



Cytotoxic Effect of ApoL1r in Bovine Epithelial Kidney Cells as an Alternative Treatment for *Trypanosoma* spp. in Cattle

Ingrid Lorena Jaramillo^{1*}, John Didier Ruiz², Piedad Matilde Agudelo³

Abstract

ApoL 1 has been considered as a good alternative to *Trypanosoma* spp treatment but is also related to kidney cytotoxicity in some mammals. The cytotoxic effect of the recombinant ApoL1 (ApoL1r) protein on bovine renal epithelial cells was evaluated *in vitro*. This cytotoxic effect led to an up to 50% decrease in cell viability. These results were comparable to those obtained through positive damage control (MM8 at 200 and 250 ug/ml). It was determined that the ApoL1r protein could not be used in experimental animal models, specifically in cattle, due to the loss of cell viability that was generated.

Keywords: *Trypanosoma* spp.; apolipoprotein ApoL1; bovine

Introduction

Parasites of the genus *Trypanosoma* are flagellate protozoa that live in the blood and tissue plasma of their hosts. *T. vivax*, *T. evansi* and *T. theileri* have worldwide distribution and cause trypanosomiasis in domestic animals, especially cattle, buffaloes, horses and camels [1,2,3]. This disease is characterized by a wide range of presentations, from subclinical to (sometimes) acute, and generates losses for the livestock industry [3, 4, 5]. It has been estimated that the financial cost of the disease caused by *Trypanosoma* spp. can give rise to loss rates of 1% through reproductive problems and up to 10% due to mortality among calves in different herds [6]. This has implied that there is a need to test vector control and therapeutic strategies.

It has been recognized that the best strategies for reducing the losses due to this disease consist of carrying out vector control activities [3]. However, because of the undeniable difficulties of carrying out vector control effectively, therapeutic strategies for altering the biological and metabolic mechanisms of the parasite should be sought [7, 8 5, 9].

For more than 35 years, isometamidium and diminazene aceturate have been used as chemotherapeutic and chemoprophylactic agents for treating trypanosomiasis in cattle, sheep and goats, but their extensive use has already begun to produce resistance [10]. In addition, although most drugs target the *Trypanosoma* kinetoplast, there have been reports of *Trypanosoma* strains isolated from wild animals and cattle that mutate and do not present kinetoplasts. Thus, it was found that animals that received treatment with trypanolytic factor relapsed into infection, probably due to a lack of point of action [7]

Currently, there are no therapeutic strategies to reduce the problem of resistance and infection [11], although strategies involving host proteins

Affiliation:

¹CES University, Medellín, El Poblado, Medellín, Antioquia, Colombia

²CES University, INCA CES Group

³CES University, PhD in Health Sciences

*Corresponding author:

Ingrid Lorena Jaramillo, CES University, Medellín, El Poblado, Medellín, Antioquia, Colombia

Citation: Ingrid Lorena Jaramillo, John Didier Ruiz, Piedad Matilde Agudelo. Cytotoxic Effect of ApoL1r in Bovine Epithelial Kidney Cells as an Alternative Treatment for *Trypanosoma* spp. in Cattle. Archives of Nephrology and Urology. 6 (2023): 85-89.

Received: June 21, 2023

Accepted: July 03, 2023

Published: July 17, 2023

naturally resistant to non-T. cruzi Trypanosoma infections have been described. These have shown promising in vitro results in animal models [12]. One of these proteins is ApoL1, which is a protein of the immune system of mammals that is responsible for lysis of Trypanosoma in naturally resistant hosts. This protein has been evaluated in terms of its activity in experimental models [13]. It has been shown that recombinant ApoL1 (ApoL1r) can form a therapeutic tool against different trypanosomes that are not resistant to its mechanism. However, it has been shown that in high quantities, this protein can generate cellular damage together with renal cell apoptosis, in relation both to epithelial and to podocyte kidney cells [14].

An effective alternative treatment approach may be of great help in controlling or eliminating the agent and avoiding associated processes. For this reason, the aim of the present study was to establish the in vitro cytotoxic effect of a recombinant ApoL1 protein on a bovine epithelial kidney cell line, prior to recommending this as a therapeutic alternative against Trypanosoma spp.

Methods

An experimental in vitro study was carried out on a culture of bovine renal epithelial cells (ATCC bovine kidney) after more than 8 days of growth. This investigation was based on cytotoxicity analysis on the recombinant protein ApoL1, in bovine kidney cells, to evaluate an adverse renal effect that had previously been described by some authors [15]. For this, an experiment was designed by means of the Minitab® software, version 21 [16]. In this, the final response variable was the percentage viability, which was expected to be higher than 90% and was derived from analysis on absorbance, using spectrophotometry. This was done in order to determine whether the ApoL1 protein would cause damage to treated cells. The running time used for the assay was 8 days, not counting the incubation time of the cells. Four factors were considered: ApoL1 protein concentration (levels of 0.1, 1 and 10 ug/ml), positive control for cell death (levels of 200 and 250 ng/ml), negative control (with and without PBS) and negative cell control (with and without cells). The trial summary is described below:

Obtaining the recombinant ApoL1-like protein

The protein came in a presentation of 100 µg from the company ABCAM, Cambridge, UK. It is a 52 kDa protein, with a total of 238 amino acids, which is produced in wheat germ. Its sequence is:

(MEGAALLRVSVLCIWMSALFLGVGVRAEEAGARVQ QNVPSTGDTGDPQSKPLGDWAAGTMDPESSIFIEDAIK YFKEKVSTQNLLLLLLTDNEAWNGFVAAAELPRNEAD ELRKALDNLARQMIMKDKNWHDKGQQYRNWFLKEF PRLKSELEDNIRRLRALADGVQKVHKGTTIANVVSGS LSISGILTLVGMGLAPFTEGGSLVLEPGMELGITAAL TGITSSTMDYGKKWWTQA) and its access number in the Uniprot database is O14791.

Cell culture

Kidney cells were obtained from the company ATCC®, Virginia, USA. with the reference MDBK (NBL-1), CCL-22. These cells originated from the kidneys of an adult male Bos Taurus animal and had been characterized as epithelial cells. For culturing, the cells were removed from the liquid nitrogen tank where they had been kept at -130 °C since purchase. Eagle's essential medium (catalog no. 30-2003) and fetal equine serum (Sigma-Aldrich) were added at a final concentration of 10%.

Once the vial of cells had been removed, it was continually shaken at 37 °C in a water bath for 2 hours. The contents of the vial were then resuspended in a 25 cm² growth vessel. This was placed in an incubator at 37 °C for 24 hours with 5% CO₂. The medium was exchanged after 8 days of growth, in order to make the evaluation.

Cell subculture: 0.25% trypsin and 0.03% EDTA were added and then the solution was stirred. It was left at room temperature for 10 minutes, dry and added to a new medium. Subculturing was performed at a concentration of approximately 10e+5 cells per ml.

Preparation of MTT solution: A stock solution of 12 mM was prepared by adding 1 ml of PBS to a vial of 5 mg of MTT. This was then vortexed for 30 seconds. If did not was filtered until could be dissolved. The solution was immediately covered to avoid the effect of light.

Preparation of HCL-SDS: 10 ml of Hydrochloric acid solution (HCL) was added to with 1 g of SDS in a tube and inverted several times.

Addition of treatments: Serial dilutions of the protein were made, with the original concentration of 100 ug/ml. The cells were then added at a concentration of 10e+5 cells per ml, in a 96-well plate. Lane 1 consisted of distilled water; Lane 2, cells at a concentration of 10e+5, with 10 ug/ml of the protein; Lane 3, cells at a concentration of 10e+5, with 1 ug/ml of protein; Lane 4, cells at a concentration of 10e+5, with 0.1 ug/ml protein; Lane 5, blank with cells; Lane 6, MM8 (methyl methane sulfonate) at 200 ug/ml; Lane 7, MM8 at 250 ug/ml; Lane 8, blank with cells; Lane 9, blank without cells; Lane 10, culture medium; and Lane 11, PBS.

MTT analysis: The medium was exchanged again as mentioned above, 100 ul of medium, and 10 ul of the MTT stock solution was added. This mixture was incubated for

Design summary:			
Factors:	4	Replications:	3
Running base:	48	Total runs:	144

4 hours and the assay was viewed using a Multiskan Sky ELISA reader (Thermo fisher Scientific®), and the readings were stored until analysis. Three replicates were made on each plate and the assay was done three times.

Cytotoxicity analysis

The cells were cultured in accordance with the manufacturer's recommendations and were kept at a standard concentration ($1-2 \times 10^5$). These cells were then seeded in a 96-well plate with a final volume of 10 μ l, with concentrations of 0.1, 1 and 10 mg/ml of ApoL1r. There was also one xxxxx lane without protein administration, as a negative control, but under the same conditions. After 12 hours of growth, the cells were treated with 50 ng/ml of tetracycline in complete Dulbecco's modified Eagle's medium (DMEM). Cytotoxicity and viability were measured at 24 hours, using a cell proliferation kit (I-MTT; Roche®).

The technique MTT is a colorimetric assay for non-radioactive quantification of cell proliferation, viability and cytotoxicity. The sample material was adherent cells in 96-well microplates. Through the colorimetric assay, the number of viable cells was analyzed according to the cleavage of tetrazolium salts that were added to the culture medium. This technique requires neither washing nor cell harvesting, and the entire assay, from microculture to data analysis using an ELISA reader, is performed on the same microplate [17].

Each treatment was run in three replications and three repetitions of this, and the data obtained were averaged to establish the point values.

Results

Evaluation of cytotoxicity in bovine kidney cells using recombinant ApoL1 For the cytotoxicity test, commercial bovine renal epithelial cells were obtained. The percentage viability of these cells was evaluated when they were subjected to three different concentrations of the recombinant protein. The viable cultured cells were subjected to interaction with three concentrations (10, 1 and 0.1 μ g/ml) in three replicates and three repetitions to determine their cytotoxicity.

Administration of an average concentration of 0.1 μ g/ml gave rise to an average percentage viability of 53%, among the cells in the tubes. The concentration of 1 μ g/ml showed an average viability of 42% and the concentration of 10 μ g/ml showed an average viability of 32%. Similar results were obtained on average regarding the positive cell death control. This showed that the therapeutic dose of 0.1 μ g/ml, and that larger or smaller doses caused viability lesions in the kidney cells used in the tests of this study. There was a statistically significant relationship in the ANOVA test (139) between administration of the treatment at all concentrations and decreased cell viability ($p < 0.05$). ApoL1 was clearly a non-viable molecule for use as a treatment for trypanosomiasis in cattle

Discussion

Among the various alternative treatments for managing *Trypanosoma* spp., use of the ApoL1 protein has shown promise. Different therapeutic alternatives have been sought around the world, given the resistance to existing treatments and the difficulties in applying the available therapies [16]. One of the strategies that has been widely investigated for treatments for humans is the use of ApoL1, but its use is known to generate kidney toxicity in humans [15]. However, its use in cattle had never been explored until now.

Bovine trypanosomiasis is responsible for economic losses in tropical and subtropical areas of Africa and Latin America. This disease is characterized by fever, anemia, loss of production and even death [18]. In the present study, the possibility of using recombinant ApoL1 in treatments for cattle was analyzed, with a view to avoiding the resistance processes that have been observed in relation to other treatments.

The recombinant ApoL1 protein was found to have high cytotoxicity, comparable to that of lethal concentrations of methyl methane sulfonate. This situation reduces the likelihood of its use for therapeutic purposes in cattle. The great association of kidney disease in humans with the different variants of ApoL1 has been widely studied [19]. However, in cattle, and even less in buffaloes, its function and possible use had not been explored.

The effect of ApoL1 on infection with *Trypanosoma brucei* was demonstrated in one study [20, 1]. This protein was considered to be a protein of the larger ApoL family, which exhibits a shared domain that forms pores (PFD), a cellular marker (MAD) and a C-terminal stem-loop-interacting RNA-binding protein (SRA) that is a binding domain [20, 21]. Its trypanolytic effect has been widely studied, as also has the mechanism of cell death associated with overexpression of the G1 and G2 variants in humans [15]. It is possible to infer that the mechanism that was observed in our study was the same as described in humans.

The bovine mechanism involves an apoptosis event in cells mediated by phosphorylation of kinases that is induced through cellular stress, and an increase in phosphorylation of protein kinase that is activated by adenosine monophosphate (AMP). This reduces intracellular potassium levels and mitochondrial respiration rates, which leads to the death of bovine renal epithelial cells [22]. These findings indicate that functions in intracellular membranes, and specifically those of the endoplasmic reticulum and mitochondria, are crucial factors in situations of cell injury mediated by the ApoL1 renal risk variant.

The information obtained in the present study will be particularly useful for veterinary pharmaceutical companies and other people involved in production of drugs for cattle

and buffaloes. Nonetheless, further studies relating to new substances for controlling trypanosomiasis in buffalo species worldwide are required. For the veterinary pharmaceutical sector, it can be recommended that the search for other molecules that might help in treating trypanosomiasis should continue. Although it is evident that ApoL1 has a good effect on Trypanosoma species that do not affect humans, its administration poses a risk to animals' kidney health.

Funding

MinCiencias-Colombia provided funding from its scholarship program, through the national call for doctorates #757.

Ethical approval

The present study was approved by the Ethics Committee of CES University, in 2018.

References

- Desquesnes Marc. Livestock Trypanosomoses and Their Vectors in Latin America. OIE (2004).
- Sobhy Hassan M., Safaa Barghash M, Tahani Behour S, et al. "Seasonal Fluctuation of Trypanosomiasis in Camels in North-West Egypt and Effect of Age, Sex, Location, Health Status and Vector Abundance on the Prevalence". Beni-Suef University Journal of Basic and Applied Sciences 6 (2017): 64–68.
- Nguyen, Quoc Doanh, Thu-Thuy Nguyen, et al. "Seroprevalence of Trypanosoma evansi Infection in Water Buffaloes from the Mountainous Region of North Vietnam and Effectiveness of Trypanocidal Drug Treatment". Journal of Veterinary Medical Science 75 (2017): 1267–69.
- Olsen, Oliver Wilford. Animal Parasites: Their Life Cycles and Ecology (1986)
- Mekata, Hirohisa, Satoru Konnai, Claro Mingala N, et al. "Isolation, cloning, and pathologic analysis of Trypanosoma evansi field isolates". Parasitology Research 112 (2017): 1513–21.
- Dargantes AP, Mercado RT, Dobson RJ, et al. "Estimating the Impact of Trypanosoma Evansi Infection (Surra) on Buffalo Population Dynamics in Southern Philippines Using Data from Cross-Sectional Surveys". International Journal for Parasitology 39 (2009): 1109–14.
- Macaraeg Bryan B, Jonathan Lazaro V, Nancy Abes S, et al. "In-vivo assessment of the effects of trypanocidal drugs against Trypanosoma evansi isolates from Philippine water buffalo (Bubalus bubalis)." Veterinarski arhiv 83 (2013): 381–92.
- Sinha Suprita, Shweta Anand, Tapan Mandal Y. "Study of plasma protein binding activity of isometamidium and its impact on anthelmintic activity using trypanosoma induced calf model". Veterinary World 6 (2013): 444.
- Babokhov Peter, Adekunle Sanyaolu O, Wellington Oyibo A, et al. "A Current Analysis of Chemotherapy Strategies for the Treatment of Human African Trypanosomiasis". Pathogens and Global Health 107 (2013): 242–52.
- Baker, Nicola, Harry P. de Koning, et al. "Drug Resistance in African Trypanosomiasis: The Melarsoprol and Pentamidine Story". Trends in Parasitology 29 (2013): 110–18.
- Geerts, Stanny, Peter H Holmes, et al. "African Bovine Trypanosomiasis: The Problem of Drug Resistance". Trends in Parasitology 17 (2001): 25–28.
- Thomson, Russell, y Alan Finkelstein. "Human Trypanolytic Factor APOL1 Forms PH-Gated Cation-Selective Channels in Planar Lipid Bilayers: Relevance to Trypanosome Lysis". Proceedings of the National Academy of Sciences 112 (2015): 2894–99.
- Perez-Morga D. "Apolipoprotein L-I Promotes Trypanosome Lysis by Forming Pores in Lysosomal Membranes". Science 309 (2005): 469–72.
- Lan Xiqian, Aakash Jhaveri, Kang Cheng, et al. "APOL1 Risk Variants Enhance Podocyte Necrosis through Compromising Lysosomal Membrane Permeability". American Journal of Physiology. Renal Physiology 307 (2014): 326-336.
- O'Toole, John F, William Schilling, et al. "ApoL1 Overexpression Drives Variant-Independent Cytotoxicity". Journal of the American Society of Nephrology 29 (2018): 869–79.
- "Army Corps of Engineers Research Laboratory. Choices (Version 4.0) (1993).
- Meerloo, Johan van, Gertjan Kaspers JL, et al. "Cell Sensitivity Assays: The MTT Assay". En Cancer Cell Culture, editado por Ian A. Cree 731 (2011): 237–245.
- Mathis, Amanda M, Jacqueline Holman L, et al. "Accumulation and Intracellular Distribution of Antitrypanosomal Diamidine Compounds DB75 and DB820 in African Trypanosomes". Antimicrobial Agents and Chemotherapy 50 (2006): 2185–91.
- Chávez-Larrea, María Augusta, Michell Lorena Medina-Pozo, et al. "First Report and Molecular Identification of Trypanosoma (Duttonella) Vivax Outbreak in Cattle Population from Ecuador". Transboundary and Emerging Diseases 68 (2021): 2422–28.
- Olabisi Opeyemi A, Jia-Yue Zhang, Lynn VerPlank, et al. "APOL1 Kidney Disease Risk Variants Cause

- Cytotoxicity by Depleting Cellular Potassium and Inducing Stress-Activated Protein Kinases”. Proceedings of the National Academy of Sciences 113 (2016): 830–37.
21. Lecordier, Laurence, Benoit Vanhollebeke, Philippe Poelvoorde, et al. “C-Terminal Mutants of Apolipoprotein L-I Efficiently Kill Both Trypanosoma Brucei Brucei and Trypanosoma Brucei Rhodesiense”. Editado por John M. Mansfield. PLoS Pathogens 5 (2009): 1000685.
 22. Granado Daniel, Daria Müller, Vanessa Krausel, et al. “Intracellular APOL1 Risk Variants Cause Cytotoxicity Accompanied by Energy Depletion”. Journal of the American Society of Nephrology 28 (2017): 3227–38.