An acute lymphoblastic leukemia cell-based preclinical assay revealed functional differences of commercial L-asparaginase administered in Colombia

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Abstract

L-asparaginase (L-ASNase) is an essential component of chemotherapy schemes due to its differential action over normal and leukemic cells. Recently, concerns on the performance of commercial formulations administered in developing countries have been reported. To address this problem, we developed a cell-based protocol to compare the activity of different L-ASNase formulations used in Colombia. We found three statistically different groups, ranging from full to no activity on leukemic cells using 0.05, 0.5 and 5.0 IU/mL concentrations. According to our results, we advise a preclinical evaluation for formulations of L-ASNase distributed in developing countries which could impact the outcome of patients.

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Abbreviations

B-ALL	B-cell acute lymphoblastic leukemia
L-ASNase	L-asparaginase
ASA	Asparaginase codified
E. coli	*Escherichia coli*
E. chrysanthemi	*Erwinia chrysanthemi*
AHA	Aspartic Acid β-hydroxamate
PEG	Polyethylene glycol
FSC/SSC	Forward Scatter/Side Scatter
NC	Negative Control
WHO	World Health Organization
UIS	Universidad Industrial de Santander
FCV	Cardiovascular Foundation

Abstract

L-asparaginase (L-ASNase) is an essential component of chemotherapy schemes due to its differential action over normal and leukemic cells. Recently, concerns on the performance of commercial formulations administered in developing countries have been reported. To address this problem, we developed a cell-based protocol to compare the activity of different L-ASNase formulations used in Colombia. We found three statistically different groups, ranging from full to no activity on leukemic cells using 0.05, 0.5 and 5.0 IU/mL concentrations. According to our results, we advise a preclinical evaluation for formulations of L-ASNase distributed in developing countries which could impact the outcome of patients.

INTRODUCTION

B-cell acute lymphoblastic leukemia (B-ALL) is a hematological disease caused by uncontrolled proliferation of undifferentiated immature lymphocytes. Medications such as Methotrexate, Cytarabine, L-ASNase, among others, are widely used in clinical practice and have been shown to be essential in the different stages of treatment, including relapse protocols. Multicenter studies around the world have shown that patients with ALL treated with combined chemotherapy schemes achieved survival rates greater than 90% in children and 70% in adults[1]. However, these reports come from high-income countries, while significantly lower levels are reported in developing countries. In Colombia, for example, it has been reported that the 5-year survival of the pediatric population is 68.9%, while in adults it is 40.3%[1]. This lower survival has been attributed to delays in getting an opportune and adequate treatment, access to health system, opportunity of diagnosis, among others[2].

Since 2020, Colombian patients with B-ALL have received diverse formulations of L-ASNase different from the original native *E. coli* L-ASNase (Kidrolase®), however alarms about their efficacy have been reported in the literature[3,4]. Recent reports have generated concerns among clinicians about the possible ineffectiveness of the drugs they are administering to their patients[5,6]. Different *in vitro* methods are used to evaluate the activity of L-ASNases, such as AHA or Nessler[7]. Although these methods determine L-ASNase activity in patient serum, they do not directly inform about the activity of the original enzyme batch. For this reason, our goal was to establish a preclinical methodology that would allow us to analyze the activity of different commercial formulations of L-ASNases distributed in Colombia on B-ALL cells.

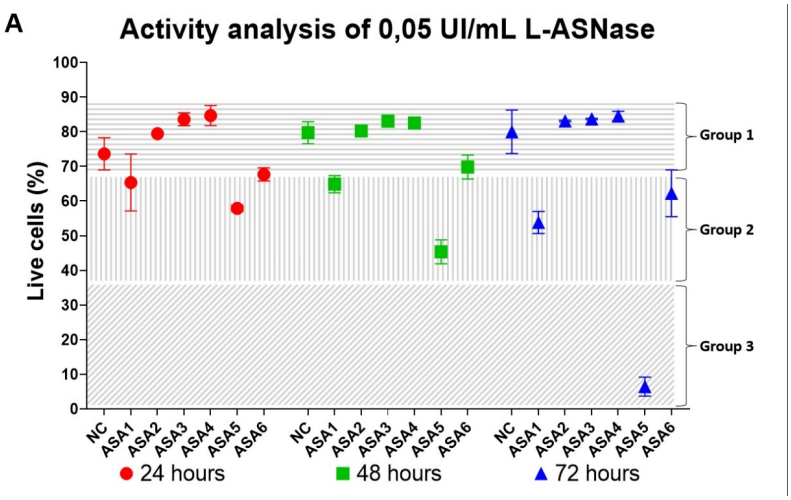
MATERIAL AND METHODS

We cultured 1x10^6 NALM-6 cells (B-ALL cells, commercially distributed by DSMZ, Germany) with concentrations (0.05, 0.5 and 0.5 IU/mL) of different commercial L-ASNases formulation, labeled ASA1-6. Formulations included native *E. coli* L-ASNases,*E. coli* PEG-asparaginase, and *E. chrysanthemi* L-ASNase. Since L-ASNase induce apoptosis in leukemic cells, cellular viability was used as indicator of this activity. Cellular viability was assessed by flow cytometry (FACSCanto II flow cell analyzer - Becton Dickinson) at 24, 48 or 72 hours of incubation using the Annexin V Apoptosis kit (Novus Biologicals) according to manufacturer's instructions. Percentage of live cells were defined as FSC/SSC homogeneous population, Annexin V and Propidium Iodide negative using Flowing Software 2 (Turku Bioscience).

RESULTS

Based on Chien W, *et al* [8] results, we use 0.05, 0.5 and 0.5 IU/mL concentrations of L-ASNase to evaluate conditions below or above therapeutic doses reported in human serum (0.1 UI/mL)[9]. As we expected, cell viability was inversely proportional to time of incubation and concentration of drug (**Fig. 1)** .

When comparing the activity of the drugs on B-ALL cells, we can define 3 statistically different groups (p <0.001) **(Fig. 1)** : Group 1 (ASA2-4), shows very low and no statistically significant differences compared to the negative control (>70-90% cell viability. p >0.05). Group 2 (ASA6) shows intermediate activity (>40 to 70% cell viability) and group 3 (ASA1 and ASA5) shows high activity (<40% cell viability). It is necessary to mention that all the drugs were reconstituted and stored according to the manufacturer's instructions, except for ASA6. ASA6 was received in the laboratory already reconstituted by the supplier.



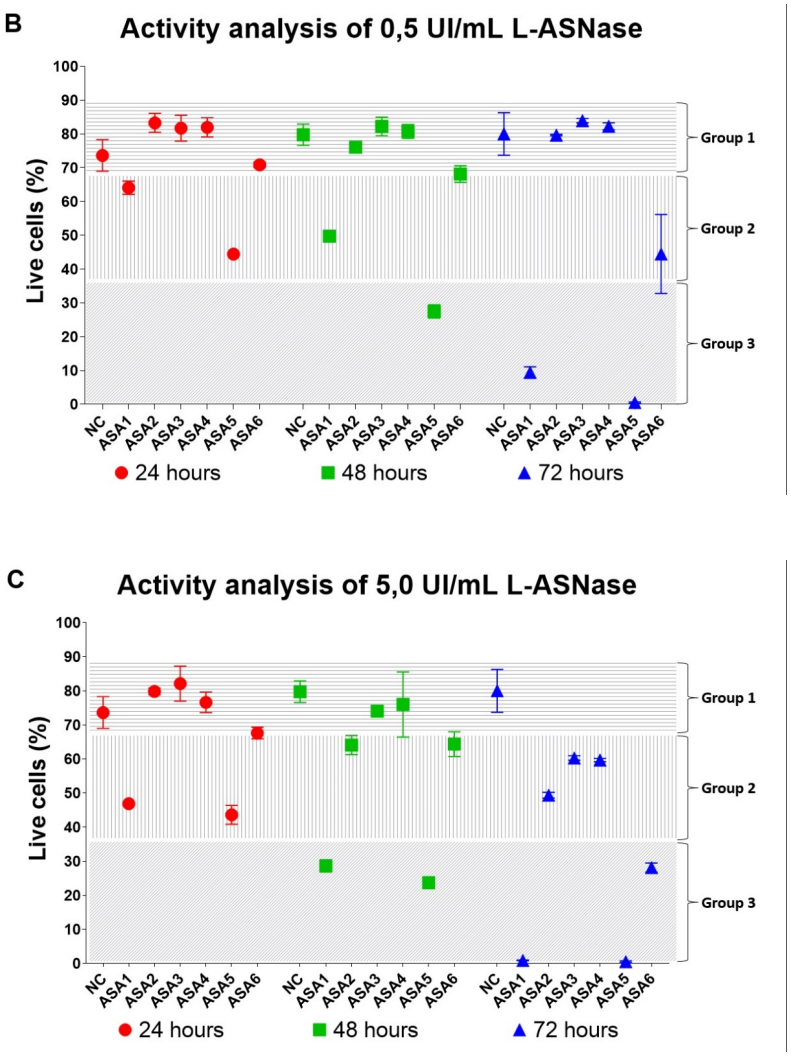


FIGURE 1 Activity analysis of 0.05, 0.5 and 5.0 IU/mL of commercial L-ASNases distributed in Colombia on B-ALL cells incubated for 24 (Red Circle), 48 (Green Square) and 72 (Blue Triangle) hours. Abbreviations: L-ASNase, L-asparaginase; NC, Negative Control.

Statistical differences (p <0.001) were identified between ASA2-4 activity and negative control just at 5.0 UI/mL with 72 hours of incubation **(Fig. 1C)** , which is a condition used in research exclusively[8]. ASA5 induced apoptosis at >90% of cells at 72 hours of incubation using the half of therapeutic dose concentration (0.05 UI/mL) **(Fig. 1A)** and ASA1 reached this percentage at the same time of incubation using 10 times more concentration (0.5 UI/mL) **(Fig. 1B)** . ASA6 induced statistically significant higher lethality of cells compared to ASA2-4 and the negative control (p <0.001), however 28.9% of cell were still viable after 72 hours with 5.0 UI/mL of this enzyme**(Fig. 1C)** .

DISCUSSION

Unlike normal cells, leukemic cells lack partially or totally of asparagine synthase activity , so they depend on importing asparagine from the extracellular medium for their proteins syntehsis[9]. Thus, a depletion of asparagine in extracellular medium by L-ASNase enzyme affect selectively tumoral cells. This is the reason for its use for more than 60 years ago to treat patients with ALL and lymphoma until today[10].

Unfortunately, some patients have important allergic reactions to L-ASNase enzyme due to their bacterial origin and it is necessary to change the formulation[11]. Diverse sources of microbial L-ASNase have been implemented and currently three are commercially available to treat patients (Native *E. coli* L-ASNases,*E. coli* PEG-asparaginase, and *E. chrysanthemi* L-ASNases)[12]. Although they all have advantages and disadvantages, their election is based on the immunological response of patients and the availability to the health system in each country, because there is no significant differences on patients outcome[13].

Nevertheless, developing countries have difficulties to acquire *E. coli* PEG-asparaginase and *E. chrysanthemi* L-ASNases because of their higher commercial prices. Here, biogeneric formulations take an important role to provide all children and adolescents with cancer a better chance of cure, described in the WHO Global Initiative for Childhood Cancer. However, there are some concerns about the quality of this drugs and our results support those worries.

Based on our results, we conclude that there are differences between the formulations of L-ASNase distributed in Colombia, which raises concerns about the quality of the treatment administered to patients. These differences may explain the limited ability of some formulations to achieve therapeutic levels in patient serum reported by groups from India and Brazil[3–6].

Inadequate transport and storage conditions, failures on reconstitution protocol, use of medicaments out of expiration date, defective reagent lot, unreported excipients, and low quality at industrial production are factors that can affect the performance of medications. Some of these factors were standardized previous to our analysis, but other factors cannot be controlled for the laboratory or health care facilities.

Consequently, we present a preclinical protocol that can be implemented to evaluate the quality of L-ASNase formulations. This methodology can analyze the activity of every batch of L-ASNase, which will allow for optimal regulation by the competent national entities, screening L-ASNase activity before being marketed and administered to patients with ALL. Thus, we present a methodology that should be included as a regulatory protocol for these drugs, which allow access to effective therapies while protecting the safety of patients.

CONFLICT OF INTERESTS

The authors declare not to have any interest conflicts.

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