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Research paper

Allopurinol encapsulated in polycyanoacrylate nanoparticles as potential lysosomatropic carrier: preparation and trypanocidal activity

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Abstract

The activity of allopurinol-loaded polyethylcyanoacrylate nanoparticles against *Trypanosoma cruzi* was compared to that of free allopurinol using in vitro cultures of epimastigotes. Ethylcyanoacrylate nanoparticles were prepared by an emulsion polymerization process, and formulations containing different concentrations of allopurinol, polyethylcyanoacrylate and surfactants were investigated and analyzed in size and amount of drug entrapped. The nanoparticles obtained were less than 200 nm in size, as measured by electron microscopy and cytometry. The peak amount of allopurinol entrapped in the nanoparticles was 62.8 ± 1.9 μg mg^{-1} of nanoparticles using 400 μl of polyethylcyanoacrylate, 200 μl of surfactant (Tween 20) and 20 mg of allopurinol in 50 ml of polymerization medium and the association efficiency was 100.7%. After 6 h of incubation at pH 7.4 the release of allopurinol from the nanoparticles was 7.4%, while at pH 1.2 only 3.1% was released after 4–6 h ($t = 42.8$, $P < 0.0001$). The in vitro studies, using cultures of *T. cruzi* epimastigotes, demonstrated considerable increases in the trypanocidal activity of the allopurinol-loaded nanoparticles in comparison with a standard solution of allopurinol (91.5 vs. 45.9%) at an allopurinol concentration of 16.7 μg ml^{-1}. In addition, it was shown that the unloaded nanoparticles, by mechanisms not completely elucidated, had a trypanocidal activity similar to that of standard solutions of allopurinol. To study cytotoxicity, increasing concentrations of unloaded nanoparticles were incubated on vero-line cell cultures. The concentration that killed 50% cells was 200 μg ml^{-1}, four times higher than that necessary to kill 50% of *T. cruzi*. It is concluded that the polyethylcyanoacrylate nanoparticles constitute a good carrier of drugs against the *T. cruzi.* The allopurinol loaded-nanoparticles significantly increased the trypanocidal activity in comparison to the free drug.

Keywords: Allopurinol; Drug carrier; Nanoparticles; Polycyanoacrylates; *Trypanosoma cruzi*

1. Introduction

The flagellate protozoan *Trypanosoma cruzi* is the causative agent of Chagas' disease, which is transmitted to the human body by insects of the family Reduviidae, specially *Triatoma infectans*. The disease occurs predominantly in Central and South America [1]. High morbidity and mortality from this disease raise very important health, social, and economic problems in Latin American countries. In the temperate zone of Chile, Chagas' disease has endemic-enzootic characteristics, with both rural and suburban distributions. In Chile human infection reaches an average of 15% in chagasic endemic areas [2]. No ideal drug is available for treating *T. cruzi* infections.

Most are chronic cases, which are asymptomatic until the later stages of the disease when visceral organomegaly and cardiopathy can occur. The acute form of the disease is treated with nitrofuran such as nifurtimox and benzmidazol. The high percentage of adverse drug reactions that occur with these drugs has discouraged many physicians from using them [3,4]. Because of this, efforts are in progress in many laboratories to find new therapeutic agents that are active against *T. cruzi* but are not harmful to the host [5]. Among these drugs, allopurinol has substantial activity against *T. cruzi* in vitro [6–11] and in experimental animals [12,13]. Convincing results using allopurinol have been obtained in treatment of chronic Chagas' disease [14–17]. The selective anti parasitic action of allopurinol is believed to be due to its incorporation into the protozoa, but not the mammalian, purine salvage pathway. This leads to the formation of 4-aminopyrazolopyrimidine ribonucleotide triphosphate, that is incorporated into ribonucleic acid [18]. The efficacy of allopurinol for the treatment of Chagas' disease has been evaluated previously using regi-

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mens of 300 and 600 mg day^{-1} [19–21]. The results showed a dose-dependent response in relation with the elimination of parasitemia (45 and 85%, respectively). If the dose of allopurinol could be reduced using a mechanism that increases their activity, it would enable their use with less risk and greater safety. In a previous study [22] it was demonstrated that nifurtimox encapsulated in nanoparticles of polyethylcyanoacrylate increased significantly the trypanosome activity in vitro and in cultures of cells infected with the parasites.

The aim of the present study was to investigate how the preparation conditions influence allopurinol loading capacity, nanoparticle size, and release profiles. The anti proliferation activity of these nanoparticles using cultures of *T. cruzi* epimastigotes was also studied.

2. Materials and methods

2.1. Chemicals

Allopurinol (1*H*-pyrazolo [3,4–*d*]-dipyrimidin-4-ol) and ethylcyanoacrylate used as the monomer for the polymerization, were obtained from Sigma Chemical Company (St. Louis, MO). Solvents were HPLC grade and other reagents were purchased from Merck Química Chilena, Santiago, Chile.

2.2. Nanoparticles preparation

Polyethylcyanoacrylate nanoparticles were prepared by polymerization according to the method described by Couvreur et al. [23]. The desired amount of allopurinol was dissolved with 3 ml of 0.1 N NaOH, and then diluted to 50 ml with an aqueous solution of 0.01 M HCl containing 0.10–0.30 ml of the non-ionic surfactant Tween 20. An appropriate amount of ethylcyanoacrylate monomer was added in a dropwise manner over a period of 10 min under mechanical stirring (1000 rev. min^{-1}). After the polymerization was complete (commonly 3 h) the colloidal suspension was adjusted to pH 7.0 with 0.2 M NaOH. The unloaded allopurinol was separated by centrifugation (Sorvall Superspeed RC2-B, Sorvall Inc, Newtown, Conneticut). Finally, the nanoparticles were suspended in water and centrifuged again to exclude drug residue from the inter-particle space.

To obtain the highest yield of entrapped allopurinol in the nanoparticles, several concentrations of allopurinol, ethylcyanoacrylate, and surfactants were assayed.

2.3. Allopurinol assay

The amount of allopurinol entrapped in the nanoparticles was determined by a UV method. Normally, 10.0 mg of dried nanoparticles loaded with allopurinol were dissolved in 25 ml of acetonitrile and the resultant organic solution was assayed by UV spectrometry. The UV apparatus consisted of a Milton Roy Spectronic 3000 spectrophotometer at 254 nm. A calibration curve between 3 and 30 μg ml^{-1} of allopurinol gave the best correlation ($y = 0.038x + 0.011$, $P = 0.999$). No interference was observed from the other components present in the nanoparticles (polyethylcyanoacrylate, Tween 20 and dimethylsulfoxide). The results were expressed as the percentage of the amount of drug contained in 100 mg of dried material. The test was performed in quadruplicate.

2.4. Morphological characterization and size analysis

Scanning electron microscopy (JEOL JSM-25-S II, 30 kV, of the Faculty of Biological Sciences of Catholic University of Chile, Santiago, Chile) was used to observe the morphology of the nanoparticles. The size and homogeneity of nanoparticles were determined using a flow cytometer (FACS flow, Becton and Dickinson). The nanoparticles were analyzed in a FACS Vantage flow cytometer (Becton and Dickinson, Immunochemistry System, San Jose, CA) fitted with an Enterprise 160 mW coherent laser at 488 nm; 60 mW at 535 nm. Data were collected from 10 000 particles for each sample, at a flow rate of approximately 500 particles s^{-1}. Readings were made at an output power of 160 mW at 488 nm. The fluorescent signal was collected with a 530/30BP band-pass filter (Fl 1 FITC), the signal being connected in logarithmic mode with the PTM Fl 1 = 555V, and analyzed using the LEASES II program (Becton and Dickinson).

2.5. Allopurinol release from nanoparticles

Allopurinol release from nanoparticles was determined at two different pH values using either Isotonic phosphate buffer (pH 7.4), and HCl (pH 1.2) according to method described by Cicek et al. [24]. For each pH, 30 mg of drug-loaded nanoparticles were suspended in 10 ml of phosphate buffer at 37°C in a thermostat bath with constant agitation. Samples were drawn at 30 min and the allopurinol concentrations were assayed by UV. For each pH, the test was performed in quadruplicate.

2.6. Parasites

T. cruzi were isolated from a chronic Chagasic patient from Northern Chile. Multiple clones were prepared, and after several passages in rats, one clone (CA-1) was finally adapted for axenic culture. Epimastigotes forms were used to evaluate the activity against *T. cruzi.*

2.7. Culture medium

Minimal essential medium (MEM; Gibco-BRL 072-110) with Hanks' salts (Gibco-BRL 041-011575) supplemented with 10% calf bovine serum was used to maintain the epimastigotes forms. Cultures were maintained in T-25 culture flasks (Sterilin) at 28°C and then sub-passage at 10^4 cells ml^{-1} every third day. For sensitivity determina-

tions, *T. cruzi* in the logarithmic growth phase were taken from these stock cultures.

2.8. Antiproliferation study in epimastigotes forms

Seventy-five microliters of unloaded nanoparticles (0.5 mg ml^{-1}), nanoparticles loaded with allopurinol (0.5 mg ml^{-1}) equivalent in concentrations to standard solution of allopurinol (50.0 μg ml^{-1}), and the standard solutions of allopurinol (50.0 μg ml^{-1}), were placed in each well of column 12 of a microtiter plate (Nunc, InterMed, Denmark). All remaining wells of the plate received 50 μl of medium. For 3-fold serial dilution, 25 μl from each well of column 12 was transferred to the corresponding well of column 11, then 25 μl was moved from column 11 to column 10; the serial dilution was carried out with a multichannel micro pipette (Titertek) and was continued up to the wells of column 3, for which 25 μl was discarded. Column 1 and 2 served as controls. Then, 50 μl medium containing 2×10^5 cells ml^{-1} was added to all wells of every second row to give a final concentration of 10^5 cells ml^{-1}. Medium alone (50 μl) was added to the remaining rows, which served as additional cell-free controls. The microtiter plates were placed in an incubator at 28°C in a 5% CO_2 atmosphere for 72 h to prevent evaporation. Parasites were counted in a Neubauer chamber (Boeco, Germany) under a microscope (Cambridge Instruments, Galen III). The test was performed in quadruplicate.

2.9. Calculations of activity

The percentage of trypanocidal activity was calculated with the equation

$$\% \text{ activity} = \frac{N_b - N_w}{N_b} \times 100 \quad (1)$$

where N_b is the number of live parasites in the blank and N_w is the number of parasites in each well.

2.10. Unloaded nanoparticles cytotoxicity assay

Vero cell-line ATCC CCL-81 were cultured at 37°C in a moist atmosphere supplemented with 5% CO_2 in RPMI 1640 medium. with 10% fetal bovine serum previously inactivated at 56°C for 30 min. The cells were washed twice and re-suspended in a complete RPMI 1640 medium. A 12×8 microtiter plate (Nunc, InterMed Denmak) was used for cytotocity assay. Each well was injected with 100 μl containing $60 \times 10-^4$ cells ml^{-1}. After incubation at 37°C for 12 h in a 5% CO_2 atmosphere, non-adhering cells were removed by rinsing them with cold medium. After a 24-h incubation period with various concentrations of unloaded nanoparticles, ranging from 1.2 to 2377.00 μg ml^{-1}, cell viability was determined by ionic intensified fluorescein diacetate method [25]. This method is based on the capacity of the fluoresceindiacetate (FDA) to enter and diffuse into the cell. Viable cells emitted a green fluorescence under UV light. Non-viable cells do not emit fluorescence. The viable cells were counted using a microscope with UV light. The percentage of cytotoxicity was calculated by the formula

$$\%C_d = 100 - \%C_v \quad (2)$$

where $\%C_d$ is the percentage of non-viable cells and $\%C_v$ is the percentage of viable cells.

2.11. Statistical test

Student's *t*-test and ANOVA test were used to compare the trypanocidal activity of the loaded and unloaded nanoparticles, and allopurinol standard.

3. Results

Fig. 1 shows a scanning electron micro graph of the spherical nanoparticles. The nanoparticles had a mean diameter of 187 ± 54 nm, using flow cytometry.

Table 1 shows the μg mg^{-1} of allopurinol entrapped in the nanoparticles after the addition of different concentrations of ethylcyanoacrylate, Tween 20, and allopurinol in the polymerization medium. The best efficiency was obtained with a 400 μl volume of cyanoacrylate (62.8 ± 1.9 μg mg^{-1} of nanoparticles; 100.7%). Using 100, 200, or 300 μl of surfactant did not lead to significant differences in the efficiency of allopurinol entrapment in the nanoparticles. Finally, the nanoparticles were prepared using the following best conditions: 50 ml polymerization medium (HCl 0.1 N), 200 μl of Tween 20, 400 μl of ethylcyanoacrylate, and 20 mg of allopurinol.

Fig. 2 shows the effect of pH on the release profile of allopurinol from the nanoparticles. At pH 1.2, the amount of allopurinol released from the nanoparticles reached 14.4 ± 0.8 μg ml^{-1} (3.1%) after 6 h incubation with the acid medium. Whereas at pH 7.4, 34.3 ± 0.4 μg ml^{-1} (7.4%)

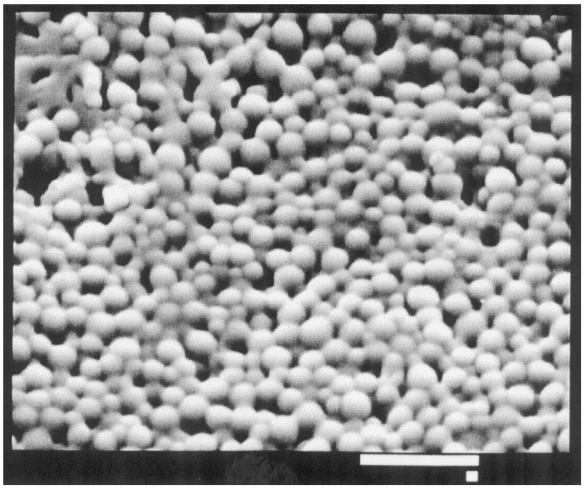

Fig. 1. Scanning electron micrograph of allopurinol-loaded nanoparticles (30 000×). Bar = 1000 nm.

Table 1
Influence of the concentrations of ethylcyanoacrylate, Tween 20 and allopurinol added in the polymerization medium on the allopurinol entrapped in the nanoparticles (μg mg^{-1} of nanoparticles)

Ethylcyanoacrylate[a]		Tween 20[b]		Allopurinol[c]	
μl	μg/mg ± SD	μl	μg/mg ± SD	mg	μg/mg ± SD
400	62.8 ± 1.9	100	68.0 ± 4.3	20	69.0 ± 3.4
500	46.9 ± 1.9	200	62.8 ± 2.3	30	70.1 ± 2.4
750	35.0 ± 1.7	300	62.7 ± 2.9	50	73.5 ± 2.6
100	27.9 ± 1.2				
F	322.8; $P < 0.0001$		0.66; $P > 0.5$		2.74; $P = 0.117$

[a] 50 ml 0.1 N HCl, 200 μl Tween 20, and allopurinol 20 mg.
[b] 50 ml 0.1 HCl, ethylcyanoacrylate 400 μl.
[c] 50 ml 0.1 HCl, 200 μl Tween 20, 400 μl ethylcyanoacrylate.

of allopurinol was released after 6 h incubation in the buffer. This difference was statistically significant ($t = 42.7$, $P < 0.0001$).

The trypanocidal activity of the nanoparticles with and without allopurinol was calculated and was compared with that of a standard solution of allopurinol equivalent to the concentration of the drug in the nanoparticles (Fig. 3).

Activity differences were observed in the three preparations investigated. The greatest trypanocidal activity, 91.5% was recorded with the allopurinol loaded nanoparticles at concentrations of 16.7 μg ml^{-1}, whereas activity at the same concentration of allopurinol in the standard solution was 45.9%. The trypanocidal activity of unloaded nanoparticles (0.18 μg ml^{-1}) was 87.2 ± 3.3%. Paradoxically this activity was greater than that of a standard solution of allopurinol. The 50% inhibitory concentration (IC_{50}) obtained from the activity–concentration curves was 37.3 ± 5.0 μg ml^{-1} for allopurinol standard solution and, 0.5 ± 0.1 μg ml^{-1} for the suspension of nanoparticles loaded with allopurinol ($t = 16.4$; $P < 0.0002$). The IC_{50} for unloaded nanoparticles was 54.7 ± 1.7 μg of nanoparticles ml^{-1}.

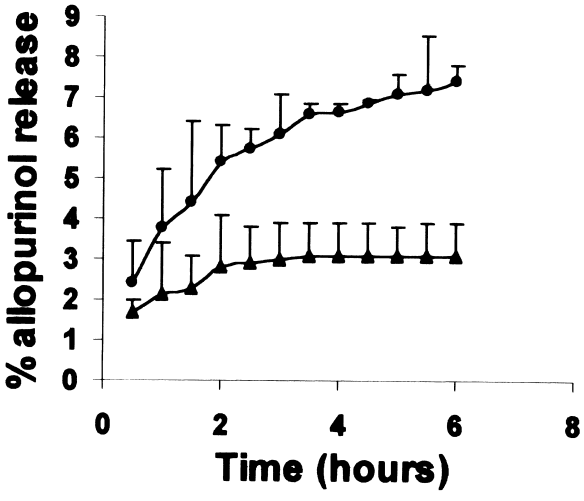

Fig. 2. The release profiles of allopurinol-loaded nanoparticles in simulated gastric fluid (pH 1.2) (-▲-) and physiological pH (pH 7.4) (-●-).

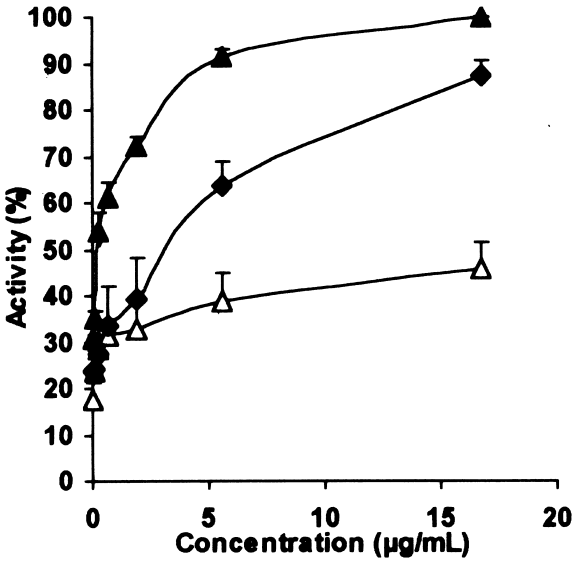

Fig. 3. Trypanocidal activity profile of allopurinol standard ( ), allopurinol-loaded (-▲-). and unloaded nanoparticles (-◆-) in free epimastigotes culture.

Table 2 shows the cytotoxicity results obtained after incubating increased concentrations of unloaded nanoparticles on vero-cells line cultures. Concentrations greater than 262.9 μg ml^{-1} of unloaded nanoparticles gave 56% cytotoxicity.

4. Discussion

In a previous study we have demonstrated the potential usefulness of polycyanoacrylate nanoparticles as colloidal carriers of nifurtimox, a drug used for treatment of Chagas' disease [22]. The most significant result of this earlier study was the increased trypanocidal activity presented by nifurtimox-loaded nanoparticles in comparison with a nifurtimox standard. Thus, dilution as low as 7.6 and 23 ng ml^{-1} registered trypanocidal activities of 75 and 90%, respectively. This trypanocidal activity was similar on infected cells and amastigote forms of the parasite.

In the present study, we obtained an adsorptive capacity for allopurinol of nearly 100% for the nanoparticles in

Table 2
Cytotoxicity of polycyanoacrylate unloaded nanoparticles ($n = 5$, mean ± SD)

Well	Unloaded nanoparticles (μg/ml)	Cytotoxicity (%) ± SD ($n = 5$)
8	1.10	0.0 ± 0.0
7	3.30	3.7 ± 4.8
6	9.80	13.0 ± 6.7
5	29.20	16.0 ± 4.8
4	87.60	22.0 ± 4.5
3	262.90	56.0 ± 5.5
2	788.90	100.0 ± 0.0
1	2366.70	100.0 ± 0.0

comparison to those obtained with nifurtimox in the previous study [22]. The adsorptive capacities of the nanoparticles mainly depend of the drugs' characteristics. Couvreur et al. [24] demonstrated the adsorptive capacity of the nanoparticles is greater when the drug is non-ionized in the solution. Probably, the high percentage of adsorption of allopurinol onto the nanoparticles could be explained by the acidic character of allopurinol ($pK_a = 9.4$). Therefore, the drug would be in a non-ionized form at pH = 2, throughout the polymerization progress. The release of allopurinol from the nanoparticles obtained at different pH values, was increased with increasing pH, as was also reported by Wade et al. [26] and Tseng et al. [27]. From this study, the degradation rate was higher at pH 7.4. According to Lenaerts et al. [28], the degradation of polyalkylcyacrylates started with an initial attack of the hydroxyl ions leading to the formation of carboanions and formaldehyde. These products react again with the hydroxyl ions to produce formaldehyde and the corresponding alcohol as the final degradation products. Therefore, an increase in the hydroxyl ion concentration causes faster degradation of the polyalkylcyanoacrylate nanoparticles as seen in the present study.

The in vitro trypanocidal activity of the allopurinol-loaded nanoparticles against the investigated *T. cruzi* was different from those obtained with the free drug. The trypanocidal activity at 5.56 μg ml^{-1} allopurinol solution was 38.6%, while the same concentration of allopurinol encapsulated in the nanoparticles was 91.5%. The IC_{50} value for allopurinol-loaded nanoparticles was 80-fold lower than those attained with the free drug.

Similar results were demonstrated by González-Martin et al. [22] for nifurtimox-loaded nanoparticles against *T. cruzi*. The activity of nifurtimox encapsulated increased by 50-fold from those presented by free nifurtimox. The enhancement of the in vitro trypanocidal activity of the allopurinol entrapped within the nanoparticles could be ascribed to a suitable interaction between the nanoparticles surface and the outer part of the bilayers of the biological membranes. In fact, the surfactant coating of the nanoparticles surface may facilitate the penetration of the loaded drug into the parasite [29].

In our study, the unloaded nanoparticles showed trypanocidal activity four to five times that a standard solution of allopurinol. This finding was observed previously by González-Martin et al. [22] and Lhern et al. [30]. The mechanism by which these unloaded nanoparticles would possess a trypanocidal activity has not been elucidated. Couvrer et al. [23] proposed that the enzymatic degradation of these nanoparticles produces formaldehyde that would be responsible for the cellular lysis. However, these authors demonstrated the contribution of the formaldehyde pathway to the degradation of polyisobutylcyanoacrylate nanoparticles was surprisingly small for all pH values tested. Conversely, isobutanol production was 85% of the theoretical quantity in the alkaline medium. Other support that would explain the high activity is the unloaded nanoparticles would be toxic for *Trypanosoma* at the concentrations used. However, we demonstrated in this study that the cytotoxic level of the unloaded nanoparticles was above 200 μg ml^{-1} to kill 50% of the cells, while the CI_{50} to kill Trypanosomes was three times less, 54.7 μg ml^{-1}. These results suggest the presence of an alternative pathway, different to those of polyalkyl chain hydrolyses, this needs to be studied further.

5. Conclusions

In vitro, the allopurinol-loaded nanoparticles are capable of offering a suitable tool for increasing the selective delivery and the therapeutic effectiveness against *T. cruzi.*

References

[1] WHO Technical Reports Series, 202 (1960).
[2] H. Schenone, F. Villarroel, A. Rojas, E. Alfaro, Factores biológicos y ecológicos de la enfermedad de Chagas en Chile, Bol. Chileno Parasitol. 35 (1980) 42–54.
[3] Z. Brener, Present status of chemotherapy and chemoprophylaxis of human trypanosomiasis in the western Hemisphere, Pharmacol. Ther. 7 (1979) 71–90.
[4] G.C. Levi, V. Amato, Neto, J.F.De Araujo, Analise de manisfestacoes colaterais devidas ao uso do medicamento Ro-7-1051 nitromidazolico especifico da doenca de Chagas. Rev. Inst. Med. Trop. Sao Paulo 17 (1975) 49–52.
[5] W.E. Gutteridge, Chemotherapy of Chagas' disease, Trans. R. Soc. Trop. Med. Hyg. 70 (1976) 123–124.
[6] J.L. Avila, A. Avila, E. Muñoz, Effect of allopurinol on different strains of Trypanosoma cruzi, Am. J. Trop. Med. Hyg. 30 (1981) 769–774.
[7] R.L. Berens, J.J. Marr, F.S. Steele, da Cruz, D.J. Nelson, Effect of allopurinol on Trypanosoma cruzi: metabolism and biological activity in intracellular and bloodstream forms. Antimicrob. Agents Chemother. 22 (1982) 657–661.
[8] R.L. Berens, J.J. Marr, D.L. Looker, D.L. Nelson, S.W. LaFon, Efficacy of pyrazolopyrimidine ribonucleosides against Trypanosoma cruzi: studies in vitro and in vivo with sensitive and resistent strains, J. Infect. Dis. 150 (1984) 602–608.
[9] W.R. Fish, J.J. Marr, R.L. Berens, D.L. Looker, D.L. Nelson, S.W. LaFon, A.E. Balber, Inosine analogs as chemotherapuetics agents for African trypanosomes: metabolism in trypanosomes and efficacy in tissue culture, Antimicrob. Agents Chemother. 27 (1985) 33–36.
[10] J.J. Marr, R.L. Berens, N.K. Cohn, D.J. Nelson, R.S. Klein, Biological action of inosine analogs in Leishmania and Trypanosome species, Antimicrob. Agents Chemother. 25 (1984) 292–295.
[11] J. Nakajima-Shimada, Y. Hirota, T. Aoki, Inhibition of Trypanosoma cruzi growth in mammalian cells by purine and pyrimidine analogs, Antimicrob. Agents Chemother. 40 (1996) 2455–2458.
[12] J.L. Avila, A. Avila, Trypanosoma cruzi: allopurinol in the treatment of mice with experimental acute Chagas' disease, Exp. Parasitol. 51 (1981) 204–208.
[13] L.E. Castrillon-Rivera, W. García-Fernández, L. Pérez-Fernández, C. Gutierrez, Efecto terapéutico de allopurinol en el ratón cepa CFW con tripanosomiasis americana (Enfermedad de Chagas), Salud Púb. Mex. 2 (1984) 146–154.
[14] R.H. Gallerano, J.J. Marr, R.R. Sosa, Therapeutic efficacy of allopurinol in patients with chronic Chagas' disease, Am. J. Trop. Med. Hyg. 43 (1990) 159–166.

[15] D.R. Almeida, A.C. Carvalho, J.N. Branco, A.P. Pereira, L. Correa, P.V. Vianna, E. Buffolo, E.E. Martínez, Chagas' disease reactivation after heart transplantation: efficacy of allopurinol treatment, J. Heart Lung Transplant. 15 (1996) 988–992.
[16] W. Apt, X. Aguilera, A. Arribada, C. Pérez, C. Miranda, P. Cortés, J. Rodríguez, Treatment of chronic Chagas' disease with itraconazole and allopurinol: preliminary report, Rev. Méd. Chile 122 (1994) 420–427.
[17] S.L. de Castro, The challenge of Chagas' disease chemotherapy: an update of drugs assayed against Trypanosoma cruzi, Acta Trop. 53 (1993) 83–98.
[18] S. Martínez, J.J. Marr, Allopurinol in the treatment of Americans leishmaniasis, N. Engl. J. Med. 326 (1992) 741–744.
[19] R.H. Gallerano, R.R. Sosa, Efecto antiparasitario del allopurinol en la enfermedad de Chagas crónico, Medicina 45 (1985) 697–698.
[20] R.H. Gallerano, R.R. Sosa, Tratamiento de pacientes chagásicos con xenodiagnóstico positivo. Efectos del nifurtimox, benznidazol, y allopurinol, Rev. Fed. Arg. Cardiol. XIV (1985) 119–123.
[21] R.R. Sosa, R.H. Gallerano, Tratamiento de la enfermedad de Chagas crónico. Efectos del allopurinol, Rev. Fed. Arg. Cardiol. 17 (1988) 234–236.
[22] G. Gonzalez-Martin, I. Merino, N. Rodríguez-Cabezas, M. Torres, R. Nuñez, A. Osuna, Characterization and Trypanocidal activity of nifurtimox-containing and empty nanoparticles of polyethylcyanoacrylates, J. Pharm. Pharmacol. 50 (1998) 29–35.
[23] P. Couvreur, V. Lenaerts, D. Leyh, M. Guiot, M. Roland, Design of biodegradable polyalkylcyanoacrylate nanoparticles as a drug carrier, in: S.S. Davis, L. Illum, J.G. Mac Vie, E. Tomlinson (Eds.), Microspheres and Drug Therapy Pharmaceutical Immunological and Medical Aspects, Elsevier, Amsterdam, 1984, pp. 103–114.
[24] H. Cicek, A. Puncel, M. Puncel, E. Piskin, Degradation and drug release characteristics of monosize polyethylcyanoacrylate microspheres, J. Biomater. Sci. Polymer. Educ. 6 (1994) 845–856.
[25] H.C. Yang, Y. Nemoto, T. Homma, H. Matsuoka, S. Yamada, O. Sumita, et al., Curr. Microbiol 30 (1995) 173–176.
[26] C.W. Wade, F. Leonard, Degradation of poly(methyl 2-cyanoacrylates), J. Biomed. Mater. Res. 6 (1972) 215–220.
[27] Y.C. Tseng, Y. Tabata, S.H. Hyon, Y. Ikada, In vitro toxicity test of 2-cyanoacrylate polymers by cell culture method, J. Biomed. Mater. Res. 24 (1990) 1355–1367.
[28] V. Lenaerts, P. Couvreur, D. Christiaens-Leyh, E. Joiris, M. Roland, B. Rollman, P. Speiser, Degradation of poly (isobutyl cyanoacrylate) nanoparticles, Biomaterials 5 (1984) 65–68.
[29] M. Fresta, G. Puglis, G. Giammona, G. Cavallaro, N. Micali, P.M. Furner, Pefloxacine mesilate and Ofloxacin loaded polyethylcyanoacrylate nanoparticles: characterization of the colloidal drug carrier formulation, J. Pharm. Sci. 84 (1995) 895–902.
[30] C. Lherm, P. Couvreur, P. Loiseau, C. Bories, P. Gayral, Unloaded polyisobutylcyanoacrylate nanoparticles: efficiency against bloodstream trypanosomes, J. Pharm. Pharmacol. 39 (1987) 650–652.