New compound with effect against parasite *Leishmania*

Parasites of the genus *Leishmania* threaten the population of 98 countries on 5 continents. Leishmaniasis is caused by intracellular protozoan parasites of the genus *Leishmania* (Trypanosomatidae) and transmitted to vertebrates by phlebotomine sand flies. Parasites infect macrophages of the mammal host. 40,000 die each year of visceral leishmaniasis, the most serious form of the disease induces by parasites' spread to liver and spleen. Effective human vaccine against the infection does not exist and the drugs in use have many undesirable side effects. In addition, parasites became resistant against these drugs in many areas. In our previous experiments we tested the library of 2500 chemical compounds for antileishmanial effect. This primary screening resulted in the identification of diphenyleneiodonium (DPI) as an effective inhibitor. The value of IC_{50} = 0.010 µM was established, which is significantly lower than the value for the current drugs such as amphotericin B (Amph. B) (IC_{50} = 0.039 µM). We also identified that the effective concentrations of the compound are non-toxic to tested human cell lines. Compound kills parasites in both mouse and human macrophages. We investigated the leishmanicidal activity of DPI *in vivo* (**Fig.1**).

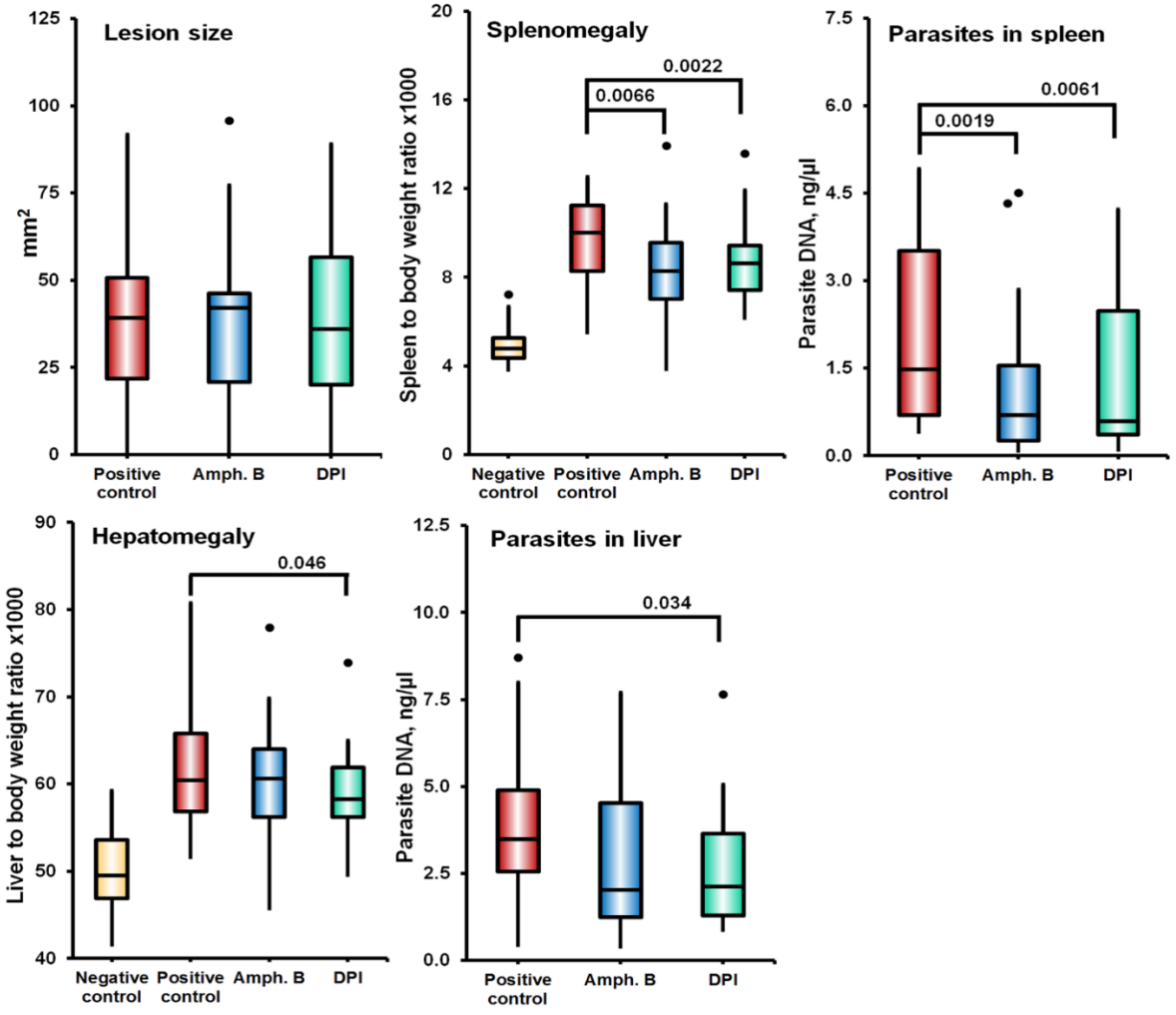

Fig. 1 Skin and visceral pathology in different experimental groups of *Leishmania*-infected susceptible BALB/c mice. The size of primary skin lesions on the 6th week of infection was measured using digital caliper. Splenomegaly and hepatomegaly were calculated as organ weight to body weight ratio multiplied by 1000. Parasite load in spleen was estimated by PCR-ELISA and expressed in ng of parasite DNA per µl.

Analysis of treatment *in vivo* established that both Amph. B and DPI reduced splenomegaly and parasite load in spleen and DPI also reduced hepatomegaly and parasite load in liver (**Fig. 1**).

We have also studied mechanism of effect of analyzed compound **(Fig. 2**): Analysis by mass spectrometry established that DPI depletes intracellular glutathione from *Leishmania* parasites.

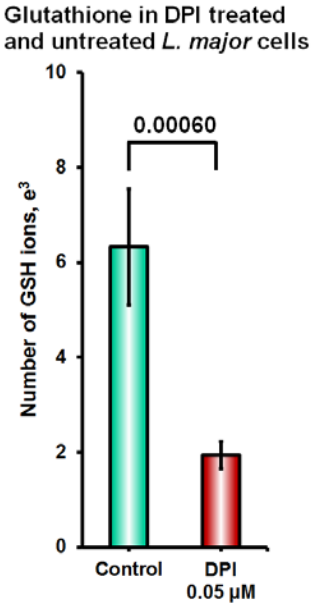

Fig. 2. Establishment of intracellular content of Glutathione (GSH) in *Leishmania* parasites after DPI treatment. The volumes of cell suspension from flasks with and without DPI containing 100 million cells were centrifuged at 2300 g for 5 minutes at room temperature. The supernatant was aspired and the pellets were resuspended in 100 ml 0.1% TRITON in PBS to permeabilize the promastigotes. The samples were incubated at room temperature for 15 minutes and centrifuged as described above. The supernatants with GSH eluted from the permeabilized cells were collected and immediately analyzed using liquid chromatography – mass spectrometry (LC-MS).

Compound was patented:
Czech patent:
Farmaceutický přípravek obsahující difenylenjodonium pro léčení onemocnění vyvolaných parazity čeledi Trypanosomatidae, Patent no. 305247, Awarded June 15, 2015, Úřad průmyslového vlastnictví České republiky.
CZ201300729-A3; CZ305247-B6

International patent:
Grekov, I; Pombinho, A; Šíma, M; Kobets, T; Bartůněk, P; Lipoldová, M. Pharmaceutical composition comprising diphenyleneiodonium for treating diseases caused by the parasites belonging to the family Trypanosomatidae).
PCT/CZ2014/000103 (published March 10, 2016)
USA patent: US 2016/0220508 A1 (published August 4, 2016)
WO2015039638-A1
Derwent Primary Accession Number: 2015-21111G

Conclusions: The results show that DPI acts as active substance against parasites of the genus *Leishmania* by depleting intracellular glutathione from *Leishmania* parasites. The compound exhibits lower side effects than currently used drugs and its antileishmanial effects *in vitro* and *in vivo* are comparable or better than effects of the most efficient antileishmanial drug amphotericin B.

To get more information about the antileishmanial compound or to buy patent for use of the compound, please contact Center for Technology Transfer, IMG AS CR, Videnska 1083, 14220 Praha 4, Czech Republic; Tel. (420-241 063 227 or 420-602 892 876).