Bacteriocin AS-48: a potent drug against sleeping sickness

African trypanosomiasis has been a historical scourge on the African continent and one of the major causes of poverty. It is responsible for sleeping sickness in humans (HAT), and it avoids the development of agriculture based on domesticated animals where it causes nagana (AAT). It is a vector-borne parasitic disease that is caused by protozoan parasites belonging to the genus *Trypanosoma*, which is transmitted to humans and animals by tsetse fly bites (*Glossina* sp.). This disease occurs in 36 sub-Saharan Africa countries in which 70 million inhabitants are at risk. Many of the affected populations live in remote rural areas with limited access to adequate health services, which complicates the surveillance and therefore the diagnosis and treatment of cases. Trypanosomiasis in domestic animals, particularly in cattle, is a significant obstacle to the economic development of affected rural areas. It is estimated that 30% of African cattle are at risk of infection. Also, the impossibility of maintaining livestock in areas of high risk also prevents their use as a source of traction and fertilizer for the cultivation of land, making these regions one of the most impoverished in the world, also from the point of view of agriculture. According to WHO, it is the neglected disease with the worst drug control. In fact, the drugs currently used present a limited efficacy, high toxicity and they are hampered by the continual appearance of resistance, so new drugs are urgently needed.

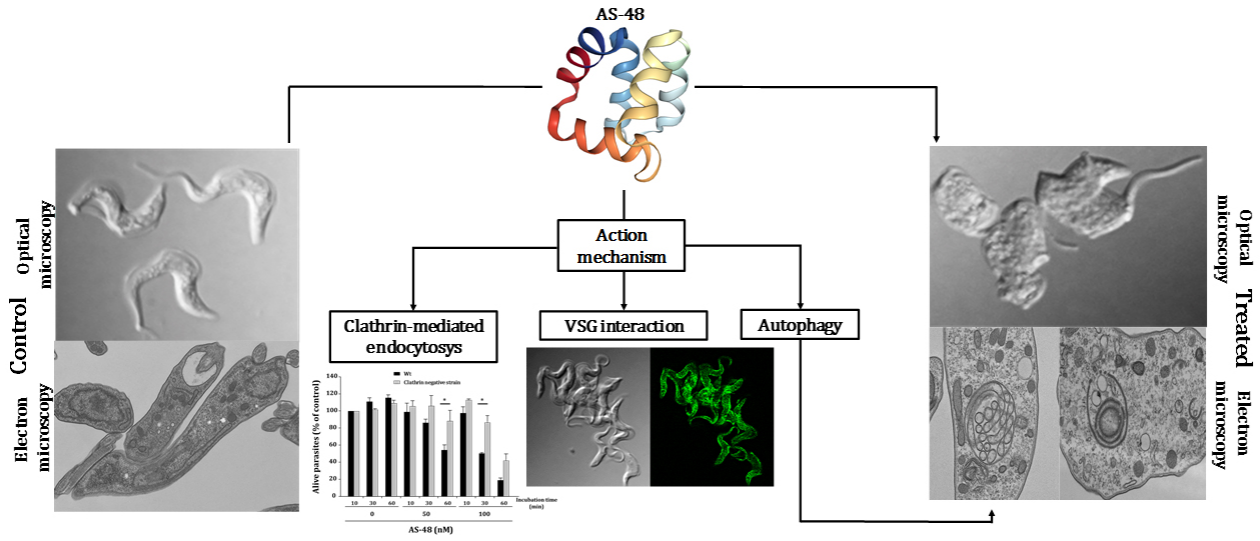

Fig. 1. Graphical abstract about AS-48 activity against Trypanosoma brucei.

Antimicrobial peptides (AMPs) have recently attracted attention for their potential as parasitical compounds. They constitute a family of small polypeptides with different spectra, modes of action, molecular weights, genetic origins, and biochemical properties. The AMPs produced by bacteria

are called bacteriocins. They are ribosomally synthesized, secreted peptides that inhibit the growth of other bacteria. Bacteriocins are biotechnologically very relevant, being used in the food industry as natural preservatives, but also they have a remarkable therapeutic potential as an alternative to antibiotics and other drugs. AS-48, a circular peptide is one of the best-known bacteriocins. Its primary target is the bacterial membrane, in which it forms pores, leading to dissipation of the proton motive force and cell death.

Although AS-48 has no activity against the majority of eukaryotes, parasites with anionic surface exposed at the external medium, allow a privileged interaction with this type of strongly cationic peptides. In fact, according to our results, AS-48 shows high parasitical activity against both, HAT- and AAT- causing trypanosomes, being the first bacteriocin reported with activity against these parasites. The EC_{50} (50% efficacy concentration) ranged from 1.7 to 3.12 nM depending on the *Trypanosoma brucei* subspecies, being the lower antimicrobial activity describe for AS-48 to date. This EC_{50} is even lower than those described for trypanocidal agents currently in use, such as suramin, pentamidine or melarsoprol. Interestingly, the action mechanism described is entirely new, since no pore-forming activity was detected. Actually, our results support that AS-48 is quickly endocytosed (via clathrin-mediated endocytosis) by the parasites after its interaction with the VSG (variant surface glycoprotein) cover. The high number of VSG copies on the parasitic surface (10^7 molecules/cell) and its extremely rapid internalization and recycling by endocytosis (the VSG coat is completely internalized in 12.5 min) could explain the AS-48's efficiency and specificity against *T. brucei. Once AS-48 is inside the cell, t*he bacteriocin could alter the normal structure of phagosomes vesicles from the endocytic pathway, producing membrane-damaging pores that could induce the observed autophagy cell death. This mode of action targeting the trypanosome's interior is considered to be an attractive drug development strategy.

Marta Martínez-García [1], ***Ruben Cebrián*** [2], ***Jean-Mathieu Bart*** [1], ***José María Pérez-Victoria*** [1], ***Mercedes Maqueda*** [3]

[1]*Institute of Parasitology and Biomedicine "López-Neyra" (IPBLN-CSIC), Granada, Spain*
[2]*Department of Molecular Genetics, University of Groningen, Groningen, The Netherlands*
[3]*Department of Microbiology, University of Granada, Granada, Spain*

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