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Toxic side effects of drugs used to treat Chagas' disease (American trypanosomiasis)

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Chagas' disease (American trypanosomiasis) is an endemic parasitic disease in some areas of Latin America. About 16–18 million persons are infected with the aetiological agent of the disease, *Trypanosoma cruzi*, and more than 100 million are living at risk of infection. There are different modes of infection: 1) via blood sucking vector insects infected with *T. cruzi*, accounting for 80–90% of transmission of the disease; 2) via blood transfusion or congenital transmission, accounting for 0.5–8% of transmission; 3) other less common forms of infection, eg, from infected food or drinks or via infected organs used in transplants. The acute phase of the disease can last from weeks to months and typically is asymptomatic or associated with fever and other mild non-specific manifestations. However, life-threatening myocarditis or meningoencephalitis can occur during the acute phase. The death rate for persons in this phase is about 10%. Approximately 10–50% of the survivors develop chronic Chagas' disease, which is characterized by potentially lethal cardiopathy and megacolon or megaoesophagus. There are two drugs available for the aetiological treatment of Chagas' disease: nifurtimox (Nfx) and benznidazole (Bz). Nfx is a nitrofurane and Bz is a nitroimidazole compound. The use of these drugs to treat the acute phase of the disease is widely accepted. However, their use in the treatment of the chronic phase is controversial. The undesirable side effects of both drugs are a major drawback in their use, frequently forcing the physician to stop treatment. The most frequent adverse effects observed in the use of Nfx are: anorexia, loss of weight, psychic alterations, excitability, sleepiness, digestive manifestations such as nausea or vomiting, and occasionally intestinal colic and diarrhoea. In the case of Bz, skin manifestations are the most notorious (eg, hypersensitivity, dermatitis with cutaneous eruptions, generalized oedema, fever, lymphoadenopathy, articular and muscular pain), with depression of bone marrow, thrombocytopenic purpura and agranulocytosis being the more severe manifestations. Experimental toxicity studies with Nfx evidenced neurotoxicity, testicular damage, ovarian toxicity, and deleterious effects in adrenal, colon, oesophageal and mammary tissue. In the case of Bz, deleterious effects were observed in adrenals, colon and oesophagus. Bz also inhibits the metabolism of several xenobiotics biotransformed by the cytochrome P450 system and its reactive metabolites react with fetal components *in vivo*. Both drugs exhibited significant mutagenic effects and were shown to be tumorigenic or carcinogenic in some studies. The toxic side effects of both nitroheterocyclic derivatives require enzymatic reduction of their nitro group. Those processes are fundamentally mediated by cytochrome P450 reductase and cytochrome P450. Other enzymes such as xanthine oxidoreductase or aldehyde oxidase may also be involved. *Human & Experimental Toxicology* **(2006) 25,** *471–479*

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The disease and its socioeconomic relevance

Chagas' disease (American trypanosomiasis) is a parasitic disease that is endemic in some areas of Central and South America and Mexico, where an estimated 16–18 million persons are infected with *Trypanosoma cruzi*. Further, more than 100 million people are living at risk of infection.[1] This disease was described first by Carlos Chagas in 1909 and was named after him. In 1993 the World Bank considered Chagas' disease as the parasitic sickness having more significant socioeconomic impact, measured as 'disability adjusted life years' (DALY), than all the other parasitic infectious diseases currently present in the region.[2–4]

Usually, the infection is due to vector insects harbouring *T. cruzi* in their intestines. These insects belong to the Triatominae subfamily, which have haematophagous habits and generally defecate immediately after biting a sleeping victim at night (frequently small children because they are defenceless). Upon awakening, the victim commonly

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rubs the itchy bite and, as a result, the infected Triatominae faeces find their way into the host bloodstream through a bite wound in the skin or via intact mucous membrane or the conjunctiva.[2–9] Infective forms of the parasite reach different tissue cells where they transform into the intracellular amastigote form. This form multiplies and finally causes a burst of the host cell, followed by the release of parasites into the circulation. There, they differentiate to the trypomastigote flagellated form, which is ready to invade new cells, thus repeating the cycle. Severe tissue lesions result from these alternative cycles of infection.

In addition to the vector-borne mode of infection, accounting for about 80–90% of transmission of the disease, there are additional modes such as blood transfusion and congenital transmission. These forms are thought to account for 5–20% and 0.5–8% of cases, respectively. There are other less common forms of infection like oral (from infected food or drink) or transplant of infected organs.[2–8]

Migration of infected Latin Americans from their original endemic rural areas to urban areas within their own countries or to the USA or European countries, looking for socioeconomic improvement, has brought into question two problems: the possible transmission of the disease through blood transfusion or by organ transplantation.[9–11] In this respect, it is important to note that already 100 000 people were reported as being infected in the USA and that in 1990 7 million people emigrated to the USA from countries where Chagas' disease is endemic.[2,3,9–11]

Transmission of *T. cruzi* infection by organ transplantation has been reported in Latin America[12] and in a non-endemic country such as the USA, where mention of three cases was made.[13]

Congenital transmission of *T. cruzi* infection might be of relevance for many years either in endemic areas or in non-endemic areas as well. In some countries, like Uruguay, Paraguay and Argentina, programmes have been implemented at the national level in which every pregnant woman is serologically examined for *T. cruzi* infection, providing diagnostic guidance at childbirth for prompt treatment.[2,3,5,8,10]

Oral transmission of *T. cruzi* infection caused by ingestion of trypomastigotes is not common but it is possible. It has been demonstrated in experimental animals and in laboratory accidents.[5]

In summary, there are multiple ways of acquiring the *T. cruzi* infection, each having a different probability of occurrence according to different situations.

The acute phase of the disease starts immediately after infection, irrespective of the specific mode of infection. It could last from weeks to months and typically is asymptomatic or associated with fever and other mild, non-specific manifestations. However, life-threatening myocarditis or meningoencephalitis can occur during this phase, particularly in young children and in immunocompromised persons. The death rate for persons caused by these severe symptoms is about 10%.[1–3,5,8,10]

After years to decades of subclinical infection, 10–50% (according to the endemic area and mode of infection) of the survivors of the acute stage develop chronic Chagas' disease, which is characterized by potentially lethal cardiopathy or megasyndromes (eg, megaoesophagus and megacolon).[1–3,5,8,10] It is important to emphasize that even persons who remain asymptomatic may be infectious for life, with low levels of parasites in blood and other tissues.[1–3,5,8,10] From the point of view of the analysis of the chronic phase, it is important to take into account how much time has elapsed since the infection was acquired. A 'recent chronic phase' is considered when infection occurred in the last 10 years or if the victims are children less than 12 years old.[8] Patients with more than 10 years of infection are considered 'late chronic cases'.[8] To decrease the risk of morbidity and mortality, the infected persons should be treated as early in the course of infection as possible with the approved available drugs. A favourable response to them critically depends on the development stage of the disease when the treatment is made.

Treatment of Chagas' disease

Since the late 1960s to early 1970s, two nitroheterocyclic drugs have been available for the aetiological treatment of Chagas' disease: a nitrofurane, nifurtimox (Nfx) [3-methyl-4(nitrofurfurilideneamino)tetrahydro-4H-1,4-thiozine-1,1-dioxide] and a nitroimidazole, benznidazole (Bz) [N-benzyl-2-nitroimidazole acetamide] (Figure 1). The use of these drugs to treat the acute phase of the disease is widely accepted. Irrespective of the mechanism of infection, the patient must be treated, as about 60% of them can be cured in this phase.[3,8,14] However, the use of these drugs in the chronic phase of the disease remains controversial.[3,8,14]

A reason for the controversy rests in the time when the treatment is being made in relation to when the infection occurred. Aetiological treatment of patients in the 'recent chronic' phase of the disease offers a better prognosis than those where the treatment is given in the 'late chronic

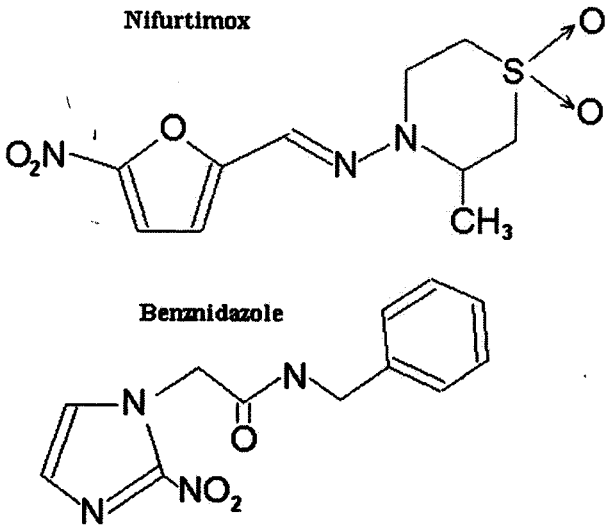

Figure 1 Nifurtimox and benznidazole chemical structures.

phase'.[3,8,14] The effectiveness of these nitroheterocyclic drugs is better for the infected extracellular forms of *T. cruzi* present in the acute phase than the intracellular forms that cause the chronic disease.[3,8,14–16]

An important issue in the treatment with these two nitroheterocyclic drugs is that their chemotherapeutic efficiency varies in patients coming from different geographic areas. This behaviour is probably due to the infection with *T. cruzi* strains having different response to the drugs.[8,14] In this respect it is also relevant to take into account that *T. cruzi* strains resistant to one of the two drugs are also resistant to the other.[3] Resistance of *T. cruzi* to Bz correlates with deletion of copies of the 'old yellow enzyme' (a NADPH flavin oxidoreductase) gene.[17] The selection of one or another drug for a given patient would not be dictated by the different response of the parasite to them but rather by the better tolerance of one of the drugs in relation to the other.[3,8] The undesirable side effects of these two drugs are a major drawback in their use, which frequently force the physician to stop treatment.[3,8,16] The analysis of these toxic side effects is the main purpose of the present review.

Clinically observed toxic side effects of Nfx and Bz

Serious undesirable side effects of Nfx and Bz have been reported during their clinical use by physicians or patients. Anorexia and weight loss, nausea and vomiting, nervous excitation, insomnia, psyche depressions, convulsions, vertigo unbalance, disorientation, forgetfulness, paraesthesias, adynamia, acoustic phenomena and intolerance to drinking alcohol were the most frequently observed effects. They were described in detail in available literature reviews.[3,8,16]

Rodriguez Coura and de Castro pointed out that the most frequent adverse effects of Nfx treatment were: anorexia, loss of weight, psychic alterations, excitability or sleepiness, digestive manifestations such as nausea or vomiting, and occasionally intestinal colic and diarrhoea.[8] According to the authors, the frequency and seriousness of Bz's clinically observed toxic effects are different in intensity and type.[8] In this case, skin manifestations were more notorious (hypersensitivity, dermatitis, with cutaneous eruptions) in addition to generalized oedema, fever, lymphoadenopathy, and articular and muscular pain. Depression of bone marrow, thrombocytopenic purpura and agranulocytosis were the most severe manifestations. Polyneuropathy, paraesthesia and polyneuritis of peripheral nerves were also reported. According to Rodriguez Coura and de Castro, the two most serious complications induced by Bz are agranulocytosis, initiated by neutropenia, sore throat, fever and septicaemia, and thrombocytopenia purpura, characterized by reduction of platelets, petechiae, haemorrhagic blisters and even mucosal bleeding.[8] In general, skin and bone marrow manifestations of toxicity are more intense for Bz, while those in the nervous system (eg, anorexia, headache, psychic excitation, loss of weight, polyneuropathy, vomiting, insomnia) are more intense for Nfx.[3,8,16]

Experimental toxicity of Nfx and Bz

By the time these two drugs were introduced to regular clinical use, the exhaustive and detailed preclinical toxicological investigations made by Bayer about Nfx were available in the literature.[18–20]

They included the acute toxicity in different species; the subchronic toxicity in the rat over 26 and 13 weeks; subchronic investigations of neurotoxicity in the chicken; chronic toxicity in the dog after oral administration for 52 weeks; and studies on the influence of Nfx on spermatogenesis in the mouse.[18] Further studies were reported related to investigations on embryotoxicity and teratogenicity in the mouse, in addition to fertility and general reproductive performance in the rat.[19]

The available published material for Nfx also includes the results of a test for carcinogenicity after oral and subcutaneous administration to rats,[20] relevant pharmacokinetic parameters,[21,22] and more importantly, the results of clinical trials in both acute and chronic infected patients.[23]

In the summary and assessment of results of the toxicological investigations, the authors reported that the LD_{50} of Nfx was between 3000 and 4000 mg po for rats and mice.[18] Interestingly, rats treated with 1000 mg/kg po had to be discontinued after only one week due to the appearance of severe symptoms of CNS toxicity. In subchronic toxicological studies, the highest dosage, of 400 mg/kg, resulted in several neurological symptoms causing the death of 6 out of 25 females rats in only the third week of treatment.[18] Histopathological studies made in those animals revealed degenerative changes, especially in the nuclei of the brain.[18] Cessation of treatment was followed by restitution. Ten weeks after the end of treatment spongiosis, glia cell proliferation, decrease in the number of nerve cells and dilatation of cerebral capillaries were evidence of defective healing.[18]

In those early studies on Nfx toxicity to mice the inhibition of spermatogenesis was observed. In the seminiferous tubules there were spermatogonia showing pyknotic nuclei but no mature sperm cells. However, the effects were described as reversible at nine weeks after treatment.[18] Further studies from our laboratory evidenced that Nfx produced intense deleterious effects in the Sertolli cells at ultrastructural level in the endoplasmic reticulum and mitochondria as well as in the perinuclear membrane, but also alterations in shape and configuration of spermatids and mature spermatozoa. Bz-induced alterations were similar in nature but far less intense and frequent.[24]

Additional experiments were done concerning the embryotoxicity of Nfx in rats and mice as well as studies on effects on fertility and general reproductive performance.[19] It was found that all dosages from 20 to 125 mg/kg po impaired the pregnant rats. In spite of the impairment, however, no malformations were induced.[19] Higher doses (50 and 125 mg/kg) resulted in a dose-dependent reduction of body weight of the rat fetuses.[19] To evaluate the effect of Nfx on general reproductive performance in young rats of both sexes, different dosage regimes of the drug were given in their food for several weeks. Doses up to 300 ppm did not damage male or female rats and did not impair the ability to reproduce.[19] However, at 600 ppm male rats became incapable of reproduction. After suspending the treatment, this effect was non-reversible, even after long periods.[19] No equivalent information was available in the literature for the case of Bz.

Our laboratory, however, did some experiments that might be relevant to the reproductive toxicology of these two drugs. In effect, we reported that the administration of either Nfx or Bz to female rats produced ultrastructural degenerative effects in the different cell types of ovaries. Specific alterations such as swelling, disruption, disorganization and loss of matrix components were observed in ovarian mitochondria.[25]

In additional studies we observed that when ^{14}C-Bz was administered orally to rats at 20 days of pregnancy, the drug was rapidly absorbed, crossed the placental barrier and reached the fetuses. Further, we also found that under those circumstances Bz reactive metabolites covalently bind not only to maternal but also to fetal proteins.[26] This suggests a potential risk for the occurrence of alterations in them. Toxicological problems for lactating newborns might also be anticipated if mothers breastfed their pups when treated with one of these drugs, as both Bz and Nfx reached breast tissue to pass via breast milk to the breast-feeding newborn.[22,27]

A no effect dose of 25 mg/kg po given during 3 weeks for Nfx was established in those early studies based on laboratory tests, macroscopical and histological findings.[18] This is of interest considering the fact that higher local concentrations of Nfx were found in the kidneys and liver as well as in the skin, lungs, adrenals, thyroid gland, aorta wall and Cowper's gland (disregarding the case of the gastrointestinal tract, where excretion was taking place after the single dose of Nfx given).[22] Our laboratory performed some distribution studies for Bz. After a single oral dose of this drug, liver, stomach and kidney had the highest concentrations at 1 h. All other organs had concentrations closely similar to that found in blood.[28]

After those early target-organ toxicity studies made for Nfx by its manufacturer and knowing no equivalent studies were available in literature by the time Bz was introduced to the market, our laboratory initiated a series of ultrastructural studies on the potential Nfx or Bz effects in different organs. For example, Bz administration to male rats caused significant subcellular alterations in the adrenal cortex involving fasciculata and reticularis zones but not in the glomerulosa. Bz-treated animals revealed cells with marked lipid accumulation and alterations in nuclei, endoplasmic reticulum and mitochondria in the reticularis and fasciculata zones.[29]

Deleterious effects involved the same adrenal zones as those altered by Bz, but the effects in them were different. No lipid accumulation was observed. However, the alterations observed involved mitochondria, nuclei, Golgi apparatus and the endoplasmic reticulum, but were more intense in the mitochondria.[30] We are not in a position at

present to decipher the toxicological meaning of these alterations observed in adrenals treated with either Bz or Nfx.

In this line of research our laboratory also studied the effects of Nfx administration on the rat colonic mucosa. Results showed intense alterations in the epithelial cells consisting of moderate dilatation of the endoplasmic reticulum but an intense dilatation of the Golgi apparatus. The latter effect suggests the potential occurrence of serious alterations in the synthesis/storage of secretory products of the colonic mucosa provoked by Nfx administration.[31] Bz also had a deleterious effect on the colon. In effect, the colon mucosa of Bz-treated rats showed intense ultrastructural alterations, abundant mucus secretion at the level of the Goblet cells and dilatation of the endoplasmic reticulum and the Golgi apparatus in epithelial cells.[32]

Our laboratory also reported the occurrence of ultrastructurally observable alterations in oesophageal tissue from rats receiving Bz intragastrically. Alterations were not very intense but were neat. They included detachment and agglomeration of polyribosomes; reduction in the presence of desmosomes and in the amount of bacteria in its surface.[33] The potential significance of these alterations in both colon and oesophageal tissue is not fully clear at present. However, it might be of merit to consider them in light of the present tendency to use these two drugs in the 'indeterminate phase' of Chagas' disease. Any deleterious effects of the drugs at this stage might be additive or synergistic with those induced by the evolution of the disease.

The early toxicological studies made by the manufacturer of Nfx included the test for carcinogenicity when the drug was administered either orally or subcutaneously.[20] The results were different in females than in males. In females the po administration of Nfx significantly decreased the percentage of malignant tumours (mammary gland carcinosarcoma, ovary adenocarcinoma) spontaneously occurring in them (from 36 to 12%) but significantly increased (mammary gland fibroadenoma, mammary gland fibroma, adrenal hemangioma) the percentage of rats bearing tumours (from 36 to 64%). In male rats the percentage of malignant tumours (adenocarcinoma of the gall bladder, bladder carcinoma) observed after po Nfx administration was significantly higher than in the control group (16 to 28%). Also, the percentage of benign tumours (adrenal adenoma, adrenal hemangioma, mammary gland adenoma) was significantly higher (4 to 24%).[20] The location of tumours modulated by Nfx po administration to female rats was the same as was already occurring spontaneously.[20] However, in the case of male rats Nfx-induced cancers or tumours involving locations not occurring in controls (adenocarcinoma of the gall bladder, adrenal hemangioma, adrenal adenoma, mammary gland fibroma, sympathogonioma of the adrenal gland) were observed.[20]

Later studies with both drugs administered to female mice evidenced significant differences in the incidence of lymphoma.[34] The increased tumorigenic/carcinogenic risk caused by these two nitrohetrocyclic drugs may not be a surprise considering the many studies available today evidencing their genotoxic properties. Most studies done concerning the matter in the past have been previously reviewed[16] and will not be considered again here. However, consideration of some results employing tests more familiar to general toxicologists were considered as relevant to be recalled. For example, the mutagenic activity of Nfx was tested in different strains of *Salmonella typhymurium* such as TA100, TA98, TA1535, TA1536, TA1537, TA1538 and in a simplified version of the Ames test known as Simultest test composed of the indicator strains TA97/TA98/TA100 and TA102.[35–37] Nfx evidenced no toxicity (His^+ reversion) in the TA100 test system in different studies and in the Simultest test.[35–37] Nfx mutagenicity was also tested in the isogenic $uvrB^+$ strain UTH8414 and led to near negative results.[35] These results would indicate that excision repair enzymes are involved in the reparation of lesions induced by Nfx.[35] Similar results were observed with Bz.[35] Further, genotoxic effects of Bz and Nfx on nitrofurazone-resistant mutants whose resistant phenotypes are considered to be attributable to its loss of nitroreductive activity were negative.[35] Those findings evidenced that the mutagenicity observed in the bacterial test systems is most probably linked to their nitroreductive activity.

The potential genotoxic effect of both Nfx and Bz was also evaluated in mammalian systems.[38–42] Navarro *et al.* reported the effect of Bz and Nfx on the induction of micronucleated erythrocytes in mice and on induction of chromosomal aberrations in human lymphocytes in an *in vitro* assay.[39] The results of both tests, microsomal analysis and chromosomal aberrations, were clearly indicative of the clastogenic effects of Bz and Nfx, in rodents *in vivo* and in human cells *in vitro*.[39] Gorla and Castro reported no significant increase in micronucleus formation in the bone marrow or splenic lymphocytes of mice treated with orally given Bz up to 2000 mg/kg bw.[38] In contrast, Nfx caused a statistically significant increase at the highest dose used, 2000 mg/kg bw, in the

micronucleus formation in the mouse bone marrow.[40] In studies on sister chromatid exchange frequency in splenic lymphocytes of mice after exposure to Nfx or Bz to po doses up to 2000 mg/kg bw, Gorla showed that Nfx but not Bz led to an increased frequency of sister chromatid exchange.[40] More importantly, Gorla *et al.* reported a 13-fold increase of chromosomal aberrations analysed from cultures of peripheral lymphocytes of two groups of chagasic children before and after treatment with Nfx.[41] Studies on two groups of chagasic children before and after treatment with Bz showed small but statistically significant levels of micronucleated interface lymphocytes and chromosomal aberrations.[42]

In the course of biochemical studies, Gorla *et al.* reported that Bz reactive metabolites bound covalently either to DNA and nuclear proteins when liver nuclei were incubated anaerobically in the presence or absence of NADPH.[43] The covalent binding to nuclear proteins involved the acidic non-histone proteins and those from the nuclear sap.[43] No identification of either the structure of the reactive metabolite formed or of the adducts produced between them and DNA bases or aminoacids was possible in those studies.

Available evidence also indicates that both Nfx and Bz significantly modify the immune response of the host. The effect depends on both the drug and the host. For example, in studies on the positive skin reactions to PPD in guinea pigs immunized with Freund's complete adjuvant it was reported that administration of Nfx impacted the specific cell-mediated immune response to PPD both *in vivo* and *in vitro*.[44] In other studies the treatment with Nfx led to a loss of resistance to reinfection with *T. cruzi*, probably associated with humoral and cell-mediated anti-*T. cruzi* immune responses respectively.[45] In the case of Bz, some workers reported a severe cell-mediated immunosuppression in *T. cruzi*-infected and BCG-immunized rabbits.[46] However, in other studies it was reported that activation of the immune system by the parasite and endogenous interferon-gamma play a major role in the efficacy of Bz against infection with *T. cruzi*.

Metabolism of Nfx and Bz and its relation to their toxic side effects and chemotherapeutic properties

The available information on the metabolism and on the mechanisms of toxicity of both Nfx and Bz was exhaustively detailed in previous reviews[16] and will be briefly summarized and updated here.

There is general consensus about the toxic effects of nitro compounds and that Nfx or Bz in particular require enzymatic reduction of their nitro group. Nitroreduction of Nfx and Bz results in activation rather than detoxication. During that bioactivation process, chemically reactive metabolites are formed. Reactive metabolites produced are considered free radical in nature. Available evidence suggests that the free radical reactive metabolites generated during Nfx or Bz nitroreduction are a nitroanion radical ($RNO^{\bullet}_{2}^{-}$) and the hydronitroxide free radical ($RNHO^{\bullet}$), respectively. The general nitroreductive process of both nitroheterocycles is depicted in Figure 2.

Transfer reactions of the hydrogen abstraction type and addition reactions are common for free radicals and they often compete and have similar rate constants. Reaction of the $RNO^{\bullet}_{2}^{-}$ with oxygen may lead by redox cycling to the formation of superoxide anion $O^{\bullet}_{2}^{-}$. This reaction might be of relevance under aerobic conditions and proved to be involved in the case of Nfx but not Bz.[16] In the case of Bz, the nitroreductive process is able to proceed even *in vivo* to the corresponding amine derivative and during this biotransformation a reactive metabolite is formed (presumably the $RNHO^{\bullet}$).

The toxicity of Bz is believed to arise because of the interaction of its reactive metabolites with DNA; proteins and lipids or other relevant cellular components.[16,26,43,47,48] This mechanisms might be involved not only in the mammalian toxicity effects but also in the deleterious actions on *T. cruzi*, which are responsible for its chemotherapeutic actions.[49]

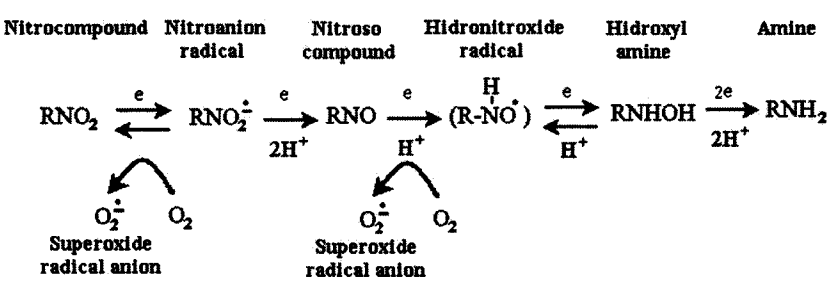

Figure 2 Nifurtimox and benznidazole possible nitroreductive metabolic pathways.

In the case of Nfx, workers in the field considered that the formation of reactive oxygen species (ROS) might be the key process involved in both the toxic and the chemotherapeutic effects of Nfx.[16] Reactions of Nfx with critical sulphydryl containing molecules with generation of nitrite was also observed but the role of these reactions in the Nfx effects is not known.[50] Available results show that lipid peroxidation occurs in liver but only after GSH levels were significantly decreased.[51,52]

Concerning the nature of the enzymes involved in the nitroreduction of Bz or Nfx the available information indicates that cytochrome P450 reductase and cytochrome P450 are the most relevant ones in the liver nitroreductive metabolism of both drugs.[16,47,48,50,53–59] Smaller fractions of the nitroreductive liver capacity were attributable to xanthine oxidoreductase and aldehyde oxidase.[50,53,54,56,57] In organs other than the liver (testes, ovaries, adrenals, colon, oesophagus) the intensity of the relative enzymatic processes may vary to some extent.[24,25,29,30,32,33] An interesting case in this respect is the case of mammary tissue, which is one of the richest sources of xanthineoxidoreductase in the entire body and which correspondingly had a relevant role in the overall nitroreductive process.[60] In organs where P450 is present not only in microsomes but also in mitochondria (steroidogenic organs) the subcellular location of the nitroreductive capacity was shown in both organelles and, more importantly, the subcellular location of cell injury changed accordingly.[24,25,29,30,32,33,60]

The practical implications of these findings proved to be significant. For example, the observation that the fetus is able to bioactivate Bz (and probably Nfx) forced avoidance of their use in pregnant women.[26] Some physicians in the very early times of the use of both drugs were tempted to prevent congenital transmission of the disease. Alternatively, the low metabolic nitroreductive capacity to bioactivate Nfx or Bz in the newborn[57] offered a rational alternative to treat the infected newborn.

At that age the nitroreductive bioactivation, which is responsible for the toxicity, is low, while the *T. cruzi* susceptibility to the drugs is the same.[49] These findings led to rational bases to conclude that young children should be treated as soon as possible after infection.[2,3,8] Chances for success are at optimum at that point.

Problems and future needs

The problem of Chagas' disease involves many related factors including public health, scientific, economical, educational, political and other factors. However the most urgent need is to find new drugs for treatment of the disease. The two available drugs are not fully effective and they have shown important toxic side effects. The challenge, however, remains in the scientific community (including toxicologists). Pharmaceutical companies have drastically reduced their investment in drug development for tropical diseases. The major reason for this is economic. The development of a new drug usually requires $200–400 million for a successful product and the prospect of a reasonable economic return is poor.[1,4,5,15]

There are currently intense efforts from the scientific community to establish new treatments or drugs.[14,15] However, both Bz and Nfx are still going to be the only drugs available for some time. This review only intends to ensure that prescribing physicians have at hand a summary of the drugs' adverse effects to allow them to make appropriate risk-benefit decisions.

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