Virtual symposium SAP 2021

Working harder, making better: novel insights in the biology and treatment of *Trypanosoma cruzi* infection

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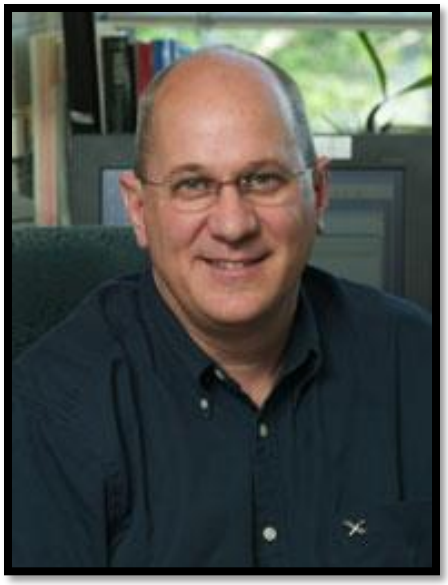
Mundo Sano

Our society has proposed to strengthen dialogue between professionals interested in basic and clinical aspects of Chagas disease (CHD). This is how we can move forward to genuine translational research aimed at improving the treatment of Chagas disease. For this aim, we programmed a virtual symposium where invited outstanding researchers will show the state of the art of basic and clinical research. Lectures will be followed by a broad discussion with the participation of invited speakers, moderators, partners, and special guests.

In this symposium we will discuss new concepts on the biology of *Trypanosoma cruzi* and its potential impact on the etiological treatment in experimental infection and clinical settings. We will also carry out an update on the knowledge of pharmacokinetics and pharmacodynamics of drugs currently used in CHD, knowledge recent understood as the disease is now included in the so-called neglected diseases. Next, we will discuss the concepts of cure in CHD in current randomized clinical trials such as BENDITA and TESEO where adult patients are treated with different doses and administration schedules of nifurtimox (NFX) and benznidazole (BZN), as well as the long term impact of treatment with NFX or BZN in children and adults followed for more than 20 years in Santa Fe Argentina.

November 2021 Adelina Riarte and Catalina Alba Soto



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Parasitological cure in Chagas disease: Parasite biology impacts treatment efficacy

The prospects for preventing human exposure to *Trypanosoma cruzi* infection is rather bleak: *T. cruzi* circulates in too many hosts to be eradicated as a human infection risk, and vector control, while effective, is expensive and labor-intensive and thus difficult to maintain. And there is scant evidence that a vaccine can be produced that will prevent infection. In contrast, there is substantial data that drug treatment can achieve parasitological cure, and more importantly, can moderate the development of severe disease.

However, drug therapies are not without their own problems as the current drugs and the treatment protocols in which they are used, often fail to achieve cure. Drug discovery methods and treatment protocol design are often formulaic, e.g. identify compounds that kill the majority of individuals in a pathogen population but don't damage the host, and deliver the compounds so as to keep concentrations near or above an (often in vitro determined) effective concentration in the host. Such approaches ignore the complexities of some pathogens, and in particular the variation among individuals in pathogen populations. For *T. cruzi*, there are at least two aspects of parasite biology that current drugs and treatment protocols do not consider: 1) the ability of *T. cruzi* to infect a wide range of host cell and tissue types (which may vary with respect to drug exposure), and 2) the propensity of a proportion of *T. cruzi* amastigotes to enter a metabolically quiescent state within which they are insensitive to currently used drugs. We believe that considering these properties in the drug discovery and treatment protocol design can lead to the discovery of better drugs and achieve better outcomes using currently available drugs.

In view of these aspects of parasite biology, we trialed a treatment protocol that used higher individual doses of benznidazole (BZN), to assure that the drug reached all tissues at a concentration that was sufficient to kill all metabolically active amastigotes, but given less frequently, thus providing windows for dormant parasites to re-enter the proliferative cycle. The latter may be particularly important for compounds such as BZN which have cytotoxic and cytostatic activity (and thus yield an **increased** fraction of non-replicating (apparently dormant) parasites in vitro). Pursuing this approach, we have found that BZN at 2- to 5-fold higher doses (than the normal daily dose) but delivered only once or twice per week, cures infections in mice that are not cured by the

standard daily dosing regimen. A similar regimen also shows promise in naturally infected, non-human primates and dogs and without increased adverse events.

We need a much better understanding of dormancy in *T. cruzi*. It is clear from both in vivo and in vitro studies that a lag in replication is quite common in *T. cruzi*, that this cessation occurs spontaneously, not requiring specific stressors or stimuli, and that it is transient. But the depth of dormancy (e.g. residual metabolic activity occurring in non-replicating/dormant amastigotes) is not yet clear. These characteristics will be important factors in determining how to attack dormancy therapeutically. Toward this end, we have designed drug-screening protocols that allow assessment of not only trypanocidal activity of compounds but simultaneously, the impact of library compounds on dormant amastigotes. Such screens should provide much-needed new options for treatment of established *T. cruzi* infections as well as tool compounds for better understanding the dormancy phenotype.

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Pharmacokinetics and pharmacodynamics of benznidazole: Getting to know an old friend in the fight against Chagas disease

Chagas disease (CD) is a worldwide problem, with over 8 million people infected. In spite of the fact that CD was first described over a century ago, there are currently only two drugs, benznidazole (BNZ) and nifurtimox (NFX), available for CD treatment. Unfortunately, even though both BNZ and NFX were developed half a century ago, their pharmacology was only partially studied until recently, and many aspects of its human pharmacokinetics and pharmacodynamics remain unknown to this day (a fact that would be unacceptable for any other drug for any other non-neglected health condition in the developed world).

Besides the obvious problems with limited clinical pharmacology information for these medications, a further, less well acknowledged problem, is the lack of pharmacological information in special populations such as infants, children, adolescents, and pregnant women. This issue becomes more acute if we consider that most CD infections are acquired during childhood (either by congenital transmission, or by early contact with the vector in childhood in endemic areas), and timely diagnosis and treatment in childhood and pregnancy, if effective, could significantly prevent later complications and congenital transmission. Unfortunately, clinical data on long-term effectiveness, and on safety of pediatric and pregnancy treatments have been scarce, and current treatment recommendations are mostly based on a few clinical trials in infants and children, with limited long term follow up. Lack of long term follow up data also prevents development of accurate biomarkers of early response that are urgently needed for further development of new medications and treatment strategies, as serology (so far, the “gold standard” for certification of treatment response) is imperfect at best and has been severely questioned as the main method to assess treatment response, with good reason.

BZN, a nitroheteroerocyclic drug developed by Roche® in the 1970’s is believed to rely on intracellular activation to reactive species that bind to parasite transcription machinery, among other cell components, and lead to parasite death. This bioactivation is believed, with little evidence, to occur mainly in the parasite and not in mammalian tissues, which would protect human tissues from significant drug toxicity. In fact, some (limited) evidence points towards BNZ activation and detoxification mainly in the liver, which would prevent further toxicity (but which also suggests

that situations in which detoxification mechanisms may be depleted, such as alcohol abuse) may increase the risk for adverse events.

Pediatric and adult BNZ pharmacokinetics studies (some incorporating quantitative PCR for parasite detection) have recently been conducted, suggesting that BNZ is not only highly and rapidly effective in elimination of circulating parasites, but that it can also lead to sustained responses in virtually all children treated, and in a large proportion of adults. Unfortunately, whether this response is enough to prevent organ involvement decades later is still a matter of debate (largely due to lack of investment in appropriate long-term follow up studies of treated patients). Treatment with BZN is considered contraindicated during pregnancy in most guidelines, due to limited evidence on safety. Unfortunately, even if pregnancy exposures seem to have taken place, their outcomes have not been reported systematically in detail, so it is not possible to judge at this stage whether actual fetal risks exist with BNZ treatment. Exposure during breastfeeding, on the other hand, has been evaluated in a small prospective clinical trial that produced evidence that BNZ is not expected to cross into breastmilk in any clinically significant amounts.

Use of BNZ in patients with renal or hepatic impairment remains controversial due to lack of pharmacokinetics and safety data, but given that BNZ is almost completely metabolized, renal elimination seems to only play a marginal role in its clearance and use in kidney failure would be possible with appropriate monitoring for adverse events. Similarly, in cases requiring emergency treatment (e.g. CD meningoencephalitis), hepatic impairment should not be an obstacle for treatment assuming that strict monitoring can be implemented, and recent preliminary data from patients with HIV-CD co-infection seems to support this suggestion.

Ongoing investigations aim to optimize BZN therapy by adjusting the current standard regimen or by combining BZN with new chemical entities. These studies are assessing alternatives that improve safety while maintaining or increasing the efficacy of BZN. Timely diagnosis and antitrypanosomal treatment are critical components of programs to eliminate CD as a public health problem and can dramatically reduce the heavy burden of morbidity and mortality caused by the disease.

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New regimens and doses of Benznidazole and Nifurtimox for the treatment of indeterminate chronic chagas disease in adult patients.

Chagas disease (CD), caused by the protozoan parasite Trypanosoma cruzi, affects between 6 and 8 million people, mainly in Latin America. CD is considered among the most neglected diseases in the world. There are still huge differences between the number of people affected by CD and those receiving treatment for this disease.

Currently approved treatments for CD are benznidazole (BZN) and nifurtimox (NFX), but the lack of convincing and consistent efficacy data and concerns about safety and tolerability profiles have limited the widespread implementation of these drugs available since the early years. 1970s, whose treatment recommendation guidelines vary significantly from one country to another.

The time of administration of treatment with these drugs has not been systematically evaluated. Current treatment regimens and dosing ranges have been derived from patient series from decades past and with very limited head-to-head comparisons.

The efficacy of both drugs in patients with T. cruzi infection is variable and depends on the stage of the disease, the dose of the drug, the age of the patients, and the infecting Trypanosoma cruzi strain or genotype.

The need for safer and more effective treatments and the availability of early biomarkers of therapeutic efficacy are the main challenges in the etiological treatment of chronic CD.

In the framework of helping to solve these challenges, two clinical trials have been carried out in three Centers of the Chagas Patient Care Platforms, Cochabamba, Tarija and Sucre: the Bendita study that has already been completed and published and the TESEO study that they will be described in this presentation.

New monotherapy regimens with benznidazole and in combination with fosravuconazole for the treatment of Chagas disease (BENDITA): a double-blind, randomized phase 2 trial. A multicenter, randomized, phase 2, double-blind, branched trial was conducted in three Chagas Patient Care Centers in Bolivia. The patients were adults aged 18 to 50 years with indeterminate chronic CD, confirmed by serological tests and positive qualitative PCR. Patients were randomly assigned to one of seven treatment groups: Participants were assigned to BZN 300 mg daily for

8 weeks, 4 weeks or 2 weeks, BZN 150 mg daily for 4 weeks, BZN 150 mg daily plus fosravuconazole for 4 weeks, BZN 300 mg once weekly for 8 weeks plus fosravuconazole or placebo, with a 12-month follow-up period.

All arms of the study, both monotherapy and in combination, showed efficacy. Eighty percent of the patients in the group that received the standard dose of BZN (300 mg / day) for two weeks, showed absence of the parasite in their blood six and 12 months after treatment, a comparable result which was observed with the group that received the standard eight-week treatment.

New chemotherapy regimens and biomarkers (BMK) for Chagas disease (TESEO study). We will determine the safety and efficacy of four BZN or NFX dosing regimens in adults with the indeterminate (asymptomatic) form of Chagas disease or with mild cardiomyopathy (Kushnir grade 1), evaluating the concomitant adverse effects and determining the number of patients that have sustained clearance of parasitaemia. evaluated by negative quantitative PCR (qPCR) at the end of treatment (EOT) and at 4, 6, 12, 18, 24, 30 and 36 months of follow-up.

In the same way, we will evaluate the changes in two different classes of BMKs: 1) derived from the host and 2) BMK derived from parasites. These BMKs will be evaluated at the beginning of the study, during treatment, at the end of treatment and at 4, 6, 12, 18, 24, 30 and 36 months of follow-up. We will determine if changes in BMK levels correlate with parasitemia, measured by qPCR.

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Impact of trypanocidal therapy in children and adults with chronic Trypanosoma cruzi infection followed for more than 20 years in the city of Santa Fe, Argentina

Nifurtimox (NFX) and benznidazole (BZL) are the only drugs in clinical use for Trypanosoma cruzi infection and were developed 50 years ago. Both drugs demonstrated high efficacy in the acute phase of the infection, but their evaluation in the chronic phase has had, and still has, significant limitations. The clinical course of chronic chagasic cardiomyopathy (CCC) is difficult to predict; it occurs in 20-30% of those infected after 1 or more decades post-infection. In addition, the serological tests used in the diagnosis of the infection remain positive for several years, even when the treatment has been effective. Parasitological tests in the chronic phase have low sensitivity and are only useful when positive, indicating treatment failure. Furthermore, there are no early markers of "serological cure" and prognostic markers of clinical evolution. In this context, randomized clinical trials including a high number of infected individuals without clinical signs or symptoms of CCC at study onset with long term follow up are needed. Such trials are expensive and difficult to carry out due to inevitable loss of patients to follow-up.

In the late 1970s and early 1980s, our research center began an observational cohort study of chronically infected children and adults. One group received treatment with NFX or BZL and another remained untreated. The dose and duration of treatment was: NFX = 12-15 mg / kg / day in children and 8-10 mg / kg / day in adults for 45-60 days; BZL = 5 mg / kg / day in children and adults for 30 days.

To evaluate the efficacy of trypanocidal treatment in children, electrocardiographic and serological evolution (3 conventional tests for detection of anti-*T. cruzi* antibodies) was compared in 82 chronically infected patients with an average follow-up of 25 years: 41 had received treatment (NFX = 8; BZL = 33) at the ages of 2 to 14 years and, 41 remained untreated.

BZL was well tolerated, requiring treatment interruption in only 2 patients due to maculopapular erythema and vomiting. Adverse effects with NFX were more frequent but children were able to complete treatment.

During the study period, negative seroconversion was observed in 92.7% of the patients who

received treatment. All children younger than 8 years became seronegative. No serological changes were observed in the untreated group.

Only 2 patients in each group had baseline ECG abnormalities. At the end of the follow-up, 9.75% of the treated patients and 21.95% of those who remained untreated showed electrocardiographic disturbances. Abnormalities that are strongly associated with CCC were only observed in the untreated group (complete right bundle branch blockade + left anterior hemiblockade; frequent ventricular extrasystoles; left anterior hemiblockade). In the treated group, one of the 2 patients who presented altered initial ECG (PR = 0.20) at the age of 6 years, developed a 1st degree AV block after 13 years of follow-up. This mild conduction disorder is not clearly associated with CCC. Using the logistic regression model, the probability of developing electrocardiographic abnormalities was significantly reduced among treated patients (OR = 0.18, 95% CI = 0.04-0.79, p = 0.023).

The therapeutic evaluation in adults with chronic infection was carried out on 113 patients without signs or symptoms of CCC at baseline, with an average follow-up of 30 years. The age at admission was between 15 and 49 years old. Fifty-two infected (NFX = 24; BZL = 28) received treatment and 61 remained untreated. Five patients had to suspend treatment due to intolerance: one treated with NFX for vomiting and 4 treated with BZL for pruritus and maculopapular erythema. All of them underwent treatment with NFX, starting 20-30 days after the suspension. The patients treated with BZL presented fewer side effects in relation to those treated with NFX (18% vs 33%), but the level of intolerance was higher.The serological evolution in the treated group was as follows: a) 67.3% persistently negative serology (NFX = 66.6%; BZL = 67.8%); b) 15.4% showed undefined serology (NFX = 20.8%; BZL = 10.7%); c) 17.3% remained unchanged in the level of Ab anti-T. cruzi (NFX = 12.5%; BZL = 21.4%). Serology of untreated patients remained positive.

During the study period, CCC developed: 5.7% vs 14.7% of treated and untreated patients, respectively. They presented anomalies in the ECG suggestive of Chagas disease but with associated pathologies (obesity, diabetes, arterial hypertension), 3.8% of the treated group and 9.8% in the untreated group. These differences were significant.

The small sample size was a considerable limitation. In addition, during 30 years of follow-up of adult individuals, a low incidence of events compatible with CCC was observed, both in treated and untreated patients. All study participants remained in the city of Santa Fe with very low or no risk of vector infection, with similar origin and socioeconomic status.

Treatment with BZL for 30 days was sufficient to achieve serological "cure" in a high percentage of children and adults. The tolerability level of NFX was slightly higher than BZL, while the efficacy profile of both drugs did not differ.

Although more evidence is needed on the benefits of trypanocidal treatment in the chronic phase of the infection, the observed findings after more than 2 decades of follow-up indicate that treatment improves clinical evolution in children and adults in relation to those who remained untreated.