Molecular Basis for Sickle Cell Disease

Adapted from Nicholas' Story by Didem Vardar-Ulu for CH373-S20

POST-CLASS WORK- VERSION B KEY

Q2A. (10 pnts) In the video you watched, Nicholas says hydroxyurea an approved drug for treating Sickle Cell Disease, changed his life. His mother explained that since he started the hydroxyurea treatment, Nicholas has been able to be more active and have a regular schedule with sports, school, and friends.

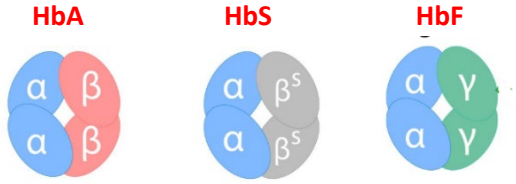
"What does hydroxyurea do and how does it help Nicholas have a more regular life?"

Search for hydroxyurea in DrugBank (https://www.drugbank.ca/drugs/DB01005), a curated resource that provides a wide variety of information of drugs and drug-like molecules. Using the information provided about what the drug does and its mechanism of action and your understanding of the molecular basis of sickle cell disease write a short explanation of how hydroxyurea help SCD patients. Your answer should provide a plausible explanation to how the interferes with the sickling process. Make sure to provide specific biochemical links between what the drug's direct effect is and the ultimate outcome (improvement in symptoms) based on the molecular explorations you did during the "in-class" part of the case study. You will be graded based on the correct and specific biochemistry you offer in your hypothesis and not for "the correct answer".

Grading Rubric:

Biochemical content needs to include:

- Statement of hydroxyurea's direct effect on the system: Increases HbF
- Structural and functional description of what HbF is: A tetramer of two alpha, two gamma subunits that carries oxygen. The gamma subunit is normally expressed in fetus as it has a higher binding affinity for oxygen than the beta subunit that replaces it in adult hemoglobin.



- Structural differences between HbS and HbF: HbS is made up of two alpha and two beta subunits like the regular adult hemoglobin. However the beta subunit of HbS carries a mutation of E6V that introduces a hydrophobic residue to the N-terminal end of the beta chain.
- Structural basis for Sickle cell disease: The association of Val6 from one beta chain with the hydrophobic patch made up of A70, F85, L88 that gets surface exposed in the "tense-deoxyhemoglobin" structure causes fiber formation across different hemoglobin tetramers and distorts (sickles) the red blood cells.

Biochemical Support needs to include:

- HbF lacks beta subunits so any mutation that impacts beta subunit will not have an effect on the structure of HbF.

- When there is an upregulation for the production of the gamma subunit and hence the preferential formation of HbF (as triggered by hydroxyl urea), there will be a relative increase of HbF tetramers over HbS tetramers in the circulating blood. Since fiber formation requires hydrophobic associations between tetramers that contain the Val6 mutation and the patch, the decrease in the concentration of the tetramers having the mutation (HbS) will decrease the likelihood of forming fibers, i.e. the chance of sickling.
- Pain is a result of the pressure of the sickled cells on the blood vessels, when there is less sickling, there will be less pressure on the vessel walls and hence decrease in pain.

Q2B. (10 pnts) In November 2019 the FDA approved voxelotor for adults and pediatric patients 12 years of age and older with sickle cell disease.

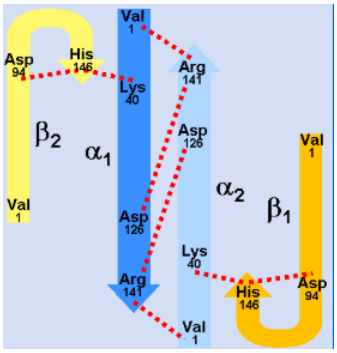
Search for voxelotor in DrugBank (https://www.drugbank.ca/drugs/DB14975), a curated resource that provides a wide variety of information of drugs and drug-like molecules. Using the information provided about what the drug does and its mechanism of action and your understanding of the molecular basis of sickle cell disease write a short explanation of how voxelotor help SCD patients. Your answer should provide a plausible explanation to how the drug interferes with the sickling process. Make sure to provide specific biochemical links between what the drug's direct effect is and the ultimate outcome (improvement in symptoms) based on the molecular explorations you did during the "in-class" part of the case study. You will be graded based on the correct and specific biochemistry you offer in your hypothesis and not for "the correct answer".

You can also access a journal article reporting Pharmacokinetics and pharmacodynamics of voxelotor through the following link:
https://drive.google.com/file/d/16GmyO9bIO3ITYjGO4YEjMI9wyGCD_f6X/view?usp=sharing

Grading Rubric:

Biochemical content needs to include:

- Statement of Voxeletor's effect: Allosteric regulator that increases hemoglobin's affinity for oxygen
- Structural and functional description of how Voxeletor regulates oxygen binding affinity: Voxeletor binds irreversibly with the amino terminus residue Valine of the alpha chain in hemoglobin.
  The deoxygenated, tense state of hemoglobin molecules is stabilized by several ionic interaction that underlie the allosteric behavior observed in hemoglobin. One of these salt bridges involves the N-terminal Valine of one of the alpha subunits and the C-terminal Arginine of the second Two salt bridges important to the allostery (communication between subunits) of hemoglobin include: one salt bridge between the N-terminal valine of one alpha subunit and the C-terminal arginine of the other alpha subunit.
  http://biomodel.uah.es/en/model1/prot/hb-salinos.htm



  When Voxelotor binds covalently with the N-terminal valines on the alpha chains of hemoglobin, the ionic interactions which stabilize the tense state are severed, thus leading to the favorability of the oxygenated state.
- Structural basis for Sickle cell disease: The association of Val6 from one beta chain with the hydrophobic patch made up of A70, F85, L88 that gets surface exposed in the "tense-

deoxyhemoglobin" structure causes fiber formation across different hemoglobin tetramers and distorts (sickles) the red blood cells.

Biochemical Support needs to include:

- In the oxygenated form of hemoglobin the conformation of the beta subunit does not expose the hydrophobic patch made up of A70, F85, L88 to the surface of the protein. Therefore, even though the blood is composed of HbS tetramers the V6 in the beta subunit cannot make the association with the hydrophobic patch on a different tetramer, needed for fiber formation.
- When there is no fiber production, the cells don't sickle.
- Pain is a result of the pressure of the sickled cells on the blood vessels, when there is less sickling, there will be less pressure on the vessel walls and hence decrease in pain.

Notes:

- The most common issue for losing point for this question was including statements taken directly out of texts without explicit connection to the underlying structural basis for the statement or conclusion.
- In other cases included statements were incorrect or vague (such as saying mutation is in V6 but not specifying that the fiber formation is due to an association of this Valine in one beta subunit of one tetramer with the hydrophobic patch in a beta subunit of ANOTHER tetramer)
- For Group 1 there was also some confusion about which of the physiological effects of hydroxyurea listed in the drug bank was relevant for this question
- For Group 2 there was also some confusion about which Valine volterex bound to (not the mutated valine in beta chain but the N-terminal valine in alpha chain)

Itemized point distribution for each idea in each question:

For Q2A.

Hydroxyurea increases HbF production +1
HbF is made up of 2alpha 2 gamma +1
HbS is made up of 2 alpha 2 beta where beta has the mutation +1
HbF does not contain the mutated beta chain +1
Fiber formation requires interaction between hydrophobic V6 of one beta subunit in a tetramer with the hydrophobic patch that is exposed in the deoxystate of the beta subunit of a different tetramer +2
When HbF goes up there is less HbS +1
When there is less HbS in the blood there is less chance for inter tetramer association (fibers) +1
Less fibers → less sickling +1
Less Sickling → less pain. +1
Each incorrect statement -0.5/ -1 depending on how fundamental the related concept is to this course

For Q2B.

Voxeletor irreversibly binds to Val on the N-terminus of alpha chain +1
Deoxy/ tense form of hemoglobin is stabilized by specific ionic interactions +1
One important ionic interaction involves N-terminal Valine of alpha subunit that voxeletor binds to +1
When voxeletor binds salt bridge is interrupted and hemoglobin tetramer favors R (oxy) state +1
In the oxy state the hydrophobic patch formed by A70, F85, L88 is not exposed +1

Fiber formation requires interaction between hydrophobic V6 of one beta subunit in a tetramer with the hydrophobic patch that is exposed in the deoxystate of the beta subunit of a different tetramer +2
When there is an increased number of tetramers that are in the oxygenated state there is less chance for inter tetramer association (fibers) +1
Less fibers → less sickling +1
Less Sickling → less pain. +1
Each incorrect statement -0.5/ -1 depending on how fundamental the related concept is to this course