# THE *A.P.P.A.*® PROJECT: FORMULATION, STABILITY AND QUALITY STUDY OF A PEDIATRIC GALENIC PREPARATION FOR THE TREATMENT OF SICKLE CELL DISEASE AT SAINT DAMIEN HOSPITAL IN HAITI

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## Sommario

Ad Haiti l'anemia falciforme è una patologia molto diffusa ed associata ad un alto tasso di mortalità. A causa di ciò, nel 2016, all'ospedale N.P.H. St Damien di Port-au-Princesi è reso necessario un medicinale a base di idrossiurea per trattare i pazienti pediatrici. Non essendoci in commercio una formulazione specifica, nell'ambito del Progetto *A.P.P.A.*®, presso l'Università di Torino, è stata studiata una formulazione galenica nonchè un metodo per controllarne la qualità e la stabilità nel tempo. Il medicinaleè stato introdotto *in loco*in seguito alla formazione del personale locale ed attualmente viene dispensato a circa 50 pazienti.

## Abstract

In Haiti sickle cell disease is a widespread disorder and it is associated with a high mortality. Because of this, in 2016, N.P.H. St Damien Hospital in Port-au-Prince required a medicine based on hydroxyurea to treat pediatric patients. Since there is no specific formulation on the market, in the context of the *A.P.P.A.*® Project at the University of Turin, a galenic formulation was studied along with a method to assess its quality and its stability over time. The medicine was introduced on site following the training of local staff and it is now dispensed to nearly 50 patients.

## Keywords

Pharmacists,Galenics, International Health Cooperation, Sickle cell disease, Hydroxyurea.

## The *A.P.P.A.*® Project

The *A.P.P.A.*® Project (*A.P.P.A.*® website) is the main activity of Aid Progress Pharmacist Agreement non-profit association; the Project is the result of the cooperation between the Department of Scienza e Tecnologia del Farmaco (Dstf) at the University of Turin and the pharmacists of the Italian community pharmacies. The Project complies with the International Health Cooperation principles and with the Italian and the laws of the guest Countries. The objective of the Project is the realization of galenic laboratories within healthcare facilities located in developing Countries. The advantages of the Project are:

- ensuring to local people medicinal products that comply with adequate quality requirements, in order also to fight the widespread phenomenon of counterfeit medicines in developing countries (Figure 1);
- customizing the dosages and pharmaceutical forms according to the actual needs of patients;
- employing local staff, to whom a profession is taught. This would encourage the opening of suitable schools;
- minimizing the financial commitment necessary to prepare these medicines.

**Figure 1: Cameroun, street pharmacists**

The Project is structured in six phases, through which it is possible to realize a galenic laboratory: from a preliminary pharmacoeconomic study meant to understand the actual local needs up to the quality control of the prepared galenic medicinal products analyzed in the lab of University of Turin. In the *A.P.P.A.*® labs, the medicinal products are chosen by physicians according to the real needs of the population and local technicians are trained by *A.P.P.A.*® volunteers to produce from raw materials high quality medicines. Several Projects are going on:two in Angola, two in Cameroun, two in Madagascar two in Chad and one in Haiti. Each lab differs from the others and for each of them, according to the different local needs, a specific handbook containing all formulas has been studied and it is constantly updated.

## The *A.P.P.A.*® Project at St Damien Hospital

Regarding the *A.P.P.A.*® lab in Haiti, it was set up in 2012 at St Damien Paediatric Hospital of "Nos Petits Frères et Sœurs" in the Tabarre district, one of the poorest neighbourhoods of Port-Au-Prince. The St Damien treats about 16,800 children and 4,500 mothers with their new-borns every year. For the laboratory established in Haiti at St Damien Hospital, given the low availability of paediatric medicines, it was necessary to study and then introduce several specific galenic formulas. The main active ingredients were identified in agreement with local Medical Doctors taking into account World Health Organization Model Lists of Essential Medicines and costs-benefits relationship. Then a formulation study was launched preferring liquid pharmaceutical forms, more suitable for children. For each preparation absorption spectrophotometry in the visible and ultraviolet was applied to the test the formulas' quality and stability respectively in accordance with European Pharmacopeia (Ph. Eur.) and European Medicines Agency (Ema) guidelines. Up today, all formulations have proved to be stable in Refrigerated Conditions (RC) and in Standard Conditions (SC) for 12 months, in Accelerated Conditions (AC) for at least 3 months. Stability tests carried out in accelerated conditions reflect the typical climatic conditions of tropical environments and therefore help predict stability in environments where conservation at controlled temperature and humidity cannot be guaranteed. Today, the galenics are made at the lab in Haiti according to specific standard procedures, their quality is constantly checked for the quality and they are used to treat patients (Figure 2).

**Figure 2: technical staff of the *A.P.P.A.*® galenic laboratory in Haiti during the preparation of medicines for oral use (left) and for injection use (right)**

## Study of a galenic preparation for the treatment of sickle cell disease

In 2016 at St Damien Hospital it was necessary to introduce a new formula for oral use containing hydroxyurea for the treatment of Sickle Cell Disease (Scd) in children. The availability of a therapy based on hydroxyurea (HU) suitable for the pediatric administration is relevant considering that this treatment is associated with an improvement of the quality of life and a decrease of mortality. In Haiti, Scd is very widespread and it has a subsequent serious impact on the population: its

prevalence in newborns appears to be greater than twice that among African Americans in the United States. A study conducted on 2013 at St Damien Hospital in fact underlined that the prevalence of Scd is 1 in 173 newborns, that is almost the double compared with that among African Americans in the Usa. (Rotz et al. 2013)

*Sickle cell disease and hydroxyurea*

The Scd is an important genetic cause of hemolytic anemia. Patients in which the disease occurs are homozygous for the HbS allele that encode the hemoglobin S (HbS) or heterozygous for the HbS allele and a second gene that code for another type of mutated-hemoglobin (hemoglobin C- HbC or thalassemia). It was observed that people heterozygous for the HbS allele are able to develop a greater resistance to malaria. In the areas where it is endemic (South-Central Asia, South and Central America and in particular Africa) the heterozygous trait is much more widespread than in other parts of the world. The presence of the heterozygous trait is greater in those countries with an important genetic influence from the African continent, such as for example Usa, Brazil and Haiti. In this latter country the individuals with African origin represents the 94% of the total population (Encyclopaedia Britannica 2016).

The distinctive clinical sign of Scd is represented by the vaso-occlusive crises (Voc). Their pathogenesis is complicated and heterogeneous: when oxygen tension is low the HbS is unstable and is prone to form spiral polymers that aggregate in voluminous and stiff particles (tactoids), responsible for the sickled shaped deformation of the red blood cells that become more rigid and obstruct the blood vessels. Moreover the sickled red cells undergo more easily to hemolysis, present an augmented adhesion to endothelium and to the other circulating cells, thus they provoke endothelial activation, the release of pro-inflammatory mediators and activation of the inflammatory cascade and coagulation pathway with a subsequent augmentation of the vasomotor tone.

Potentially all the conditions that lead to a reduction of the Hb oxygenation can trigger a Voc. The other Scd clinical signs are: intense painful events to bones and joints, cerebrovascular events, elevated risk of infections, priapism, lung diseases among which the most important is the Acute Chest Syndrome (Acs), defined as the appearance of a new pulmonary infiltrate during an X-ray examination in association with one of the following: fever, dyspnea, chest pain, desaturation, splenic or hepatic entrapment.

The standard treatment of patients affected by this disease consists in the administration of analgesics (NSAID and opioids), in the antibiotic prophylaxis during the first 5 years of life, in several vaccinations such as pneumococcal, meningococcal, Haemophilus Influenzae b type and blood transfusions.

Hydroxyurea, an inhibitor of ribonucleotide reductase, largely used in the treatment of the myeloproliferative disorders, represents the only approved drug that cause a reduction of the vaso-occlusive events typical in the serious forms of the disease. It is listed in the WHO Model list of Essential Medicines, (WHO 2017) both for the adults and the children.

*Mechanism of action of hydroxyurea*

It is known that the fetal hemoglobin (HbF) levels are one of the most important factors able to modify the clinical expression of the Scd.

High levels of HbF are associated with a less frequency of the painful crisis, a lower number of episodes of Acs and a reduced premature mortality. These clinical and epidemiological observations are supported by *in vitro* studies that demonstrated that the high levels of HbF inhibit the polymerization of the HbS through the formation of hybrid molecules ($\alpha 2\beta^S\gamma$), that go between the polymers and interrupt their growth. The pharmacological induction of HbF was tested by using myelosuppressive drugs, cytokines and short-chain fatty acids. Nevertheless, among all the studied molecules, HU is the only one that demonstrated effective and not very toxic. (Russo et al. 2011)

*Clinical evidence*

The clinical evidence and the poor toxicity of HU has been broadly demonstrated by several clinical studies thus the drug represents a valid therapeutic option for lots of patients with Scd. Since HU is a cytotoxic and cytostatic drug, initially its use in pediatric age was restricted only to children affected by severe forms of the disease. Afterwards, numerous clinical trials demonstrated that the efficacy and the toxicity of HU in children are similar to the ones in adults. The most frequent adverse effect observed, the myelotoxicity, is transitional and after the discontinuation of the treatment a rapid recovery of the hematological values takes place. Moreover, no delay in the growth has been observed. HU could also have an effect on prevention of stroke, one of the major complications in children with Scd and represents an important cause of morbidity and mortality. (Russo et al. 2011)

*Toxicity*

The adverse effects reported by using HU are rare; the eventual discontinuation of the drug takes place for lack of efficacy or compliance of the patient. The most frequent dose-dependent adverse effect is marrow aplasia, reversible when the drug is discontinued; this effect demands a follow-up of hematological values during the therapy. Until today considering the incidence of a leukemia transformation in patients affected by myeloproliferative diseases no difference was observed between the ones treated with HU and the ones not treated. (Russo et al. 2011)

*Hydroxyurea and pregnancy*

Since HUis an inhibitor of synthesis of Dna, all cells with elevated mitotic index are susceptible to its action. Thus, it is necessary to inform the patients, both men and women, about the potential risks of HUon conception and that when they would like to start a pregnancy it will be necessary to stop the treatment some months before. (Russo et al. 2011)

*Posology and routes of administration*

The initial dose of HUis of 10-15 mg/kg/day during 6-8 weeks. In absence of the clinical and hematological response it is necessary to increase the dose of 5 mg/kg/day every 4 weeks until a maximum of 35 mg/kg/day. A continuous oral therapy is recommended in a only daily administration. (Russo et al. 2011)

*Study of a galenic formulation for pediatric use*

In collaboration with the Akron Children's Hospital in Ohio, Usa, at St Damien Hospital in Haiti, during 2016 a health policy study was started with the purpose to assess the results obtained by the treatment with HU of pediatric patients affected by Scd. The results to be evaluated are not in terms of efficacy, since it has been widely demonstrated by numerous studies, but in terms of applicability of the therapeutic protocol already in use at their health facility. The applicability of a therapeutic protocol in a developing country like Haiti could be very difficult considering both the low level of education of the population and the reduced availability of infrastructures. For example, having to go monthly to the hospital to collect the medicine or understanding that it is necessary to take it daily can represent significant obstacles that risk undermining the continuity and thus the success of the therapy.

Considering the great diffusion of Scd in Haiti and the affordability of the therapy, the implications of this study could have a great impact on the quality of life of lots of Haitians.

On the market there is no pediatric formulation based on HU. The literature provides a procedure of preparation of a liquid formulation based on HU obtained from the capsules of industrial origin (Heeney et al. 2004). This procedure presents nevertheless the following several critical issues:

- the final volume of the preparation (to achieve by adding syrup) is initially measured by pouring in the immediate packaging a quantity of water corresponding to the final volume and by marking externally on the packaging the level to achieve. Certainly this is not a precise method;
- the quantity of powder (active ingredient and excipients) contained in the capsules of industrial origin to be weighed is not checked;

- the syrup chosen to achieve the final volume contains ethanol, excipient that must be avoided, if not strictly necessary, in the pediatric formulations;
- the stability of the preparation under environmental conditions typical of Haiti has not been studied.

Underlined this critical issues, it was decide to conduct an *ad-hoc* formulation study. Since the active ingredient is not available on the market for the preparation of galenic preparations it was necessary to use the powder contained by the Italian industrial medicinal product called Oncocarbide®. This consists in capsules that contain 500 mg of HU; the excipients are: lactose, calcium citrate, sodium citrate dibasic and magnesium stearate.

Considering the necessity to facilitate the pediatric administration, the good water solubility of the active ingredient and the kind of the excipients, it was decided to produce a liquid aqueous preparation. In particular, a preparation based on sucrose syrup was chosen, since it is constituted by inexpensive and easily available on site raw materials and able to give a good palatability to the preparation. The composition of the syrup for 500 ml resulted as follows:

| | |
|---|---|
| Hydroxyurea | 50 g (contained in 100 cps) |
| Sodium methyl p-hydroxybenzoate | 0,25 g |
| Purified water | 250 g |
| Saccharose syrup | q.b. a 500 ml |

Considering the equipment available on site it was decided to prepare 500 ml as the maximum quantity pro batch, to be sure to guarantee the quality of the final product.
The developed procedure indicates to calculate the average weight of the powder contained by a single capsule (active ingredient + excipients) and to empty a sufficient number of capsules to obtain the intended dose of active ingredient; afterwards a solution of HU is prepared by dissolving the powder in purified water and sodium methyl p-hydroxybenzoate and by stirring. The solution must be filtered and weighed, and subsequently the quantity of sucrose syrup necessary to achieve the final volume must be added. Finally, the pH of the preparation must be measured as a preliminary control on the galenic medicine.

*Quality control and stability test of the syrup of hydrxyurea*

When the procedure of preparation of the new formulation was defined, a method of analysis to perform the quality control and to test the stability over time was chosen.

In the literature we found different techniques to quantify HU but the majority of them required expensive and hardly available on site equipment and reagents. Thus, it was decide to take as reference the analytical method reported in the article "Assay for Hydroxyurea" by J.F. Alicino in 1970 that is aiodometric titration. This method is inexpensive and easy to reproduce, important conditions that must be considered when, in accordance with the objectives of the Project and of the International Cooperation, we would make the laboratory independent by introducing the analytical method on site too.

J. F. Alicino reported two analytical procedures of which we chose the most reproducible even if less sensible in order to introduce the analytical method on site. The analytical method was validated in our experimental conditions through the analysis of HUPh.Eur standard and 139 tests on 15 different samples were performed. All the samples satisfied the assay uniformity of content (Ph.Eur., assay 2.9.6) (EDQM 2010): the results obtained were in the range of ±10% of the expected (nearly 90% of the results ranged between ± 5% of the expected).

To assess the stability of the preparation over time, at Dstf several samples were prepared and were analyzed after storing them in different environmental conditions established by Ema guidelines for the stability tests of the industrial medicinal products: this procedure was routinely applied for the galenic preparations introduced at St Damien Hospital in Haiti in the last 6 years. In particular, the stability test involves the preparation of the medicine and its analysis with the purpose to obtain a value that represents the starting point or time zero ($T_0$). After the analysis at $T_0$ the formulation is partitioned in different aliquots and each of them stored in different conditions of temperature (T) and relative humidity (RH %), based on the Ema guidelines (Ema website). The storage conditions of the samples detailed in the Table 1.

**Table 1 – Storage conditions of the samples for the stability tests**

| Storage condition | T (°C) | RH % | Period covered by data |
|---|---|---|---|
| SC | 25±2 | 60±5 | 12 months.<br>Analysis at time zero (T0), every 30 days for 3 months (SC-T1 to SC-T3), after 6 months (T6), after 12 months (T12) |
| RC | 5±3 | / | 12 months.<br>Analysis at time zero (T0), every 30 days for 3 months (RC-T1 to RC-T3), after 6 months (T6), after 12 months (T12) |
| AC | 40±2 | 60±5 | 3 months.<br>Analysis at time zero (T0), every 30 days for 3 months (AC-T1 to AC-T3), after 6 months (T6), after 12 months (T12) |

When it is scheduled, the preparation is analyzed while $T_0$ is used as the reference value, not only in the stability test but also in the routine quality control on medicines produced in the *A.P.P.A.*® laboratories.

The SCare applied to define the stability of samples under storage conditions accepted by the Ph.Eur; the RC are applied to evaluate the need for this type of storage. The AC allow firstly to reduce the time of analysis, since a month of conservation in these conditions is the equivalent to four months of conservation in SC (Cooperativa Farmaceutica 1996). Secondly, they help to predict the stability in the tropical environments typical of the countries where the *A.P.P.A.*® laboratories are active and the conservation of the medicinal products at controlled temperature and humidity often cannot be guaranteed.

The results achieved by the stability test for the HU syrup are listed in Table 2. The result was considered acceptable when ranged between ±10 % in comparison with $T_0$ value. The table reports the average percentage error obtained by the analysis of the samples stored in envisaged environmental conditions. The preparation studied as above described is stable in SC up to 6 months, in RC up to 12 months and in AC up to 3 months.

**Table 2 – Results of the stability test of the hydroxyurea syrup 100 mg/ml**

| Storage conditions | T0 | T1 | T2 | T3 | T6 | T12 |
|---|---|---|---|---|---|---|
| | Δ% | Δ% | Δ% | Δ% | Δ% | Δ% |
| SC | -2,01% | +2,12% | +1,04% | -2,85% | -7,50% | -11,49% |
| RC | / | +3,96% | +0,61% | +0,57% | -4,76% | -5,42% |
| AC | / | +2,85% | -2,17% | -9,11% | -21,22% | -45,48% |

In order to label the preparation with a correctly defined expiry dates necessary to guarantee the security of the patients, we took the results of the stability tests into account. We also took into account the fact that the medicines will be used in places other than the hospital, such as houses, where the environmental conditions cannot be controlled and thus the correct conservation of the medicine cannot be guaranteed. As a precautionary measure it was thought appropriate to give a validity period of three months for the prepared syrup. This is the same period for which it was tested in AC, condition that reflects the environmental conditions of Haiti.

**Figure 3 - Technicians during the filtration of thehydroxyurea syrup**

*Introduction of the formula on site*

At St Damien Hospital local staff was trained and specific procedures were introduced concerning both the preparation (Figure 3 and 4) and the dispensing of the medicinal product. Considering the potential mutagenicity and teratogenicity of hydroxyurea, during the training a particular attention was given to all phases of its manipulation, giving precise guidelines to who prepares the medicinal product, to who is dispensing it and to who would teach the relatives of the patients how to use it.
The instructions were created in French language and enriched by clear images to be understood by everyone, considering that the average level of education in Haiti is quite low (Figure 5 and 6).
The local personnel responsible for the preparation of the medicine was considered capable and autonomous to operate. In fact all the results obtained by its control of quality were positive. The process is constantly checked in order to guarantee the quality of the medicinal product in accordance with the Ph.Eur.. At Dstf we also started a new study of the HU syrup 100 mg/ml prepared using as raw materials the industrial medicinal products available on the local market, with the objective to make the galenic laboratory and thus the health facility more independent, in accordance with the International Cooperation and with the *A.P.P.A.*® Project.

**Figure 4 – Technician during the preparation of hydroxyurea syrup**

Thanks to the strong collaboration among all the institutions involved in the Project, in order to meet the needs of the St Damien Hospital the new formula was studied, it was successfully introduced on site and about 3 litre of syrup are prepared every week at the *A.P.P.A.*®galenic laboratory. The medicinal product is monthly dispensed to nearly 50 patients involved in the health policy study conducted by the Akron Children's Hospital, Usa. At the moment the study is giving promising results: this confirms the

quality of the galenic product and, given the great need to treat the patients affected by Scdin Haiti, the applicability of the therapy based on hydroxyurea that will improve the quality of life of many Haitians.


**Figure 5 – Procedure for the manipulation of the hydroxyurea syrup meant for the personnel responsible for the dispensing of the medicine**


**Figure 6- Procedure for the manipulation of the hydroxyurea syrup meant for the relatives of the patients**

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## Abbreviations

| | |
|---|---|
| AC | Accelerated Conditions |
| Acs | Acute Chest Syndrome |
| *A.P.P.A.*® | Aid Progress Pharmacist Agreement |
| Dstf | Department of Scienza e Tecnologia del Farmaco |
| Ema | European Medicine Agency |
| HbC | Hemoglobin C |
| HbF | Fetal hemoglobin |
| HbS | Hemoglobin S |
| HU | Hydroxyurea |
| Ph. Eu. | European Pharmacopoeia |
| RC | Refrigerated Conditions |
| SC | Standard Conditions |
| Scd | Sickle cell disease |
| Voc | Vaso-occlusive crises |