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

Non-profit organization Aid Progress Pharmacist Agreement®, Turin, Italy

Department of Scienza e Tecnologia del Farmaco, University of Turin, Italy
THE A.P.P.A.® PROJECT

✓ International Health Cooperation
✓ It is based on voluntary work
✓ Its aim is the realization, within the health facilities of Developing Countries, of laboratories for the preparation of galenic medicines on the basis of the local therapeutic needs.

The medicinal products prepared must meet the requirements of:

QUALITY       SAFETY       EFFICACY

The advantages are:
✓ Customizing the dosages and pharmaceutical forms according to the actual needs of patients
✓ Employing local staff, to whom a profession is taught
✓ Minimizing the financial commitment necessary to prepare the medicines
✓ Discouraging the use of counterfeit medicines
COUNTERFEIT MEDICINES

“A counterfeit medicine is one which is deliberately and fraudulently mislabeled with respect to identity and/or source. Counterfeiting can apply to both branded and generic products and counterfeit products may include products with the correct ingredients or with the wrong ingredients, without active ingredients, with insufficient active ingredients or with fake packaging” (WHO)

“IMPERFECT” COUNTERFEITS

«These products contain the right components, with an incorrect concentration and/or formulation resulting in defective quality specifications. In the vast majority of cases, they are devoid of any therapeutic efficacy»

“CRIMINAL” COUNTERFEITS

«They are apparently similar to the original medicinal product, but do not contain any active ingredient and can even include harmful or toxic substances. They are usually sold at high prices and for the treatment of serious pathologies. Consequences for users of criminal counterfeits can be fatal»

Di Giorgio D. Counterfeit drugs. The phenomenon and enforcement activities. Milano:Tecniche nuove; 2010.
## PHASES OF A.P.P.A.® PROJECT

<table>
<thead>
<tr>
<th>Phase «ZERO»</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Preliminary pharmaco-economic study</strong> which implies a trip on site to evaluate the local situation. Some industrial medicines should be purchased in local pharmacies and sent to the laboratory of the University of Turin to evaluate if these medicinal products, present on the local market, meet the requirement of quality.</td>
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<table>
<thead>
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<tr>
<td><strong>Choice of the medicines and the related pharmaceutical forms, based on the local need.</strong></td>
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<tr>
<td><strong>Learning of the preparation techniques of the pharmaceutical forms by the student of pharmacy.</strong></td>
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<tbody>
<tr>
<td><strong>Internship in Italy of an operator coming from the country of destination of the Project. Purchase and delivery on site of the equipment and of the raw materials required.</strong></td>
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<tbody>
<tr>
<td><strong>Mission on site of the trained student with the purpose of setting up the laboratory and transmitting, in coordination with the operator that was trained in Italy, to the others local operators the acquired knowledge.</strong></td>
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<th>5</th>
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<tbody>
<tr>
<td><strong>Preparation of medicinal products and related quality control; moreover, routinely, some samples are sent to University of Turin, where they are tested to verify their quality.</strong></td>
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<tbody>
<tr>
<td><strong>Periodical missions of Pharmacy's students at new lab are performed each year to give a continuous supervision in the production of medicinal products and eventually to introduce new formulas according to the requests of medical doctors responsible of the health facility.</strong></td>
</tr>
</tbody>
</table>
ANGOLA – Cubal, Nossa Senhora de Paz hospital, Companhia de Santa Teresa de Jesus.
ANGOLA – Funda, A.M.E.N. ONG health care facility.
CAMEROUN – Douala, La Bethanie hospital – Kribi – Saint Joseph hospital
CAMEROUN – Garoua, Notre Dame des Apôtres hospital, Djamboutou.
TCHAD – N’Djamena, Le Bon Samaritain hospital.
TCHAD – Biobé, Le Bon Samaritain hospital.
HAITI – Tabarre Chateaublond, N.P.H. Saint Damien paediatric hospital.
MADAGASCAR – Vohipeno, Henintsoa hospital.
MADAGASCAR – Ihosy, Eglise Catholique Apostolique Romaine medical center.
HÔPITAL SAINT DAMIEN

- Located in Tabarre, suburb of Port-au-Prince
- Facility of excellence
- The biggest paediatric hospital of the Caribbean
- Realized thanks to the big contribution of the Fondazione Francesca Rava – N.P.H. Italia ONLUS
- In 2015, 13300 children were visited and 3400 hospitalized

Offered services:
Emergency room, surgery, laboratory of analysis, outpatients ward, cancer ward, dental clinic, recovery program for malnourished children, vaccinations and screening ward, maternity ward, PHARMACY

St Damien hospital
GALENICS IN HAITI: WHY?

- LOW AVAILABILITY ON SITE OF PEDIATRIC MEDICINAL PRODUCTS
- POOR QUALITY OF MEDICINAL PRODUCTS

THE A.P.P.A. ® GALENIC LABORATORY IN HAITI

2011: pharmacoeconomic study

2011-2012: formulation and stability study and elaboration of the A.P.P.A. ® formulas handbook

2012: initiation of the galenic laboratory and of the laboratories for the preparation of sterile products

2012-2017: annual missions for the control and the development of the laboratory

PERIODIC QUALITY CONTROL OF THE PRODUCED MEDICINES

MONTHLY

- 100 LITRES OF LIQUID PREPARATIONS
- 4000 CAPSULES
INTRODUCTION OF HYDROXYUREA SYRUP 100 mg/ml

SICKLE CELL DISEASE (SCD)
Group of inherited red blood cell disorders. People with SCD have abnormal hemoglobin that makes the red blood cell sickle shaped and stiff
- Oxygen delivery problems
- Vascular occlusions

PREVALENCE
Prevalence worldwide → 1: 23 333
Prevalence among the African Americans → 1: 365
Prevalence in Haiti → 1:173

Rotz et al - Prevalence of sickle cell disease, hemoglobin S, and hemoglobin C among Haitian newborns, American Journal of Hematology, 2013

Global map of the presence or absence of the sickle cell mutation

Tewari et al - Environmental Determinants Of Severity In Sickle Cell Disease, Haematologica, September 2015
**INTRODUCTION OF HYDROXYUREA SYRUP 100 mg/ml**

**WHO Model list of Essential Medicines for children**

**HYDROXYUREA**

- Increase in the quality of life
- Decrease of the mortality

**POSOLOGY:** 10-30 mg/kg/die *per os*
Max. dose: 35 mg/kg/die

- **IDROSSIUREA**
  - ↑ HbF synthesis (fetal hemoglobin)
  - ↓ polymerization of the HbS (emoglobina mutata) chains
  - ↓ vaso-occlusive crises

In collaboration with the *Akron Children’s Hospital* of Ohio, USA, study on 50 patients:

- Valuation dell’APPLICABILITY OF THE THERAPEUTICAL PROTOCOL in a DEVELOPING COUNTRY
INTRODUCTION OF HYDROXYUREA SYRUP 100 mg/ml

Formulation request by the medical staff:
LIQUID PREPARATION FOR ORAL USE based on sucrose syrup (100 mg/ml) – paediatric use

Hydroxyurea as active ingredient is not available on the market

Preparation by using the CAPSULES OF INDUSTRIAL ORIGIN

Formula for 500 ml

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxyurea capsules 500 mg</td>
<td>100 cps</td>
</tr>
<tr>
<td>Sodium methyll p-hydroxybenzoate</td>
<td>0,25 g</td>
</tr>
<tr>
<td>Purified water</td>
<td>250,00 g</td>
</tr>
<tr>
<td>Sucrose syrup</td>
<td>296,00 g</td>
</tr>
</tbody>
</table>

1. Preparation of an aqueous solution of hydroxyurea
2. Filtration
3. Addition of sucrose syrup
ANALYTICAL METHOD
TO PERFORM THE STABILITY TEST AND THE QUALITY CONTROL

- LOW COST
- SIMPLE TO REPRODUCE

IODOMETRIC TITRATION

- The sample is treated with NaHCO$_3$ 10% and NaH$_2$PO$_4$ 20% solutions
- Reaction between HYDROXYUREA and 0,01 N IODINE
- The excess iodine is titrated with 0,01 N THIOSULPHATE
  \[ 2 S_2O_3^{2-} + I_2 \rightarrow S_4O_6^{2-} + 2 I^- \]
- Conversion factor: 1 ml of iodine 0,01 N corresponds to 0,360 mg of hydroxyurea
- Maximum reactivity with basic pH

EMA Guideline on stability testing: stability testing of existing active substances and related finished products, 2003, CPMP/QWP/122/02, rev 1 corr

**STORAGE CONDITION**

<table>
<thead>
<tr>
<th>STORAGE CONDITION</th>
<th>T (°C)</th>
<th>RH %</th>
<th>PERIOD COVERED BY DATA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard (SC)</td>
<td>25±2</td>
<td>60±5</td>
<td>12 months. Analysis at time zero (T0), every 30 days for 3 months (SC-T1 to SC-3), after 6 months (T6), after 12 months (T12)</td>
</tr>
<tr>
<td>Refrigerated (RC)</td>
<td>5±3</td>
<td>/</td>
<td>12 months. Analysis at time zero (T0), every 30 days for 3 months (RC-T1 to RC-3), after 6 months (T6), after 12 months (T12)</td>
</tr>
<tr>
<td>Accelerated (AC)</td>
<td>40±2</td>
<td>60±5</td>
<td>12 months. Analysis at time zero (T0), every 30 days for 3 months (AC-T1 to AC-3), after 6 months (T6), after 12 months (T12)</td>
</tr>
</tbody>
</table>

T0: time 0; T1: 30 days; T2: 60 days; T3: 90 days; T6: 180 days
T: temperature; RH: relative humidity

**RESULTS OF THE STABILITY TEST OF THE HYDROXYUREA SYRUP 100 mg/ml**

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

*The results represents the average of the analysis of 15 samples
Δ% = percentage error compared with the expected concentration value
Stability is demonstrated when: -10% < Δ% < +10%

Stability was demonstrated up to 3 months in tropical conditions

STABILITY TEST  Evaluation of the expiration date
In galenics, in accordance with the European Law (Ph Eur), “the quality as a fundamental support to the security and the efficacy” must be ensured.

**QUALITY CONTROL AND QUALITY ASSURANCE**

**PHARMACEUTICAL FORMS TESTS**

- Ointments for skin application; Suppositories:
  - Verification of observation of the procedures
  - Control of aspect
  - Control of the amount to be sold
  - Control of the resilience of the packaging

- Stiff capsules:
  - Verification of observation of the procedures
  - Control of aspect and solidity of capsules
  - Control of the number of capsules prepared
  - Uniformity of mass

- Liquid preparations:
  - Verification of observation of the procedures
  - Control of the amount of product to be sold
  - Control of the resilience of the packaging

**RAW MATERIALS**

- Organoleptic control
- Melting point

**STABILITY TESTS** (EMA)

**ITALY**

**PHARMACEUTICAL FORMS TESTS** (Ph Eur)
- Uniformity of content (2.9.6)
- Uniformity of mass (2.9.5)
- Disaggregation (2.9.1)
- Friability (2.9.7)
- Hardness (2.9.8)
- Sterility (2.6.1)
INTRODUCTION OF MEDICINAL PRODUCT ON SITE

PROCEDURES FOR THE PREPARATION AND THE MANIPULATION OF THE HYDROXYUREA SYRUP

Training to:

➤ Technical personnel of the galenic laboratory
➤ Technical personnel of the external pharmacy
➤ Nursing staff

Avertissements pour l’emploi de l’hydroxyurea

Pendant toutes les opérations il faut:

- Opérer sous la hotte chimique
- Utiliser les gants, le masque anti poussière, les lunettes de protection
  N.B. le masque anti poussière est personnelle.
- Il faut écrire le nom de l’opérateur et la date de première utilisation des gants et la garder dans
  la hotte.
- Il faut la utiliser pendant 6 mois au maximum.

À la fin du travail il faut:

- Nettoyer soigneusement le matériau et le plan de travail avec alcool 70°
- Éliminer les déchets dans un conteneur adapté sous hotte.
- Se laver soigneusement les mains.

Les risques associés à l'utilisation de la substance sont les suivants:

- Sensibilisation respiratoire, catégorie 1
- Muragénicité sur les cellules germinales, catégories 1A, 1B, 2
- Carcinogénicité, catégories, 1A, 1B, 2
- Toxicité réproductrice, catégories 1A, 1B, 2
- Toxicité spécifique pour un organe cible à la suite d'une exposition unique, catégories 1, 2
- Toxicité spécifique pour un organe cible à la suite d’une exposition répétée, catégories 1, 2
- Dangere d'aspiration, catégorie 1

Instructions pour la manipulation du sirop de hydroxyurea

1. Travaillez dans une salle protégée.
2. Prênez le bon état d'esprit.
3. Portez tous les gants.
4. Lavage soigneux des mains et du visage.
   - Lavage mains et du visage à l'eau et au savon.
   - Lavage sous la GSP et la serviette utilisée.
5. La manipulation est interdite à tous les hommes enceintes.
The new formula was studied, its quality was demonstrated and its stability was tested.

The local personnel was trained to produce the medicinal product.

During 18 months about 40 liters of hydroxyurea syrup were prepared and dispensed to nearly 50 patients involved in the study.

The study is giving promising results: the treatment of sickle cell disease with hydroxyurea will improve the quality of life of many haitians.
AKNOWLEDGEMENTS

• Fondazione Francesca Rava – NPH Italia onlus non-profit association that have requested and financed the realization of the A.P.P.A.® Project in Haiti

• Akron Children’s Hospital, Ohio, USA

• The NPFS - Saint Damien Hospital, Haiti. In particular all the local technicians which in these years have been working in the lab

FOR FURTHER INFORMATION
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WRITE AT: appa.onlus@unito.it