

Pediatric Clinical Pharmacology Studies in Chagas Disease

Focus on Argentina

Facundo Garcia-Bournissen,¹ Jaime Altcheh,² Norberto Giglio,³ Guido Mastrantonio,⁴ Carlos Omar Della Védova⁴ and Gideon Koren¹

1 Division of Clinical Pharmacology and Toxicology, Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada

2 Division of Parasitology, Hospital de Niños 'Ricardo Gutierrez' de Buenos Aires, Buenos Aires, Argentina

3 Division of Epidemiology, Hospital de Niños 'Ricardo Gutierrez' de Buenos Aires, Buenos Aires, Argentina

4 LaSeISiC (UNLP-CIC-CONICET), Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Buenos Aires, Argentina

Abstract

Chagas disease is a neglected parasitic disease endemic in the Americas. It mainly affects impoverished populations and the acute phase of the infection mostly affects children. Many cases have also been detected in nonendemic countries as a result of recent migratory trends. The chronic phase is relatively asymptomatic, but 30% of patients with chronic infection eventually develop cardiac and digestive complications that commonly lead to death or disability. Only two drugs are available for the treatment of Chagas disease, benznidazole and nifurtimox. These drugs have been shown to be effective in the treatment of both acute and early chronic phases in children, but the pharmacokinetics of these drugs have never been studied in this population. We have set out to conduct a pharmacokinetics study of benznidazole in a pediatric population with Chagas disease. The results of this study are expected to allow better estimation of the optimal doses and schedule of pharmacotherapy for Chagas disease in children.

Chagas disease is one of the most neglected diseases in the world. It is a zoonosis caused by infection with the parasite *Trypanosoma cruzi*. The geographic distribution of this disease is wide, from southern USA to southern Argentina, with endemic characteristics seen from the north of Mexico to the north of Argentina.^[1] Many cases have been detected in nonendemic countries in the last decade due to recent migratory trends and improvements in screening techniques.^[2]

There are currently about 15 million people infected in Latin America; approximately 200 000 new cases and over 15 000 deaths as a result of complications of the disease occur every year.^[1,3] *T. cruzi* can be transmitted by hematophagous arthropod vectors (triatomines), blood transfusions,^[4] or vertical transmission (i.e. mother to child).^[5,6] Most of the new infections occur in children, either by vector or vertical transmission,^[7] with an estimated mortality of approximately 5%, mainly as a result of myocarditis and meningoencephalitis.^[1,7]

The acute phase of the disease lasts approximately 4 weeks, and is followed by a chronic asymptomatic stage that eventually leads to irreversible heart disease in up to 30% of infected patients many years after the initial infection.^[1,7] The chronic stage is when most of the mortality and disability associated with Chagas disease occurs. The most common complications observed in this phase include dilated cardiomyopathy, arrhythmias, heart failure, sudden death, megaesophagus, and megacolon.^[1,7]

Estimated economic costs associated with Chagas disease in Latin America are estimated to be in the order of \$US6 billion per year. The majority of the costs are associated with healthcare expenses and with the loss of work productivity in the young population affected.^[3]

In Argentina, where 89% of the population lives in urban areas, in particular in or around the country's capital Buenos Aires, most of the patients infected with Chagas disease live in an urban setting. The prevalence of Chagas disease in children and women

attending primary care centers in Buenos Aires has been estimated to be about 2%.^[8] Most new infections in urban areas are due to vertical transmission and occasionally due to blood transfusions.^[5]

Pharmacology, Efficacy, and Tolerability of Treatments for Chagas Disease

Pharmacotherapy of Chagas Disease

There are only two drugs currently available for the treatment of Chagas disease, nifurtimox and benznidazole.^[1]

Benznidazole, a nitroimidazole compound, was developed almost 40 years ago.^[9] The mechanism of action of benznidazole is still not clear, but it has been shown to induce reductive stress leading to covalent modification of proteins and other macromolecules in *T. cruzi*.^[1,10] Benznidazole, and possibly its metabolites, induces the production of free radicals that can damage the DNA of the parasite, as well as inhibit RNA and protein synthesis.^[9,11]

Benznidazole followed one-compartment model pharmacokinetics in healthy men who volunteered to receive oral benznidazole 100 mg.^[12-14] The peak plasma concentration of 2.2–2.8 mg/L was observed 3–4 hours post dose. The estimated elimination half life ($t_{1/2}$) was 12 hours, and the apparent volume of distribution (Vd) was 0.56 L/kg.^[13] In a study of multiple doses of benznidazole in adults with Chagas disease,^[14] doses of 7 mg/kg produced overall plasma concentrations similar to those estimated from the pharmacokinetic parameters in the previous study in healthy volunteers.^[13] Benznidazole is eliminated by the liver, with <20% of the drug excreted unchanged by the kidney. There are virtually no studies on the metabolism of benznidazole in humans, and very little information is available from animal studies.^[10,15-20]

Nifurtimox leads to the production of free radicals and reductive damage of parasite cellular components such as proteins and nucleic acids. Inhibition of the enzyme tripanotionine reductase is believed to also play a role in the parasiticide action of nifurtimox through inhibition of tripanotionine metabolism; tripanotionine is a molecule that is important for the detoxification of reactive intermediate products of the metabolism of the parasite.^[9-11] Similar to benznidazole, the pharmacokinetics of nifurtimox seem to follow a one-compartment model.^[21,22] Pharmacokinetics studies on this drug published to date were carried out on adult healthy volunteers and in patients with chronic renal failure. Healthy adults who received a dose of oral nifurtimox 15 mg/kg had a median peak plasma concentration of 0.7 mg/L that occurred between 3 and 4 hours after administration. The estimated $t_{1/2}$ of the drug was 3.5 hours and the Vd was 529 L.^[21] Nifurtimox is eliminated mainly by the liver, with <1% leaving the body un-

changed.^[23,24] It has wide tissue distribution, as reflected by the large Vd. Studies on the mechanisms of biotransformation of nifurtimox in the liver suggest that cytochrome P450 enzymes are likely to be responsible for most of the metabolism.^[23-25]

Clinical Trials in Pediatric Chagas Disease

A small number of pediatric clinical trials in Chagas disease have been conducted, which have consistently shown that the treatment of children in the acute or in the indeterminate (quiescent) phase leads to a cure.^[26-34] These results have prompted the release of guidelines for the pharmacologic treatment of pediatric Chagas disease indicating that all children <15 years of age with Chagas disease should be treated for 60 days with one of the two available drugs, irrespective of the phase of the infection.^[35,36]

Pharmacologic treatment of Chagas disease in the early chronic phase is effective and carries a high cure rate.^[3,26-33,35,37-40] It was previously thought that treatment of adults in the chronic phase was not effective, but this concept has been challenged by small clinical trials suggesting some degree of clinical improvement. Trials to evaluate the effectiveness of long-term treatment in reducing complications in adult patients in the chronic phase are currently underway.^[41,42]

In spite of the evidence of the effectiveness of benznidazole and nifurtimox in children, the information available on the pharmacokinetics and pharmacodynamics of these drugs in children is extremely limited.^[35] To date, dosing decisions in children have been made on the basis of the scarce data available from the adult population, and have not taken into account any pharmacokinetic characteristics of these drugs in children. To make matters worse, no appropriate pediatric formulations (e.g. liquid formulation) are available, which all too frequently forces healthcare providers to resort to pill fractionation, a methodology fraught with difficulties and risks.^[43-45]

The most commonly observed adverse drug reactions are very similar for both benznidazole and nifurtimox in pediatrics and include nausea, anorexia, headache, gastrointestinal discomfort, and arthralgias.^[26] Maculopapular rash and pruritus are also relatively frequent, and are a common reason for treatment discontinuation. More severe reactions associated with benznidazole treatment, such as severe cutaneous reactions, peripheral neuropathy, and granulocytopenia, are rarely observed in children, but can be relatively frequent in adults.^[26] It is not currently known whether there is a correlation between these adverse reactions and drug plasma levels, mainly because there is virtually no information on plasma levels in children. Nevertheless, some adverse drug reactions associated with these drugs, such as peripheral polyneuropathy and gastrointestinal complications, seem to be dose-dependent

which would suggest a correlation to plasma levels. Peripheral polyneuropathy, in particular, is a frequent observation in the adult population (about 20% of treated patients), but is rare in children.^[29,46,47] Its incidence and severity in adults are dose-dependent; this has been confirmed in experiments in dogs.^[47,48]

In response to this dearth of information on benznidazole and nifurtimox in the pediatric population, the Scientific Working Group on Chagas Disease, Special Programme for Research and Training in Tropical Diseases (TDR), WHO/Pan American Health Organization (PAHO) has signaled the study of the pharmacokinetics of these agents in children as a research priority.^[49]

Pediatric Pharmacology Studies

Pharmacokinetic studies have traditionally involved a limited number of patients, usually adult healthy volunteers. The pharmacokinetic profile of the studied drug would usually be characterized extensively in each individual participant, which requires a large number of samples to be obtained. The requirement for extensive sampling makes these studies very difficult to perform in pediatric populations due to ethical and logistical limitations (e.g. blood volume restrictions, inordinate number of required punctures).

In the last few decades, a new approach to pharmacokinetics, population pharmacokinetics, has gained momentum as the method of choice when studying vulnerable populations such as children. This method changes the focus away from the complete estimation of the pharmacokinetic parameters in each individual participant towards the estimation of the parameters (and their variability) in the whole population. In order to estimate the pharmacokinetics of a drug from a population perspective, a larger number of individuals is required to participate, but the amount of samples per individual is greatly reduced (to as few as two samples in some study designs).^[50-55] This milder burden on the individual patient makes population pharmacokinetics the ideal choice for studies in children. The US FDA and the European Medicines Agency recommend this method as the standard for pharmacokinetic studies in children.^[56-59]

Study of the Pharmacokinetics of Benznidazole in Children with Chagas Disease in Buenos Aires, Argentina

Given the absolute absence of data on the pharmacokinetics of benznidazole in the pediatric population, we set out to perform a population pharmacokinetics study in children receiving this drug for the treatment of Chagas disease. Benznidazole was chosen over nifurtimox because of accessibility issues; benznidazole was relatively easier to obtain in Buenos Aires at the time that the study was started.

The study^[60] was designed as an observational, prospective, population pharmacokinetics study, enrolling children with Chagas disease of both sexes who were 2–12 years of age and eligible for treatment with benznidazole, as per current treatment protocols.^[5,26,27,29] The diagnostic criteria of Chagas disease required for patient inclusion in the study includes at least two positive serologic tests for *T. cruzi* infection (ELISA, hemagglutination, and particle agglutination tests). Informed consent signed by the caregivers and the assent of the patient is required. Patients with a history of hypersensitivity to benznidazole or any of the drug excipients, immunocompromised patients, and patients with altered hepatic or renal function are excluded from participation.

Patient recruitment is currently taking place at the Parasitology Division, Children's Hospital 'R Gutierrez' of Buenos Aires, Buenos Aires, Argentina, where these patients are routinely treated and followed up. Recruitment is carried out by a team of pediatric investigators headed by Dr Jaime Altcheh, the principal investigator. A total of 50 patients will be enrolled in the study.

The patients will receive, as per the routine treatment protocol of Chagas disease, oral benznidazole (RADANIL®, Roche, Buenos Aires, Buenos Aires, Argentina) 5–8 mg/kg/day twice daily for 60 days. Treatment will occur in an outpatient setting and will follow the usual management protocol for children with Chagas disease. Benznidazole is provided free of charge by the National Agency of Medications, Food, and Technology (ANMAT), National Ministry of Health, Buenos Aires, Argentina. Benznidazole is extracted and measured (by high-performance liquid chromatography) according to published protocols.^[17-21]

We expect the recruitment phase of the study to take an estimated 24 months. The study is being conducted according to the guidelines of the International Conference on Harmonization (ICH6), Good Clinical Practice guidelines (GCP), the declaration of Helsinki, the UNICEF convention on the rights of children, and the regulations of the ANMAT.

Conclusion

Chagas disease is a chronic infection that is usually acquired in infancy but that leads to deleterious consequences for the patient and society many years after the initial presentation. Many aspects of the treatment of this disease still remain unstudied, in particular the optimal doses and treatment schedules. In spite of the currently available drugs (benznidazole and nifurtimox) being effective in children, there is still a dire need for new, safer drugs for the acute phase and drugs that are effective for the late chronic phase of the disease in both children and adults. In spite of the public health and economic implications of this disease for Latin America, very few

efforts are being directed towards the development of new medications.

We have attempted to carry out the first pharmacokinetics study of benznidazole in the pediatric population. We expect that the results of this study will lead to a better understanding of, and eventually significant improvements in, the pharmacotherapy of Chagas disease in children.

Acknowledgments

No sources of funding were used to assist in the preparation of this article. The authors have no conflicts of interest that are directly relevant to the content of this article.

References

- Urbina JA, Docampo R. Specific chemotherapy of Chagas disease: controversies and advances. *Trends Parasitol* 2003; 19 (11): 495-501
- Schmunis GA. Epidemiology of Chagas disease in non-endemic countries: the role of international migration. *Mem Inst Oswaldo Cruz* 2007; 102 Suppl. 1: 75-85
- WHO Expert Committee on the Control of Chagas Disease, World Health Organization. Control of Chagas disease: Second report of the WHO Expert Committee. 905ed. Geneva: World Health Organization, 2002
- Schmunis GA. Risk of Chagas disease through transfusions in the Americans [in Spanish]. *Medicina (B Aires)* 1999; 59 Suppl. 2: 125-34
- Freilij H, Altcheh J. Congenital Chagas' disease: diagnostic and clinical aspects. *Clin Infect Dis* 1995; 21 (3): 551-5
- Gurtler RE, Segura EL, Cohen JE. Congenital transmission of *Trypanosoma cruzi* infection in Argentina. *Emerg Infect Dis* 2003; 9 (1): 29-32
- Teixeira AR, Nascimento RJ, Sturm NR. Evolution and pathology in Chagas disease: a review. *Mem Inst Oswaldo Cruz* 2006; 101 (5): 463-91
- Berenstein A, Tarlovsky A, Siniawsky S, Delliafonte A, Ibarra S, Callapa J, Moscatelli G, Biancardi M, Folini ME, Garcia M, and Freilij H y Altcheh J. Estudio de prevalencia de enfermedad de Chagas en Centros de Salud de la ciudad de Buenos Aires. 34to Congreso Argentino de Pediatría, ciudad de Córdoba, Argentina. 2006
- Raether W, Hanel H. Nitroheterocyclic drugs with broad spectrum activity. *Parasitol Res* 2003; 90 Suppl. 1: S19-39
- Moreno SN, Docampo R, Mason RP, et al. Different behaviors of benznidazole as free radical generator with mammalian and *Trypanosoma cruzi* microsomal preparations. *Arch Biochem Biophys* 1982; 218 (2): 585-91
- Docampo R. Sensitivity of parasites to free radical damage by antiparasitic drugs. *Chem Biol Interact* 1990; 73 (1): 1-27
- Lau AH, Lam NP, Piscitelli SC, et al. Clinical pharmacokinetics of metronidazole and other nitroimidazole anti-infectives. *Clin Pharmacokinet* 1992; 23 (5): 328-64
- Raaflaub J, Ziegler WH. Single-dose pharmacokinetics of the trypanosomicide benznidazole in man. *Arzneimittelforschung* 1979; 29 (10): 1611-4
- Raaflaub J. Multiple-dose kinetics of the trypanosomicide benznidazole in man. *Arzneimittelforschung* 1980; 30 (12): 2192-4
- Richle RW, Raaflaub J. Difference of effective antitypanosomal dosages of benznidazole in mice and man: chemotherapeutic and pharmacokinetic results. *Acta Trop* 1980; 37 (3): 257-61
- Workman P, White RA, Walton MI, et al. Preclinical pharmacokinetics of benznidazole. *Br J Cancer* 1984; 50 (3): 291-303
- Lee FY, Workman P. Altered pharmacokinetics in the mechanism of chemosensitization: effects of nitroimidazoles and other chemical modifiers on the pharmacokinetics, antitumour activity and acute toxicity of selected nitrogen mustards. *Cancer Chemother Pharmacol* 1986; 17 (1): 30-7
- Workman P, Walton MI, Lee FY. Benznidazole: nitroreduction and inhibition of cytochrome P-450 in chemosensitization of tumour response to cytotoxic drugs. *Biochem Pharmacol* 1986; 35 (1): 117-9
- Lee FY, Workman P, Cheeseman KH. Misonidazole and benznidazole inhibit hydroxylation of CCNU by mouse liver microsomal cytochrome P-450 in vitro. *Biochem Pharmacol* 1987; 36 (8): 1349-55
- Walton MI, Workman P. Nitroimidazole bioreductive metabolism: quantitation and characterisation of mouse tissue benznidazole nitroreductases in vivo and in vitro. *Biochem Pharmacol* 1987; 36 (6): 887-96
- Gonzalez-Martin G, Thambo S, Paulos C, et al. The pharmacokinetics of nifurtimox in chronic renal failure. *Eur J Clin Pharmacol* 1992; 42 (6): 671-3
- Paulos C, Paredes J, Vasquez I, et al. Pharmacokinetics of a nitrofurantoin compound, nifurtimox, in healthy volunteers. *Int J Clin Pharmacol Ther Toxicol* 1989; 27 (9): 454-7
- Medenwald H, Brandau K, Schlossmann K. Quantitative determination of nifurtimox in body fluids of rat, dog and man. *Arzneimittelforschung* 1972; 22 (9): 1613-7
- Duhm B, Maul W, Medenwald H, et al. Investigations on the pharmacokinetics of nifurtimox- 35 S in the rat and dog. *Arzneimittelforschung* 1972; 22 (9): 1617-24
- Masana M, de Toranzo EG, Castro JA. Studies on nifurtimox nitroreductase activity in liver and other rat tissues. *Arch Int Pharmacodyn Ther* 1984; 270 (1): 4-10
- de Andrade AL, Zicker F, de Oliveira RM, et al. Randomised trial of efficacy of benznidazole in treatment of early *Trypanosoma cruzi* infection. *Lancet* 1996; 348 (9039): 1407-13
- Sosa ES, Segura EL. Treatment of *Trypanosoma cruzi* infection in the indeterminate phase: experience and current guidelines in Argentina [in Spanish]. *Medicina (B Aires)* 1999; 59 Suppl. 2: 166-70
- Altcheh J, Biancardi M, Lapena A, et al. Congenital Chagas disease: experience in the Hospital de Niños, Ricardo Gutiérrez, Buenos Aires, Argentina [in Spanish]. *Rev Soc Bras Med Trop* 2005; 38 Suppl. 2: 41-5
- Sosa ES, Segura EL, Ruiz AM, et al. Efficacy of chemotherapy with benznidazole in children in the indeterminate phase of Chagas' disease. *Am J Trop Med Hyg* 1998; 59 (4): 526-9
- Moya PR, Paolasso RD, Blanco S, et al. Treatment of Chagas' disease with nifurtimox during the first months of life [in Spanish]. *Medicina (B Aires)* 1985; 45 (5): 553-8
- Russomando G, Figueredo A, Almiron M, et al. Polymerase chain reaction-based detection of *Trypanosoma cruzi* DNA in serum. *J Clin Microbiol* 1992; 30 (11): 2864-8
- Streiger ML, del Barco ML, Fabbro DL, et al. Longitudinal study and specific chemotherapy in children with chronic Chagas' disease, residing in a low endemicity area of Argentina [in Portuguese]. *Rev Soc Bras Med Trop* 2004; 37 (5): 365-75
- Streiger M, Fabbro D, del Barco M, et al. Congenital Chagas disease in the city of Santa Fe. Diagnosis and treatment [in Spanish]. *Medicina (B Aires)* 1995; 55 (2): 125-32
- Altcheh J, Corral R, Biancardi MA, et al. Anti-F2/3 antibodies as cure marker in children with congenital *Trypanosoma cruzi* infection [in Spanish]. *Medicina (B Aires)* 2003; 63 (1): 37-40
- Sosa-Estani S, Segura EL. Etiological treatment in patients infected by *Trypanosoma cruzi*: experiences in Argentina. *Curr Opin Infect Dis* 2006; 19 (6): 583-7
- Sosa ES, Segura EL. Treatment of *Trypanosoma cruzi* infection in the undetermined phase: experience and current guidelines of treatment in Argentina. *Mem Inst Oswaldo Cruz* 1999; 94 Suppl. 1: 363-5
- Schijman AG, Altcheh J, Burgos JM, et al. Aetiological treatment of congenital Chagas' disease diagnosed and monitored by the polymerase chain reaction. *J Antimicrob Chemother* 2003; 52 (3): 441-9
- Viotti R, Vigliano C, Armenti H, et al. Treatment of chronic Chagas' disease with benznidazole: clinical and serologic evolution of patients with long-term follow-up. *Am. Heart J* 1994; 127 (1): 151-62
- Fabbro DL, Streiger ML, Arias ED, et al. Trypanocide treatment among adults with chronic Chagas disease living in Santa Fe city (Argentina), over a mean follow-up of 21 years: parasitological, serological and clinical evolution. *Rev Soc Bras Med Trop* 2007; 40 (1): 1-10
- Fabbro DS, Arias E, Streiger M, et al. Evolutionary behavior towards cardiomyopathy of treated (nifurtimox or benznidazole) and untreated chronic chagasic patients. *Rev Inst Med Trop Sao Paulo* 2000; 42 (2): 99-109

41. Viotti R, Vigliano C, Lococo B, et al. Long-term cardiac outcomes of treating chronic Chagas disease with benznidazole versus no treatment: a non-randomized trial. *Ann Intern Med* 2006; 144 (10): 724-34
42. Marin-Neto JA, Rassi Jr A, Morillo CA, et al. Rationale and design of a randomized placebo-controlled trial assessing the effects of etiologic treatment in Chagas' cardiomyopathy: the BENznidazole Evaluation For Interrupting Trypanosomiasis (BENEFIT). *Am Heart J* 2008; 156 (1): 37-43
43. Wilson JT. An update on the therapeutic orphan. *Pediatrics* 1999; 104 (3 Pt 2): 585-90
44. Nahata MC. Lack of pediatric drug formulations. *Pediatrics* 1999; 104 (3 Pt 2): 607-9
45. The therapeutic orphan: 30 years later. Proceedings of a joint conference of the Pediatric Pharmacology Research Unit Network, the European Society of Developmental Pharmacology, and the National Institute of Child Health and Human Development. Washington DC, USA, May 2, 1997. *Pediatrics* 1999; 104 (3 Pt 2): 581-645
46. Faria CR, Souza SEM, Rassi A. Polineuropatia periférica induzida por benzonidazol no tratamento da doença de Chagas. 129 [letter]. *Arq Neuropsiq* 1986; 44 (2): 125
47. Flores-Vieira CL, Barreira AA. Experimental benznidazole encephalopathy: I. Clinical-neurological alterations. *J Neurol Sci* 1997; 150 (1): 3-11
48. Flores-Vieira CL, Chimelli L, Franca Fernandes RM, et al. Experimental benznidazole encephalopathy: II. Electroencephalographic and morphological alterations. *J Neurol Sci* 1997; 150 (1): 13-25
49. WHO Expert Committee on the Control of Chagas Disease, World Health Organization. Report of the WHO Expert Committee on the Control of Chagas Disease, 2005. 905ed. Geneva: World Health Organization, 2005 [online]. Available from URL: http://www.who.int/tdr/publications/publications/pdf/swg_chagas.pdf [Accessed 2008 Aug 28]
50. Sheiner LB, Rosenberg B, Marathe VV. Estimation of population characteristics of pharmacokinetic parameters from routine clinical data. *J Pharmacokinet Biopharm* 1977; 5 (5): 445-79
51. Sheiner LB, Benet LZ, Pagliaro LA. A standard approach to compiling clinical pharmacokinetic data. *J Pharmacokinet Biopharm* 1981; 9 (1): 59-127
52. Sheiner LB. The population approach to pharmacokinetic data analysis: rationale and standard data analysis methods. *Drug Metab Rev* 1984; 15 (1-2): 153-71
53. Sheiner LB, Ludden TM. Population pharmacokinetics/dynamics. *Annu Rev Pharmacol Toxicol* 1992; 32: 185-209
54. Duffull S, Waterhouse T, Eccleston J. Some considerations on the design of population pharmacokinetic studies. *J Pharmacokinet Pharmacodyn* 2005; 32 (3-4): 441-57
55. Duffull SB. Design of clinical pharmacology trials. *Clin Exp Pharmacol Physiol* 2001; 28 (11): 905-12
56. Meibohm B, Laer S, Panetta JC, et al. Population pharmacokinetic studies in pediatrics: issues in design and analysis. *AAPS J* 2005; 7 (2): E475-87
57. Roy A, Ette EI. A pragmatic approach to the design of population pharmacokinetic studies. *AAPS J* 2005; 7 (2): E408-20
58. Sheiner LB, Beal SL. Evaluation of methods for estimating population pharmacokinetics parameters: I. Michaelis-Menten model: routine clinical pharmacokinetic data. *J Pharmacokinet Biopharm* 1980; 8 (6): 553-71
59. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER). Guidance for industry: population pharmacokinetics. 1999 [online]. Available from URL: <http://www.fda.gov/CDER/guidance/1852fnl.pdf>. [Accessed 2008 Aug 28]
60. Clinicaltrials.gov registry number NCT00699387 [online]. Available from URL: <http://www.clinicaltrials.gov> [Accessed 2008 Nov 18]

Correspondence: Dr *Facundo Garcia-Bournissen*, Division of Clinical Pharmacology and Toxicology, Hospital for Sick Children, University of Toronto, 555 University Ave, Black Wing, 8th floor, Room 8232, Toronto, ON M5G 1X8, Canada.

E-mail: Facundo.garciabournissen@sickkids.ca