# Decoding redox stress responses in African trypanosomes

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## 1. Introduction

- ***Trypanosoma congolense*** and *T. vivax* are the main cause of **livestock trypanosomiasis** in sub-Saharan Africa.
- Most understanding of trypanosome biology is derived from **the human infectious**, ***T. brucei***, with few studies on *T. congolense.*
- Macrophages are **the key innate immune** cells at early stages of trypanosomiasis. Macrophage-induced **oxidative stress** is important in clearing trypanosome infections but its roles is not fully resolved.
- Redox molecules, such as **nitric oxide** (NO) and **reactive oxygen species** (ROS), are **key immune mediators** that elicit oxidative stress – a potent defence mechanism against trypanosomiasis.
- Therefore this study analysed **host-pathogen interactions** between *T. brucei* and *T. congolense* using **oxidative stress-inducing compounds** and **macrophages**.

## 2. Aims

- To **optimise *in vitro* conditions** to characterise nitric oxide release from a nitric oxide donor, S-nitroso-N-acetylpenicillamine (SNAP), as a foundation for studying the interaction between trypanosomes and NO.
- To determine **differential sensitivity** between *T. brucei* and *T. congolense* bloodstream-forms (BSFs) to SNAP under *in vitro* conditions.
- To elucidate the **survival rates** between *T. brucei* and *T. congolense* BSFs in presence of LPS-stimulated macrophages under *in vitro* conditions.

## 3. Temperature impacts NO release from SNAP

- ***T. congolese* IL3000** grows at **34ºC** and ***T. brucei*** grows at **37ºC**, *in vitro*.
- SNAP is used as **a long term source** of **NO** in experimental studies and was used to understand **trypanosome response** in this study.
- **Differences** in **nitric oxide release** between these **two temperatures** were assessed.

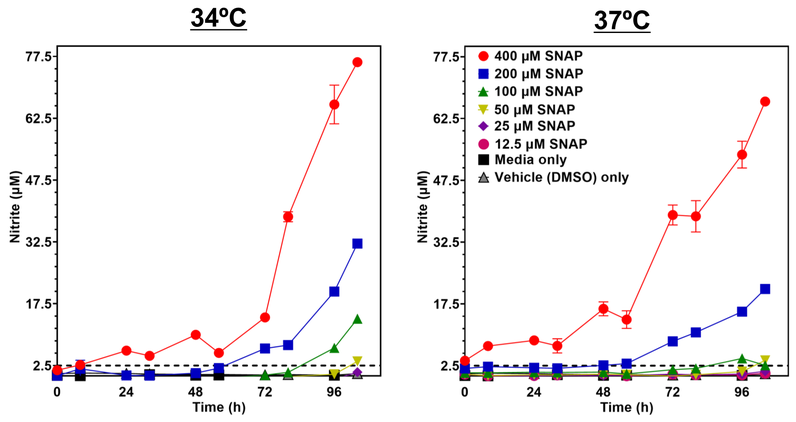


**Figure 1: Different SNAP concentrations incubated at either 34 or 37ºC over time and NO levels assessed in the media by determining the levels of stable end-product, nitrite by Griess assay.**

- **Temperature** impacts **nitric oxide release** from SNAP. These data necessitated **the shift of future experiments** to one temperature (**physiological relevant**; 37ºC).
- *T. congolense* IL3000 isolate from a collaborator which remains viable at 37ºC was explored for **downstream experiments**.

## 4. Differential parasite sensitivity to SNAP

- To **quantify** differential sensitivity of the two species to SNAP, **half maximal inhibitory concentration** ($EC_{50}$) of trypanosome growth were determined.
- The $EC_{50}$ or trypanosome proliferation *in vitro* was determined by the Alamar Blue (AB) assay.

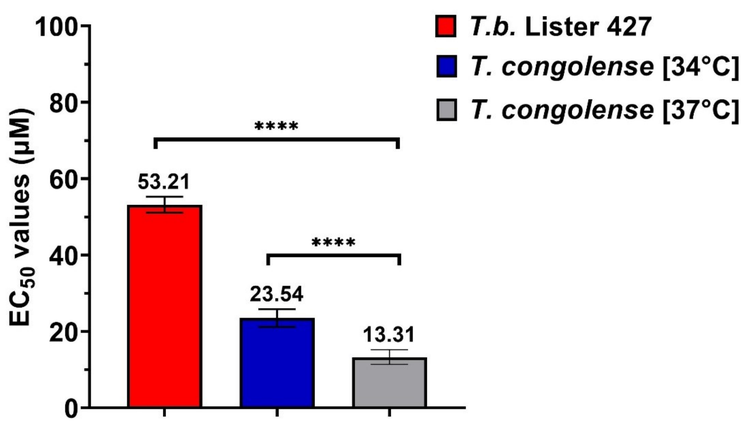


**Figure 2: The bar graphs show 50% inhibitory concentrations for *T. brucei* and *T. congolense* drugged with oxidative stress-inducing compound, SNAP determined by a resazurin based technique, Alamar blue assay.**.

- *T. brucei* is **less sensitive** to SNAP exposure compared to *T. congolense* (34ºC), but similar to *T. congolense* (37ºC).
- With this foundation of data, it was critical to determine **nitric oxide release** from LPS-stimulated **macrophages** (key innate immune cells**)** under **different temperatures** prior to trypanosome co-culture.

## 5. Assessment of NO release from macrophages in two different media

- RAW macrophage-like cells are maintained in the lab at **37ºC** using **RPMI media**.
- **Growth of macrophages** in the **Morrison's Universal Media; MUM (a minimal media)** optimised for **trypanosome growth** was assessed.
- Subsequently, **nitric oxide levels** were **compared** upon LPS-stimulation for macrophages grown in RPMI and minimal media.

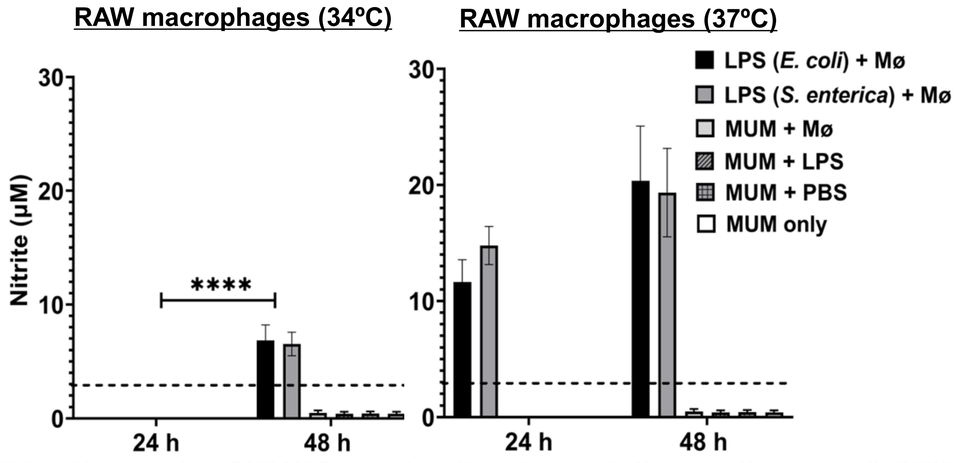


**Figure 4: Nitric oxide release from RAW 264.7 macrophage-like cells grown in Morrison's Universal media (MUM) and RPMI media in response to different strains of LPS stimulation.**

- **Differences** in media composition **did not affect NO release** in response to LPS-stimulation of RAW macrophage-like cells.
- **Different temperatures** resulted in **different levels of NO release** in response to LPS-stimulation of RAW macrophage-like cells.
- Hence, trypanosome-macrophage co-culture experiments were carried out in **MUM** at **37ºC** in the presence of LPS-stimulated macrophages **to assay differential parasite sensitivity**.

## 6. Parasite survival in macrophage co-cultures

- **Two critical hurdles** had been overcome:-
  a) Growth of **multiple trypanosomes species** under the **same media and temperature.**
  b) Growth of **macrophages** in the **same media as the trypanosomes.**
- This enabled the investigation of **differential sensitivity of the two trypanosomes species** in the presence of LPS-stimulated macrophages, *in vitro*.

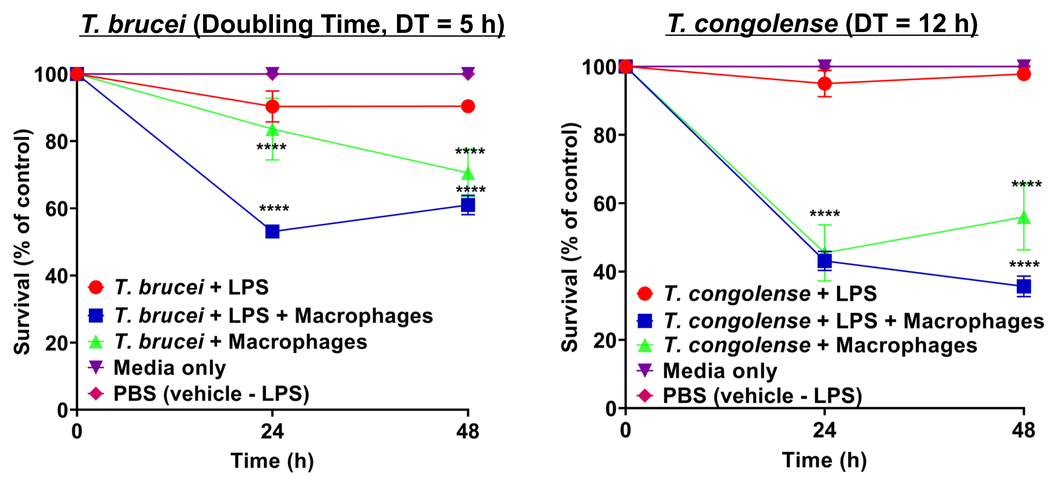


**Figure 5: Survival plots showing differential parasite sensitivity between *T. brucei* and *T. congolense* BSFs in presence of LPS-stimulated RAW macrophage-like cells**.

- *T. brucei* growth is **less affected** by LPS-stimulated macrophages compared to *T. congolense*, *in vitro*. Albeit, the **differential parasite growth rates** should be taken into consideration.
- However, the **underlying biology** behind this phenomenon **requires further studies**.
- Future experiments will aim to investigate **differential sensitivity** of these two trypanosome species in presence of the **clinically relevant bovine macrophages**, *in vitro*.

## 7. Summary

- We investigated the **effect of nitric oxide on** two trypanosome species and **optimised the *in vitro* conditions.**
- *T. brucei* appears to be **less sensitive** to a nitric oxide donor (SNAP) compared to *T. congolense.*
- Similarly, ***T. congolense*** is **less viable** when co-cultured with LPS-stimulated RAW macrophage-like cells compared **to *T. brucei***, *in vitro*.

## 8. Impact

- This research will broaden our knowledge on **the parasite metabolism in the clinically relevant species,** potentially informing on **drug and vaccine target discovery.**
- The **macrophage-trypanosome interaction studies** will inform on **strategies** to **boost the mammalian innate immune system** to facilitate **effective parasite control.**

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