Biochemical Pharmacology, Vol. 36, No. 10, pp. 1750–1751, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00

Effect of 3-aminobenzamide on the frequency of antigen switching in *Trypanosoma brucei in vitro*

(*Received* 8 *August* 1986; *accepted* 24 *November* 1986)

African trypanosomes avoid elimination by the immune responses of the hosts through antigenic variation of the glycoprotein surface coat [1, 2]. In this process, a gene encoding an antigenically different variant surface glycoprotein (VSG) is activated, and the original VSG gene is no longer expressed. The newly expressed gene may be duplicated from a basic copy gene and inserted into either the expression site of the previously expressed gene or another expression site on a different chromosome. Alternatively, the basic copy may be activated without duplication if it is already located at a telomere [2]. There is no evidence that the route of gene activation is dependent on the previously expressed VSG gene. For gene insertion, DNA strand breaks and ligation are necessary, and it is possible that DNA repair mechanisms are involved when VSG gene rearrangements occur.

ADP-ribosyl transferase (ADPRT) mediated polyADP-ribosylation has been implicated in the ligation stage of DNA repair [3]. To determine whether this reaction is required for *Trypanosoma brucei* VSG switching, Cornelissen *et al.* [4] examined the effect of inhibition of polyADP-ribosylation by 3-aminobenzamide on antigenic variation *in vivo*. They concluded that the antigen switching rate of *T. brucei* in rats continuously perfused with 3-aminobenzamide was lower than in the control animals. However, there are a number of potential problems with these experiments. First, the method chosen to determine the frequency of switching is invalid. The proportion of trypanosomes expressing different antigens at any time is dependent on several factors, including the relative growth rates of the new and old variants and the length of time since the population was homogeneous, as well as the switching frequency [5]. Second, the animals in this study showed other effects of the 3-aminobenzamide treatment including significant weight loss. The effect of 3-aminobenzamide and its metabolites on the immune response of whole animals is not known, although mitogen-induced differentiation of isolated human lymphocytes is reduced if 3-aminobenzamide is added before the mitogen [6]. It is possible that the treated animals had a diminished immune response while the control animals would be expected to have produced specific anti-trypanosome antibody in the 6–7 days before being killed. Thus, the greater proportion of switched trypanosomes in the control animals could reflect several changes in the physiological status of the host and the host–parasite relationship rather than a difference in the switch frequency of the antigen. Furthermore, the difference in the values obtained for the switching frequency may not have been significant as a wide range of values has been obtained for the switching frequency in a single antigen type [5].

The problems presented by using whole animals to analyze the effect of 3-aminobenzamide on the frequency of switching in *T. brucei* can be avoided with an *in vitro* system that is not affected by trypanosome growth rates or any immune response. To determine whether 3-aminobenzamide has an effect on the switching frequency, we used an *in vitro* system and a method of analysis that allow accurate determination of the switching frequency. Contrary to the findings of Cornelissen *et al.* [4], we did not find a significant effect of 3-aminobenzamide on the switching frequency.

Methods and results

The effect of 3-aminobenzamide (Sigma) on the antigen switching frequency of the *T. brucei* variant clone 117 (Molteno Institute Trypanozoon antigen type 1.4) [7] was investigated. The gene coding for this antigen type is in the same expression site as the 118 gene in the clone used by Cornelissen *et al.* [4]. The switching frequency was determined by the method described by Lamont *et al.* [5]. Trypanosomes were cultured *in vitro* with an irradiated *Microtus montanus* embryo fibroblast feeder layer [5]. After limiting dilution cloning, a clone was divided into forty-eight separate 1-ml cultures. Freshly prepared 3-aminobenzamide (1.5 mM final concentration) was added to twenty-four of the cultures immediately and also after 3 days. Six days after the clone was split, all the trypanosomes and the feeder layer cells were carefully harvested from the wells and the trypanosomes were counted (control mean, 7.0×10^4; 3-aminobenzamide-treated mean, 6.4×10^4). To ensure that all trypanosomes expressing the original 117 antigen type were eliminated, two steps were taken. First, 10 μl of hyperimmune rabbit antiserum raised against purified 117 variant antigen was added to the cells from each well and incubated at 23° for 20 min. Second, the cells were injected into mice that had been immunized with 117 VSG. Trypanosomes expressing the original antigen type have never been observed at any time during an infection when this double selection is used [5]. The mice were monitored for 3 weeks, and trypanosomes were obtained from all infected mice for determination of the antigen type by immunofluorescence [5].

Two mice receiving either the control or the 3-aminobenzamide-treated cultures developed a patent parasitemia 6–7 days after inoculation. Immunofluorescence assays revealed that the trypanosomes were not expressing the original antigen type. There was no significant effect on the switching frequency of *T. brucei* variant antigen type 117 (Table 1), calculated as described [5]. The difference observed was small, and both values were within the range previously found for this antigen type in similar switching experiments [5]. Similar results were obtained in another experiment using 3-aminobenzamide. The antigen type(s) of the switched trypanosomes could not be determined by immunofluoresence assays with the available antisera.

To ensure that the 3-aminobenzamide was active throughout the incubation period, the inhibitory activity of 3-aminobenzamide was tested after incubation with trypanosomes. Trypanosomes at an initial concentration of 1×10^5/ml were incubated with 10 mM 3-aminobenzamide for 3 days at 37°. Activity was compared with a freshly prepared 10 mM solution using the ADPRT assay of Farzaneh *et al.* [8]. The two solutions were diluted such that the concentrations spanned the published K_i [8]. There was no apparent inhibitory effect of 10 mM 3-aminobenzamide on trypanosome growth or on the feeder cell layer. At each concentration tested, the effect of the inhibitor on the maximum incorporation of NAD and the initial velocity of the reaction was the same for the freshly prepared solution as for the inhibitor that had been incubated in the presence of trypanosomes for 3 days (data not shown). Therefore, the inhibitory activity of 3-aminobenzamide did not decrease with time and throughout the period of the exper-

Table 1. Effect of 3-aminobenzamide on the rate of antigen switching in *Trypanosoma brucei*

Concentration of 3-aminobenzamide (mM)	Trypanosomes injected per mouse	Number of mice infected/ injected	Frequency of switching (switches/tryp/ generation)
0	7.0×10^4	2/24	1.8×10^{-6}
1.5	6.4×10^4	2/24	9.4×10^{-7}

iment was considerably higher than the K_i of 3-aminobenzamide for trypanosome ADPRT, which has been estimated to be 4.3 μM [3].

Discussion

We did not find any significant difference in the frequency of antigen switching *in vitro* in the presence or absence of 3-aminobenzamide at concentrations that exceeded the K_i, contrary to previously published results using an *in vivo* assay [4]. The *in vitro* system used here enabled us to determine the frequency of antigen type switching in a controlled environment free from potential artifacts, including effects of the immune response. The level of 3-aminobenzamide used had no effect on the growth of the parasites.

Clearly for the gene rearrangement necessary for expression of some VSG genes, the parasite must be able to insert DNA into another chromosome and DNA repair mechanisms may be involved. However, the results presented here show that inhibition of ADP-ribosylation does not decrease the frequency of anitgen switching, suggesting several alternative explanations. It is possible that (1)gene insertion in trypanosomes does not use conventional repair mechanisms, (2) ADP-ribosylation is not necessary for DNA repair in trypanosomes, or (3) if gene insertion is inhibited, the parasite preferentially activates telomeric genes, thereby obviating the requirement for gene insertion.

DNA repair systems in trypanosomes have not been studied. Although inhibitors of polyADP-ribosylation inhibit morphological differentiation of *T. cruzi* [9], it is not known whether polyADP-ribosylation is important in DNA repair in trypanosomes. ADP-ribosyl transferase-mediated polyADP-ribosylation has been implicated in the ligation stage of DNA repair [3] in other eukaryotes, although the actual function of polyADP-ribosylation is not clear [10, 11]. However, it has been suggested that ADP-ribosylation is not, in fact, required for DNA repair and, although the role is unclear, it may instead by involved in cellular recovery from DNA damage [10, 11]. If this is true, then the lack of inhibition of VSG gene switching in *T. brucei* observed here is not surprising. It would be possible to investigate whether there is preferential activation of telomeric VSG genes when polyADP-ribosylation is inhibited by 3-aminobenzamide. However, antigenic typing and characterization of the gene rearrangements occurring in our experiments were beyond the scope of the current study.

In summary, we have shown using an *in vitro* system that, contrary to previously published results using *in vivo* analysis, 3-aminobenzamide does not have an effect on the frequency of antigen switching in *T. brucei*. The activity of the 3-aminobenzamide was not diminished under the conditions used in the study.

Acknowledgements—This work was supported NIH Grant AI 21729. We would like to thank Dr Kasturi Haldar for critically reading the manuscript.

Laboratory of Molecular Parasitology
The Rockefeller University
New York, NY 10021, U.S.A.

GRETEL S. LAMONT
GEORGE A. M. CROSS*

REFERENCES

1. J. C. Boothroyd, *A. Rev. Microbiol.* **39**, 475 (1985).
2. J. E. Donelson and A. C. Rice-Ficht, *Microbiol. Rev.* **49**, 107 (1985).
3. K. Ueda, *A. Rev. Biochem.* **54**, 73 (1985).
4. A. W. C. A. Cornelissen, P. A. M. Michels, P. Borst, W. Spanjer, J. A. M. Versluijs Broers, C. Van der Meer, F. Farzaneh and S. Shall, *Biochem. Pharmac.* **34**, 4151 (1985).
5. G. S. Lamont, R. S. Tucker and G. A. M. Cross, *Parasitology* **92**, 355 (1986).
6. A. P. Johnstone and G. T. Williams, *Nature, Lond.* **300**, 368 (1982).
7. G. A. M. Cross, *Parasitology.* **71**, 393 (1975).
8. F. Farzaneh, S. Shall, P. Michels and P. Borst, *Molec. biochem. Parasit.* **14**, 251 (1985).
9. G. T. Williams, in *ADP-Ribosylation of Proteins* (Eds. F. R. Althaus, H. Hilz and S. Shall), p. 358. Springer, Berlin (1985).
10. W. F. Morgan, M. C. Djordjevic, K. M. Milam, J. L. Schwartz, C. Borek and J. E. Cleaver, in *ADP-Ribosylation of Proteins* (Eds. F. R. Althaus, H. Hilz and S. Shall), p. 277. Springer, Berlin (1985).
11. E. L. Jacobson, J. Y. Smith, V. Nunbhakdi and D. G. Smith, in *ADP-Ribosylation of Proteins* (Eds. F. R. Althaus, H. Hilz and S. Shall), p. 244. Springer, Berlin (1985).

* Address all correspondence to: Dr. George A. M. Cross, Laboratory of Molecular Parasitology, The Rockefeller University, 1230 York Ave., New York, NY 10021.