com
Methanolic extract of green tea was prepared by the filariasis animal model.

The WHO estimated herbal medicine as the mainstay particularly for the developing countries. Such traditional use of plants are cost effective and socio-culturally well accepted. Earlier study with herbal extracts of Vitex negundo, Aegle marmelos and Butea monosperma showed promising antifilarial activity against the B. malayi parasite and we found the polyphenolics as the active principles in most of these extracts. Off late, our data with the polyphenol-rich green tea extract has shown promise as a novel therapeutic agent against the lymphatic filariasis (in communication). Incidentally, tea contains a large number of potentially bioactive chemicals, including flavonoids. The polyphenolics derived from green tea were reported to induce apoptosis through possible inhibition of the folate metabolism. Our recent bioinformatic approach targeting folate metabolism suggested dihydrofolate reductase (DHFR) to be a potential candidate drug. Further, thiazolidineone compounds are also known for their versatile therapeutic properties including the anticancer, antibacterial, antiproliferative and antimalarial actions. In our earlier studies, the initial screening with a series of thiazolidine derivatives against the filarial parasite showed potent antifilarial activity of those compounds and our results support a possible involvement of apoptosis in their antiparasitic action.

With this perspective, here, we tried to evaluate green tea extract and Im8, a thiazolidine derivative, against the filarial parasite using the in situ micropore chamber method and compared with the standard antifilarial drug (DEC) and the untreated control in mouse as the suitable filariasis animal model.

Materials and Methods

Materials and reagents

All reagents and chemicals were obtained from commercial sources (Himedia Laboratories Pvt. Ltd, Mumbai and Sigma Aldrich Chemicals Pvt. Ltd, Mumbai). The synthetic thiazolidine derivative (Im8) was synthesized and purified in our laboratory. Methanolic extract of green tea was prepared by the method described by the International Organization for Standardization (ISO) 14502-1. Briefly, 0.200±0.001g of green tea powder (Tetley brand) was weighed in an extraction tube, and 5 mL of 70% methanol at 70°C was added in it. The extract was mixed and heated at 70°C for 10 min with intermittent vortexing. After cooling at 25°C, the extract was centrifuged at 200 × g for 10 min. The supernatant was decanted in a graduated tube. The extraction step was repeated once again. Both extracts were pooled and the volume was adjusted to 10 mL with cold 70% methanol. Extract obtained was stored at 4°C for further use.

Ethics statement

All animal experiments included in this study were approved by the Institutional Animal Ethics Committee (IAEC), MGIMS, Sevagram which follows the norms of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) in India. BALB/c mice, 6-8 wk old, were inbred and kept under the suitable environment (12 h light/dark cycles with temperature range of 18-23°C and 40-60% humidity). They were fed on pelleted animal diet and water ad libitum in a CPCSEA registered Central Animal House.

Infection and parasites

The human filarial parasite B. malayi life cycle was maintained in Jirds (Meriones unguiculatus), mastomys (Mastomys coucha) using mosquitoes (Aedes aegypti) as vectors by standard methods. Jirds were inoculated i.p. with 100 L3 larvae as described previously. Microfilariae (Mf) at 180 d post-infection were obtained from the peritoneal cavity of the Jirds. Mf were washed with RPMI 1640 medium (containing 20 µg mL⁻¹ gentamycin, 100 µg mL⁻¹ penicillin, 100 µg mL⁻¹ streptomycin) plated on the sterile plastic petri dishes and incubated at 37°C for 1 h to remove the peritoneal exudate cells of the Jirds. The microfilariae were collected from the petri dishes, washed with RPMI 1640 medium and used for further experiments.

In situ efficacy

Administration of test agents and DEC as a standard drug

The test agents (Im8 solubilized in DMSO and tea extract prepared in 70% methanol) were suspended in 0.1% Tween-80 in distilled water. DEC was prepared in distilled water. In BALB/c mice, the green tea herbal extract, Im8 thiazolidine derivative and DEC were administered at 25 mg/kg body wt. through s.c.
route as four doses at 12 h intervals. The doses and route of administration were pre-optimized. All the suspensions/solutions of the test agents and the standard drug DEC compounds (reference drug) were prepared fresh daily before administration\textsuperscript{23}.

**Test agent and standard drug treatment**

The efficacy of green tea extract and Im8 were analyzed using the micropore chamber technique following the standard method\textsuperscript{24,25}. In the therapeutic treatment protocol, BALB/c mice were divided into six different groups (n=5 per group) as follows: (i) control (mice without treatment); (ii) control-green tea extract vehicle (mice treated with methanol); (iii) control-Im8 vehicle (mice treated with DMSO); (iv) green tea extract (mice treated with green tea extract); (v) Im8 (mice treated with Im8 derivative); and (vi) DEC (mice treated with DEC in distilled water).

BALB/c mice were administered s.c. with the test agent and reference drug in four doses with 12 h gap after implantation of the micropore chamber containing Mf (Fig. 2). The BALB/c mice were treated with the drug twice a day (12 h) to maintain the drug levels in the circulation of mice. Micropore chambers were assembled using 14 × 2 mm plexi glass rings having a small hole in the rim and 3.0 µM nucleopore polycarbonate membranes (Millipore Corporations, Bedford, MA). Membranes were attached to the plexi glass rings with cyanoacrylic adhesive and dental cement. The chambers were sterilized overnight at 80°C. The chambers were then loaded through the hole with 50 Mf suspended in RPMI 1640 medium supplemented with 10% heat inactivated Fetal Calf Serum (FCS) and sealed with dental cement. Chambers were then implanted into the peritoneal cavity of each BALB/c mice under anaesthesia (Thiopen tone sodium i.p., 50 mg/kg). Strict aseptic conditions were followed for the surgical procedures.

**Assessment of antimicrofilarial efficacy**

After 48 h of implantation, the chambers were taken out from peritoneal cavity and washed in normal saline. Contents of each chamber were examined microscopically for the assessment of loss of Mf motility. The antiparasitic action was considered to be achieved, if Mf was found to be non-motile and limpid and had several adherent cells on the surface. Anti-microfilarial efficacy of the test agents and the standard drug, DEC was expressed as percent (\%) loss of Mf motility.

**Statistical analysis**

The results of loss of Mf motility for each group were expressed as mean ± SD. The results from the suitable groups against respective vehicle controls were compared by Mann Whitney’s ‘U’ test. For test of significance, \(P\) value <0.05 was considered.

**Results**

**Determination of antimicrofilarial activity of green tea extract and thiazolidine compound by in situ micropore chamber method**

The green tea extract and the thiazolidine derivative i.e. Im8 were selected for the in situ testing using the micropore chamber technique in BALB/c mice to record the survival of the parasites in a simulated physiological environment. The percentage (\%) loss of motility of Mf (Mean ± SD) obtained from BALB/c mice treated with the green tea extract was found to be maximum (64 ± 18.46%) followed by the loss of motility of Mf recovered from the vehicle control group of animals and thiazolidine compound (Im8, 25 mg/kg) and DEC (25 mg/kg). [Each histogram is represented as an average ±S.D. of 5 independent observations (n=5). *Significantly different at \(P\) <0.05 as compared to the green tea extract-treated and DEC-treated groups]
showed 4 ± 0.24% and 6 ± 1.31% loss of motility from the green tea and Im8 vehicle control, respectively. The differences from the respective control values were significant (P <0.05). Mf recovery from those mice which received standard drug control (DEC) showed 15 ± 6.37% of loss of Mf motility. This was also significantly low as compared to that obtained with the green tea treatment and also lower than thiazolidine drug treatment (although insignificant) (Fig. 3).

Discussion

Enormous suffering and socioeconomic burdens of human lymphatic filariasis constantly remain challenging in spite of the mass drug administration programme. The WHO mandated research towards development of antifilarial agents against the parasite which needs sufficient experimental evidence(s) from the animal model study for further clinical trials. Towards this end, in this study, we used the BALB/c mice for testing the therapeutic efficacy of green tea extract and the synthetic thiazolidine derivative (Im8) following the promising results observed from our in vitro pharmacological actions of these tested agents. 

Oxidative stress has been found responsible for observed the apoptotic action of these synthetic drugs. Although the free radicals and ROS are important for cell cycle signaling regulation, excess of oxidative stress induces apoptosis. Role-reversal of otherwise well-known anti-oxidant effect of polyphenol into conditional pro-oxidant effect at higher dose range has been reported. High flavonoid content of green tea might be suggestive of such rationale in operation behind the observed therapeutic action(s). In our earlier in vitro studies with green tea, we reported apoptosis in the parasite (data presented elsewhere). Similar apoptosis in cancer cells by green tea has been shown by others.

Result of the present study revealed significantly higher antifilarial effect as compared to their respective vehicle controls. Both these agents also displayed better efficacy as compared to the DEC (positive drug control) treatment alone within the specified period of the study. Green tea apparently caused notable antifilarial action in the animal study which was even better than that exhibited by the synthetic compound. This observation is probably suggestive of a synergistic action(s) of the myriad active molecules present in the whole green tea extract than the action(s) of a single compound. Our earlier results also support this observation.

Although the standard drug DEC exhibited effective antifilarial action as expected, its impact was considerably low as compared to both of these agents, green tea extract and Im8, employed in this study. The DEC is known for its indirect action through stimulation of the host inflammatory response rather than by its direct effect, which presumably needs more time to exert its effect. Hence, it appears that during the short experimental period, green tea and the thiazolidine compound (Im8) surpassed the pharmacological efficacy of DEC owing to their direct action on the parasite. Such direct antiparasitic effect with these agents in contrast to that of DEC, which was totally ineffective in in vitro cell free culture conditions, was observed in our earlier studies, as mentioned before.

This study revealed that both green tea extract and the thiazolidine compound (Im8), which were already shown to be effective in in vitro condition, were also effective in a simulated in situ environment of BALB/c mice as a suitable animal model of filariasis and may serve as new effective leads for the development of novel antifilarial agent.

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Conflict of Interest

The authors declare no conflicts of interest in this work.

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