IMPROVEMENT OF THE MASS PRODUCTION OF NICOSAN™/XICKLE™ AN HERBAL MEDICINE THAT CONTAINS STRONG ANTISICKLING AGENTS

Toshio Asakura M.D., Ph.D.[1], Qiukan Chen M.D.[1] , Swami Nathan Ph.D.[2], Prema Tripathi[2] and Robert Swift, Ph.D.[2] [1]Division of Hematology, Children's Hospital of Philadelphia, Philadelphia, PA, USA
[2]Xechem Inc., Edison, NJ, USA

Objective of Purpose: NIPRISAN (NICOSAN™/Xickle™), an extract of 4 dried plants, has been used for many years by traditional health providers in Nigeria to treat patients with sickle cell disease (SCD). Early basic studies and Phase I/II clinical trials of NIPRISAN performed at the National Institute for Pharmaceutical Research and Development (NIPRD) in Nigeria showed that administration of NIPRISAN to children with SCD significantly reduced the frequency of painful episodes and increased school attendance. The purpose of this study was to establish a method of production of NIPRISAN (NICOSAN™/Xickle™) that shows similar antisickling activity among different batches.

Method: The SCD Reference Laboratory (Ref Lab) at The Children's Hospital of Philadelphia performs extensive preclinical *in vitro* and *in vivo* evaluation of each candidate drug. In the *in vitro* evaluation, we test each drug using the following 11 tests and determine whether a candidate drug has one or more of the following beneficial or adverse effects:

1. inhibition/promotion of sickling of sickle erythrocytes (SS cells)
2. hydration/dehydration of SS cells
3. prolongation/shortening of the delay time prior to hemoglobin (Hb) S polymerization,
4. increase/decrease in the solubility of deoxy-Hb S
5. shift of the oxygen equilibrium curve (OEC) to the left/right
6. increase/decrease in membrane ion (Na^+, K^+) transport
7. reduction/increase in the adhesion of red blood cells (RBCs) to endothelial cells
8. decrease/increase in methemoglobin formation,
9. decrease/increase in hemolysis
10. decrease/increase in the amount of intracellular denatured Hb S.

Drugs that show a beneficial effect in the *in vitro* evaluation protocol without significant adverse effects are further tested *in vivo* using transgenic (Tg) sickle mice. The usefulness of this new multi-evaluation drug discovery method is its capacity to evaluate the effectiveness/adverse effect of each drug accurately. The method of production of NIPRISAN was varied at each step in an effort to establish a NIPRISAN product that has consistently high antisickling activity among different lots. A small sample was collected at each step of production at the factory of Xechem, Inc. and the samples were sent to the SCD Ref Lab for evaluation of antisickling activity.

Results: After testing many samples of NIPRISAN (NICOSAN™/Xickle™) that were produced by different methods, we could establish a drug production method by which we can produce NIPRISAN (NICOSAN™/Xickle™) that shows maximum antisickling activity with minimum adverse effects. We also found that NIPRISAN (NICOSAN™/Xickle™) contains various aromatic aldehydes that combine with sickle hemoglobin and inhibit polymerization of deoxy-Hb S and sickling of sickle hemoglobin. Oral administration of NIPRISAN to transgenic mice that produce human sickle hemoglobin prevented hypoxia-induced formation of sickled cells in the venous blood and vaso-occlusive events in a dose-dependent manner.

Conclusion: One problem with herbal medicines has been the difficulty in producing drugs that have consistently strong activity among different batches. Each step in the production of NIPRISAN was optimized, and we have now found a method of production of NIPRISAN (the final product is called NICOSAN™ in Nigeria and Xickle™ in the USA) with consistently high antisickling activity among different batches. Swift et al. found that the anti-sickling activity varied depending on the location where the component plants were grown (which had different growing conditions) and the age of the plants. The relationships between these two factors and antisickling activity are presented by Swift et al. at this meeting.