Sugar transporters as potential drug targets for malarial control biology essay

Science, Biology

Introduction and Background

Malaria is a vector borne disease caused by the protist microorganism of the genus Plasmodium. It is carried by the female anopheles mosquito and therefore is a mosquito vectored disease. An infected mosquito will introduce Plasmodium via its saliva once it has bitten its host. Plasmodium then enters the blood stream and circulates to the liver where it matures and reproduces. Malaria affects the tropical and the sub tropical areas, in particular the Americas, Asia and Africa. Malaria poses a global health threat worldwide with approximately one million deaths each year from this disease alone [K. Slavic, et al, 2011]. Majority of the deaths occur in Sub Saharan Africa amongst young children and also ninety percent of the overall deaths caused by malaria occur in this region. The reason why malaria is common in these areas is due to the warm climate and rainfall as this creates stagnant pools, which provide a prime breeding ground for mosquito larvae. The disease affects both humans and animals. The most common species that causes malaria is Plasmodium falciparum, this particular species causes the most deaths worldwide and is the most life threatening. Other species include P. vivax, P. malariae, and P. ovale these are not as life threatening, but cause a mild version of malaria. The symptoms of malaria are fevers, chills, headaches, vomiting and lethargy. In some cases sufferers become nauseous, have dry coughs, diarrhoea and also jaundice. The fever and chills occur in cycles of every few days. The more severe complications are liver failure, central nervous system failure and comas. These are generally caused by the P. falciparum species along with a long list of other symptoms and complications it causes. Malarial transmission is via the female

anopheles mosquitoes. This has to be infected prior to transmission; once it bites a host human it transmits the Plasmodium parasite into the blood stream shown below. Figure 1: Showing the transmission of malaria. Ankur Chakravarthy, 2011 http://exploreable. files. wordpress. com/2011/04/malaria_lifecycle. jpgFigure 1 briefly shows the transmission of malaria. Once the parasite is in the body it rapidly goes to the liver were it starts reproducing and is where it subsequently enters into the blood stream. This causes the bursting of red blood cells, which depletes the oxygen capacity in the body causing symptoms like fevers anaemia and jaundice. Once another mosquito ingests the blood of an infected human, the subsequent mosquito is infected with the malaria causing parasite and the cycle starts again with this infected mosquito carrying it to its next host. Areas that have malaria generally have a high population of humans for mosquitoes to feed from. Also, there will be a significant mosquito population and thus there will be transmission between the mosquito vectors and human hosts. Vector control is a method used to prevent malaria and its subsequent effects. The main aim of this is to prevent mosquito bites. Techniques like indoor residual spraying (IRS) use the spraying of insecticides within homes, which subsequently kills mosquitoes and prevents further transmission [Ossè, R. A., et al, 2013]. This has been quite an effective control measure especially in areas with endemic malaria and is also recommended by the World Health Organisation (WHO). A downside is that there is resistance to this technique from mosquitoes as a result of evolution. Another control measure is the use of mosquito nets; this prevents transmission by creating a barrier, which prevents bites and are often coated

with insecticides. This also reduces transmission of malaria, but is not considered as the best preventative measure. Treating stagnant pools or still water with chemicals helps prevent a breeding ground for mosquitoes. There are chemical prophylaxes taken in drug form which are only capable of killing the parasite in selected stages in its life cycle. One of the first to be used was quinine, which is currently used to treat severe cases of malaria and resistant cases of malaria. Chloroquine, mefloquine, and a few others were up until recently the preventative drugs. Chloroquine was the most commonly used anti malarial drug as was it was very effective and cheap. But as these monotherapies face resistance the WHO recommends Artemisinin based Combination Therapy (ACT) [Majori G, 2004]. Which combine existing monotherapies i. e. mefloquine, sulfadoxine, pyrimethamine and others with artemisinins. This proves effective in treating P. falciparum malaria. The reason why drugs are combined in this therapy is that it reduces the likelihood of resistance and is also more effective than the conventional monotherapies. The ACT’s are now the preferred anti malarial drugs presently. There are no vaccines that are currently available for malaria. There is now a need to look for new alternatives to ACT because there are cases of resistance in Eastern Asia [Slavic K et al, 2011]. Therefore scientists are looking for new control measures and treatments as a result of this. One such concept is to target the sugar transporters in the Plasmodium parasite. As sugar transporters are the main routes for energy into Plasmodium the aim is that targeting these transporters will block or destroy the sugar transporters and therefore reduce or prevent the uptake of glucose. This in theory will work because it will destroy a glucose transport

system, which in theory will kill of the Plasmodium parasite. The targeting of sugar transporter is not only being looked at for malaria it is also being looked at in cancers. In cancers it would be theoretically harder because you would target the tumour sugar transporters. In the terms of drug targeting of sugar transporters, it is fairly unconventional but seems promising. The reason why sugar transporters have emerged as potential targets is because they supply energy in the form of sugars within the parasite. If targeting sugar transporters in the future is successful it could be used as a new treatment for malaria, especially if there are emerging cases of resistance to ACT's. In this literature review I will be looking at sugar transporters as potential drug targets for malarial control. This will enable me to conclude the viability of sugar transporters as potential targets for malarial control.

Sugar transporters in diverse biological systems

Sugar transporters are part of a group of membrane bound proteins which are involved in the binding and the transport of molecules across membranes in all types of animals and plants. They come under a group of membrane transporters called major facilitator superfamily (MFS) [Pao S S, et al, 1998]. Sugar transporters exist in all phyla and are used to transport sugars such as glucose across membranes to provide energy to cells. The most common sugar transporters in mammals are the GLUT/ SLC2A family. In humans glucose is taking up from the extracellular fluid into the cell. This is via membrane bound proteins and there are two types of structurally related glucose transporters. One of them as mentioned before is the GLUT/ SLC2A family GLUT is the protein name and SLC2A is the gene name. These

come under the family of the facilitative glucose transporters in humans. These mediate a two way energy independent process of glucose transport into cells or tissues [Feng-Qi Z, et al, 2011]. The other type is sodium glucose co transporters with SLC5A as the gene name and with a protein name of SGLT. Which use a sodium linked transport mechanism, that transports substrates against the electrochemical concentration gradient. Both types play a crucial role in transporting substrate molecules across membranes, particularly glucose as this is the primary energy source in humans. But they differ as one is sodium dependent and the other is sodium independent in the terms of mechanism of transport. Glucose is obtained via the diet coming from the lumen of the small intestine in which glucose transporters are the key gateways as they bring about an energy source into the cells. Sodium dependent glucose transporters, transport primarily glucose and other sugars like galactose. This is done through an active transport process to get molecules across the other side of the membrane with the aid of the sodium potassium ion pump and going against the electrochemical gradient. These are mainly associated with the small intestine and they transport glucose across luminal membrane of cells found lining the small intestine. They also transport glucose across the proximal tubules of the kidneys. There are two known types of this glucose transporter and they differ in affinity for glucose uptake. The first to be effectively cloned is SGLT1 this is a high affinity transporter this was done using a rabbits intestine as the source [Hediger 1987]. The human intestinal transporter was also cloned after. SGLT1 is located in the small intestinal absorptive cells on the membranes and also the proximal tubules of the nephron. The other type of sodium dependent

glucose transporter is SGLT2; this is found in the membranes of the convoluted tubules in the kidneys. This transporter has a low affinity for glucose. As SGLT2 is involved in transporting larger quantities of glucose and SGLT1 has a higher affinity to uptake any glucose which SGLT2 has not already transported. The difference in affinity means greater efficiency in the uptake of glucose and reduction in loss with regards to it being wasted. Therefore this avoids the loss of glucose to the urinary tract and prevents it being excreted. They also differ in capacity as one has to transport large quantities of glucose and the other smaller and more specific quantities. Facilitative glucose transporters are independent of the use of sodium. They mainly use diffusion as the primary mechanism of transport, unlike the sodium dependent glucose transporters which use active transport which requires energy. These primarily transport glucose along other sugars also. Facilitative glucose transporters have the protein symbol GLUT in mammals. In humans there are thirteen known versions of this GLUT family and they are further divided into three different classes. The first to be successively discovered is GLUT 1. The naming of the structures has caused much debate by scientists previously, but they have come together to agree that there are twelve GLUTs and one HMIT. [reference] There are three different types of classes respectively, the first is class one, the second is class two and the third is class three. These have been put in these classes on the basis of function, structure and tissue expression. Also, including other features like substrate specificity and transport mechanism. As mentioned before sugar transporters are present in all phyla. If we look at yeast for example at the species Saccharomyces cerevisiae this is the most commonly found yeast

species in foodstuffs like bread and is used in the winemaking process. The main sugar transporters in this species belong to the Hxt family [Özcan S and Johnston M, 1999]. Besides, the Hxt family there are a few other transporters outside this family in this species. The glucose transport mechanism occurs mainly by facilitative diffusion, there are thirteen known sugar transporters with their functions and their sugar uptake affinities being known. Hxt 1 is an example of one of the transporters found in Saccharomyces cerevisiae, this particular transporter is a low affinity glucose transporter. Induction of this transporter occurs when there are high levels of glucose. Hxt 2 transporter is the opposite type of transporter in the terms of role. Hxt 2 has a high or immediate level affinity for glucose and it is induced by low levels of glucose. Other transporters in this family include Hxt 3, Hxt 4, Hxt 5, Hxt 6, Hxt7, Hxt 8, Hxt 9 and Hxt 11 and each of one individually varies in the levels of glucose affinity. Other transporters outside the Hxt family are Snf3, Rgt2 and Gal2. Snf3 and Rgt2 are glucose sensors and the Gal 2 is a galactose transporter. Recently the genome of bacteria Oenococcus oeni had been sequenced, this species uses hexose sugars which are six carbon sugars i. e. glucose and fructose as part of its metabolism [Ok Bin Kim, 2011]. This gram positive bacterium can be found in grapevine in which it ferments these hexose sugars. The significance of sequencing the genome meant that any existing transporters within this bacterium can be identified. The potential genes that code for transporter proteins have been identified within the bacteria, an example of a sugar transporter identified is COG0580 (gene name) this is involved in glycerol and related permease uptake. Further study shows that there are two transporters for glucose uptake they vary on

structure and function. Glucose is uptake in this particular species is by transporter proteins 1574 and 0819 [Ok Bin Kim, 2011]. These are not considered to be major glucose transporters in this species, as bacteria generally use a phosphotransferase system to uptake sugars and other pathways which are there to degrade sugars. But this study [Ok Bin Kim, 2011] does show that transporters are used in the secondary transport of glucose. The study shows that transporter 1574 is a secondary transporter with its structure being accounted for. Transporter 0819 is also a secondary glucose transporter which is also glucose inducible. Both are thought to be part of a MFS transporter group. Although information on glucose transport is known sufficiently in this bacterium as the phosphotransferase system is the primary system to uptake sugars. The glucose transporters play a secondary role in sugar transport in this species and most bacteria. In plants the major energy yielding sugar is sucrose, which is a vital photosynthetic synthesis product. This is synthesized in the leaves and needs to be transported over long distances to organs. Sugar transporters have a highly invaluable role in transport and regulation of sucrose. Sucrose transporters are apparent within the sources i. e. the leaves where they aid the initial transport of sucrose to the sinks which are plant organs in the tissues. The initial role they play is transporting sucrose from the chloroplasts to the apoplasts using sucrose transporters and then to transport sucrose from the apoplasts to the companion cells. The companion cells transport sucrose to the phloem, which in turn using mass flow transports sucrose to the sinks. They play a crucial role in plants in which they allocate sucrose on a cellular level and at an organism level. Sucrose transporters also belong to the MFS. Sucrose

transporters in particular belong to the SUC/SUT family overall there are three different divisions of this family, which are classified by structural differences. The first of the three is SUC2/SUT1, then SUC3/SUT2 and SUC4. Within the sub families they are very close structurally. There is indeed a difference between the three sucrose transporter families; the way all three of these structures differ is in the size of the exons and expression location. Looking at the Arabidopsis genus, this has its sugar transporters well documented. Studies have shown that there are approximately 69 sugar transporters and 9 of these belong to the SUC/SUT family in this particular plant [Katsuhiro, 2007].

Sugar transporters in human systems

Sugar transporters in humans consist of sodium independent and dependent transporters, the sodium dependent have been described earlier. The sodium independent transporters belong to the GLUT family in which they are separated into three classes. Class one facilitative transporters consists of four transporters GLUT 1, 2, 3 and 4. GLUT 1 is expressed predominantly in the red blood cells in humans. It is expressed in the brain in particular as part of the blood brain barrier in the endothelial cells. It is also expressed in lower levels in other areas like muscles and adipose tissue for respiration. This is the primary transporter for the erythrocytes, which relies on glucose from the blood plasma for an ongoing supply. The average concentration of glucose in the blood plasma is maintained at five millimolar which provides a constant supply. This particular transporter increases its presence on the erythrocyte plasma membrane when there are lowered levels of glucose and

decreases its presence when there are high levels of glucose. GLUT 2 is also a transmembrane glucose transporter that passively allows the transport of glucose. The distribution of this particular transporter is primarily in the liver, but is also found in the beta cells of the pancreas, the kidney tubules and the small intestine. In the liver it plays a role in glucose transport on the membrane of the hepatocytes. In the beta pancreatic cells it is known to play a role in glucose sensing, where it is one of the high quantity and low glucose affinity transporters of glucose. GLUT 3 is considered to be a high affinity transporter particularly in times of low glucose concentration and that is partly due to the location it is commonly found. It is the primary supplier of energy to the brain in particular the neurons. It also supplies glucose to other locations that include the placenta. It supplies the brain with glucose along with the GLUT 1 transporter. GLUT 4 is different to the others in this class as it is regulated by the hormone insulin. It is found most commonly in the heart in the cardiac muscle, also on a number of different locations in the skeletal muscle and the adipose tissue. Both muscle and fat tissues are the major tissues that are sensitive to insulin. Once insulin is present it stimulates GLUT 4 to be in place on the membrane and to uptake more glucose. Class one transporters have been studied more compared to class two and three. Therefore there is more known on a structural and functional basis, in class one compared to the others. If we look closely at class two transporters in particular the first one GLUT 5. This transporter is a known fructose transporter. The next three transporters GLUT 7, GLUT 9 and GLUT 11 there is evidence of their functions which has been recently made clear. GLUT 7 plays a role in glucose transport out of the endoplasmic

reticulum. GLUT 9 has been known to be expressed in the kidneys and the liver. It is thought to play a role transporting uric acid. GLUT 11 the information is scarce regarding this particular transporter, but is a known solute transporter. Class three transporters comprise of five transporters. The transporters locations have all been accounted for, but most transporters in this class have been functionally accounted for. Except for one, but there is indication of its function made clear recently. Determining the location of each and every transporter is difficult, but determining the function takes a lot of research and time. In this case GLUT 12 function is relatively unconfirmed although there is indication of it playing a secondary role in insulin dependent glucose transport in various tissues like the adipose tissues. But the rest have known functions, GLUT 6, GLUT 8 and GLUT 10 are known to be functional glucose transporters. The fifth transporter is an H+/myoinositol transporter is found in the brain were it is used as a neuronal transporter. The GLUTs are the main transport proteins for sugars in mammals. Overall, the transporters have in some cases completely different roles in different locations and they also vary in affinity to their sugars. Individually, they differ functionally i. e. some are found to supply glucose to key organs like the brain or prevent loss of glucose in the urine. Another difference in the human GLUT family is the locations as they are all located in different regions of the body. But all function in transport of a particular substrate across a range of different locations throughout the body. [add reference]

Sugar transporters in plasmodial systems

When plasmodium is in the red blood cell stage it relies on two particular transporters for glucose. These are sugar transporters in humans and on the Plasmodium’s plasma membrane. GLUT 1 is the glucose transporter that allows glucose transport from the blood plasma into the red blood cell. This transporter is vital to the life cycle of the Plasmodium and is the most commonly found on the membrane of the red blood cells. The Plasmodium sugar transporters are therefore reliant on the uptake of glucose into the red blood cells by GLUT 1. The key sugar transporter identified in P. falciparum is PfHT (plasmodium faciparum hexose transporter) [Slavic et al. 2011]. This is the primary supplier of hexose sugars including glucose and fructose in the P. falciparum species. This PfHT transporter also belongs to the MFS. There have been other potential transporters that have been identified but they are not considered as significant sugar transporters compared to PfHT. Studies show that this transporter does not require the use of sodium and therefore is sodium independent. Two other transporters have been identified one is a putative sugar transporter PFI0955w which belongs to the MFS. The other sugar transporter is PFI0785c. On the basis of their substrate transport they have only been recognised to be sugar transporters. More focus has been on the PfHT transporter as this has arisen as a potential drug target for malaria due to the fact that it is the main sugar transporter in the plasmodial system. Therefore more research has been focused on PfHT and information is more readily available compared to the other mentioned transporters. Research shows that PfHT is undoubtedly essential for the development of the parasite in the red blood cell stage. In other species of Plasmodium other sugar

transporters have been recognised but because P. falciparum causes the most fatalities the focus has been on that. Plasmodium berghei is another species of Plasmodium mainly found to infect rodents; the sugar transporter gene that encodes this particular transporter has been named as PBANKA 082040[reference]. The transporter for this species is PbHT. This species of Plasmodium causes malaria in rodents and the PbHT has been confirmed to have the same importance as PfHT does in P. falciparum. PbHT has been confirmed as the primary sugar transporter in the rodent species. Other species like Plasmodium vivax have also had their key sugar transporter indentified as PvHT encoded by this PVX 099390 gene [Joët, T., et al, 2004]. Many other species have their genes that encode the sugar transporter and the actual transporter identified.

Structural features of both human and plasmodial sugar transporters

Structure is important as it generally enables functional roles in proteins. If we look at the structure of both Plasmodium and human sugar transporters, this can indicate how the structure of the protein relates to the relative function of the protein. Looking at GLUT 1 this is commonly found in the erythrocytes and provides a constant source of glucose to them. The glucose binds to the GLUT 1 protein on the plasma membrane of the erythrocyte. The GLUT 1 protein must be open to allow glucose to bind, shown in Figure 2. Once glucose is bound, GLUT 1 changes its conformational structure to allow the glucose to pass into the intracellular environment. This is done by subsequently opening the protein on the intracellular side of the protein and closing the protein in the extracellular environment. Then, releasing the

glucose into the intracellular environment and at the same time closing the extracellular entrance. This type of transport is facilitated diffusion as the substrate has to bind and change the structure on a conformational basis of the carrier protein to allow diffusion, moving against the concentration gradient. Figure 2: Showing the mechanism of transport by GLUT 1 into the cytosol of cells (pink carrier protein), date accessed 20th march 2013 http://jpkc. scu. edu. cn/ywwy/zbsw(E)/pic/ech5-6. jpghttp://jpkc. scu. edu. cn/ywwy/zbsw%28E%29/pic/ech5-6. jpgGlycosylated loopCytoplasmic loopC terminusN terminusFigure 3: Showing the predicted structure of GLUT 1Trista K, 2011GLUT 1 overall is hydrophobic, but does have certain polar regions. It is made up of 492 amino acids and more than half of these amino acids make it the hydrophobic in nature. Which is not uncommon in membrane bound proteins, especially them that are part of the MFS. This means that on the hydrophobicity scales the amino acid residues are positive, thus hydrophobic. The GLUT 1 molecule consists of 12 membrane spanning alpha helices with cytoplasmic N and C termini [Trista K, 2011]. The N or NH2-terminus is found on the bottom left hand side shown in Figure 2 sticking out of the membrane. The C or COOH (carboxylic acid) terminus is also shown in Figure 2, as the long molecule that loops at the end, on the bottom right hand side. In the middle of Figure 2 is a long loop like structure connecting one transmembrane protein to another. This is called a cytoplasmic loop and is found in the intracellular environment. There is also another loop structure called the glycosylated loop connecting the first two transmembrane proteins found on the top left hand side of Figure 2. This loop is based within the extracellular environment. Figure 2 shows a predicted model of the GLUT

1 protein as the actual detailed structure is unknown. But this predicted structure is very likely to resemble the actual structure. The GLUT 1 protein is considered amphipathic, although mainly hydrophobic overall. The amphipathic qualities arise from hydrophobic and polar regions, these particular regions are within the membrane helices. The glucose will generally pass through the polar region via a channel. The conformational change described previously is generally specific for glucose molecules, but is sensitive to other molecules including inhibitors. GLUT1 is a primary transport protein for vitamin C in humans. Humans cannot synthesize this vitamin so GLUT 1 plays a vital aspect in delivering this vitamin to cells that require it. The transmembrane protein arrangement is that twelve of them sit side by side made up of their constituent amino acids. There are six of these transmembrane proteins are thought to be adjoined together, and then there is a channel for the substrate to pass through. This is thought to be water filled and then on the other side of this channel, there are the other six transmenbrane proteins which make up the twelve in total. Structurally it is a very basic integral membrane protein that is specific to certain substrates and inhibitors unlike other transporters; it does not require the use of sodium or other factors. Overall the relative structure is fairly simple, which enables it to be effective in its relative function. Figure 4: PfHT predicted structureAsha Parbhu Patel et al, 2008The PfHT is found on the parasites plasma membrane in P. falciparum. Unlike the human GLUT 1 it can facilitate the transport of both glucose and fructose, thus the name P. falciparum hexose transporter. This is important to the survival of the organism because when glucose levels are low, it can turn to fructose as the

major energy sugar. As a result of the human body being more complex than the parasites, this requires wider range of specific sugar transporters that occupy a certain role. The specific fructose transporter in humans is GLUT 5. The GLUT family in some cases is generally singled out to be specific to one sugar. Like the GLUT 1 transporter, PfHT it is also sodium independent, therefore is not influenced by ions. As PfHT is the primary sugar transporter in Plasmodium more research is focused towards it, rather than other potential sugar transporters in Plasmodium. Therefore the structure of PfHT has been predicted unlike the other potential sugar transporters. http://ars. els-cdn. com/content/image/1-s2. 0-S1477893908000069-gr3. jpgAbove is the predicted two dimensional structure of the PfHT transporter. By briefly looking at both GLUT 1 and PfHT transporters in Figures 3 and 4, they appear fairly similar structurally. This is documented in many research journals also. In fact the closet human equivalent is the GLUT 1 transporter to PfHT [reference]. Ideally, this would be the case because they both have similar functions, but most importantly the location i. e. when the parasite is in the erythrocyte having a similar structure to primary erythrocyte sugar transporter GLUT 1 is likely to show similar function and substrate specificity. The parasite may have adapted a sugar transporter like the GLUT 1 transporter to uptake sugars with the same efficiency as GLUT 1 within the erythrocyte stage. PfHT is made also from 12 transmembrane proteins that are arranged side by side shown by the two dimensional depiction above. The bottom left hand side in the intracellular environment in the cytoplasm shows an N (amino) terminal, which is also apparent in the GLUT 1 structure. On the right hand side in the intracellular environment there is the C

(carboxylic acid) terminal, which is apparent in GLUT 1 also. But the C and N termini are found in similar locations, but differ slightly in shape GLUT 1s N termini was a straight line of amino acids constituents and C termini was shaped like a fishing hook. Whereas, the C and N termini are both identical in the PfHT in a way they both look like a test tube. In the middle there is a long loop like structure connecting two adjacent transmembrane proteins, this is also apparent in GLUT 1. The structures found on the extracellular side are likely to be glycosylation sites and the others are residues. The structures itself is a prediction currently as there is no fully confirmed model, the GLUT 1 model is more of an accurate prediction then PfHT, as it is better documented.

Homology sequences and key differences and similarities of human and plasmodial sugar transporters

DNA analysis of the genome of P. falciparum has identified the PfHT gene. It shows that PfHT transporter exhibits relatively similar homology to the GLUT family. The level of homology between the GLUT families is between fifty to sixty percent; this is with regards to sequence analysis of the PfHT [Derbyshire, E. T. et al, 2008]. Showing that PfHT exhibits particular homology to the GLUT 1 transporter compared to the rest of the GLUT family. The expression of the PfHT was studied using a frog germ line this has been crucial in examining the sequences of the P. falciparum genome [Slavic et al. 2011]. As described before PfHT predicted structure is very similar to the GLUT 1 structure. It also has 12 transmenbrane proteins that have C and N terminal on either side of the transmembrane proteins in the intracellular environment, which is also seen in GLUT 1’s structure. The way

it differs is GLUT 1 has a mechanism of up taking glucose into the cytosol as described before, where it changes its conformational shape to allow glucose into the cell. It is known that PfHT has a different mechanism in place compared to GLUT 1, but a confirmed mechanism of action itself is unknown. This can be crucial to know when selecting inhibitors to block the substrate transportation within the parasite. What is known is that PfHT is a hexose transporter and GLUT 1 is a glucose transporter. PfHT specialises for two substrates glucose and fructose, whereas GLUT 1 is only specific to single substrate glucose. PfHT has a far greater affinity to glucose in comparison to GLUT 1. They are both sodium independent transporters which apparent in both sets of sequence analysis data. On the basis of sequence homology the GLUT family differs from PfHT only by about forty to fifty percent and both show similar structure depicted by Figure 3 and 4. This is considerably significant as they are both sugar transporter found in different organisms one is found in complex mammals and the other is found in a parasitic protist. They both have twelve transmembrane proteins, but the terminals on the transmembrane proteins and other structures differ structurally.

Identifying the specific sugar transporters to targets

PfHT is the major sugar transporter in the P. falciparum species and it is the key supply route for glucose into P. falciparum. As this is crucial for the life cycle of stage within the erythrocyte, this has made it as a genuine target for potential drugs. Other transporters have been identified in P. falciparum but these are not key facilitative transporters so research has not really focused into them. Theoretically, if you can prevent any hexose sugars from being

used in the parasite this can kill of the organism, as it cannot produce any energy yielding ATP to survive. The transporter itself is a high affinity facilitative transporter which the parasite relies solely for energy and therefore is fundamentally important to the survival of the parasite. PfHT has been genetically validated in such a way to confirm the validity of this transporter and to prove that targeting this transporter can effectively kill the parasite [Ksenija Slavic, 2010]. This study genetically confirms PfHT is functionally essential to the parasite and gives the go ahead for new anti malarial drugs that specifically target this hexose transporter. This genetic approach confirmed this by targeting the gene that encodes the transporter in a genetically modified parasite. The gene was removed from the genome of the parasite, to show whether removing this transporter can actually kill the organism. In this case having no expression of this transporter showed its loss of function, thus effectively killing the parasite. The gene that encodes this transporter is a single copy and is not expressed elsewhere in the genome. The Achilles heel has been found in this malaria causing parasite, because the parasite has no backup plan to losing PfHT. The genetic validity puts this study on the next stage, whether it can be targeted by potential chemicals. The genetic approach also could be used to target PfHT as it did effectively work in this study Ksenija Slavic, 2010.

The techniques of targeting these specific sugar transporters in plasmodium.

The genetic approach is the method that was used to validate the essentialness of the PfHT transporter. Although from the researchers point of view this was only used to validate PfHT as a potential drug target at the

same time it did in fact remove PfHT and kill the parasite. There are two ways that the genetic method can work one is to target the genes encoding the PfHT with the aim it will limit the expression of it or remove it completely, as described previously [Ksenija Slavic, 2010]. The other potential genetic method would entail the use of an inhibitor if discovered, by over expressing the gene that encodes the PfHT and using the inhibitor to block all the expressed transporters. This could work as the PfHT will be over expressed and the inhibitor will inhibit PfHT. This means that the parasite cannot express anymore transporters as it will already be over expressed. This method would not give the parasite a backup plan by expressing more PfHT and will limits its survival. But this approach requires genetically modified parasites and may not work in normal parasites. One approach would require the use of an inhibitor. The length of the approach and the costs of genetically targeting the parasite are infeasible. Also, the way the parasite was genetically targeted was in an in vitro environment rather than in the erythrocyte stage which it would be normally found in. The chemical approach entails the use of inhibitor molecules to inhibit the glucose uptake into the parasite . The inhibitor should specifically target the parasites sugar transporter without attacking host proteins. It has to be specific to PfHT as GLUT 1 and the other GLUT’s are fairly similar in structure. The inhibitor should work in an artificial environment just as much as in the body. Like every drug in the form of an inhibitor it has to be developed on the basis of concentration of the inhibitor and target specificity to keep in vivo conditions safe. These along with being specific to target proteins and without attacking host proteins are important aspects in selecting inhibitors. Especially,

considering GLUT 1’s structure is very similar to PfHT. The chemical approach would be more feasible cost wise and if an effective inhibitor is identified it could be a lot more effective time wise. At the moment the current drugs that are used take many hours to take effect, whereas these potential drugs that are inhibitor based are predicted to be more immediate. This is why researchers are focusing on finding an inhibitor of PfHT. The chemical technique involves testing out a variety of potential inhibitors. Only certain inhibitors would work due to the specificity of the PfHT transporter as it is specific to certain isomers. It would prove beneficial for these inhibitors to be glucose derived. Two potential inhibitors have been identified one is called compound 3361 which is a glucose derivative [K Slavic, Et al, 2011]. The other potential inhibitor identified is catechin, which is not a glucose derivative but a plant derivative [K Slavic, Et al, 2009]. The compound 3361 is likely to be the most effective inhibitor. This glucose derivative shows high levels of specificity to PfHT and is reported not to interfere with the GLUT transporter family including GLUT 1. This inhibitor compound is effective in killing the parasite organism at fifty percent inhibitor concentration in a controlled artificial environment [Ksenija Slavic, Michael J. Delves Et al, 2011]. It was also tested against P. berghei within a rodent in which it was effective in killing the rodent species P. berghei and thus proves effective in vivo conditions. Compound 3361, has also been used against other species of Plasmodium and has been effective in killing them also. This proves that compound 3361 is a potential inhibitor that can target PfHT and other Plasmodial sugar transporters. This compound 3361 could be at the heart of new anti malarial drugs in the future, with it not only being specific to

humans but also animals. Also it can be used to fight a variety of Plasmodium species that cause malaria in humans. Although not used in humans it targets PfHT in vitro with a very high specificity and is proposed not interfere with the host sugar transporters like GLUT 1 which would be highly present in the erythrocyte stage of the parasites life cycle. It only targets the specific sugar transporters which supply the parasite with energy. It also has the specific isomerism for the effective inhibition of PfHT. The other potential inhibitors discovered were the catechins. These were in fact discovered earlier than the compound 3361. Catechins are a type of phenol molecule which differs in structure compared to compound 3361. Like compound 3361, catechins have the ability to inhibit sugar transporter, in which they do inhibit the PfHT transporter [Ksenija Slavic et al, 2009]. One type of catechins inhibited PfHT effectively, whereas another type was not effective in inhibition. The type that did inhibit PfHT also inhibited GLUT 1 and 5, the glucose and fructose transporters in humans. The key inhibitory property of the specific catechins is down to the structure which is also the case for compound 3361. These also show that they have anti malarial potential as inhibitors. But saying this they do in fact inhibit GLUT 1 and 5, which would be potential dangerous as it would block sugars transport to the host cells. This is not what we would expect to achieve as a key feature for the inhibitor was not to interfere with host transporters. If they can be modified in way that they can only target the PfHT without inhibiting the GLUT 1 and 5 transporters in humans, they can potentially be used as anti malarial drugs. Also they have the advantage of being naturally available because they are found in green tea. Catechins prove to be potential

inhibitors of PfHT, but as the researchers who first investigated them and published a study of them in 2009 found that they had their limitations [Ksenija Slavic et al, 2009]. This is why in 2011 compound 3361 was identified as a better alternative as it is specific to the parasites key sugar transporter. Compound 3361, appears currently to be the best potential inhibitor.

Discussion

The current state of knowledge I believe is promising. Research show far has found a so called Achilles heel in the most deadly malaria parasite Plasmodium falciparum. This is the key sugar transporter PfHT in this particular species. The current research has proved that this is the key supplier of sugars to the parasite once in the erythrocyte stage and cutting of the supply of sugars via the PfHT sugar transporter proves that the organism cannot look to any other transporters or mechanism to bring glucose into the parasites cells. This reduces the ATP levels and subsequently the organism runs out of energy and dies. Other factors that make targeting PfhT viable is once PfHT has been targeted the organism cannot in its genome activate anymore PfHT to be expressed, even when its chemically targeted with compound 3361. This inhibitor proved effective in an in vivo environment in which it managed to inhibit PbHT and kill of the rodents species Plasmodium. In the human species in vitro has the same affect. P. falciparum cannot prevent inhibition by inhibitors or turn to any other sugar transporters. In the fact parasite dependence is highly on the PfHT and once the primary sugar glucose is in short supply the transporter

can use fructose sugar as an alternate energy source. In the terms of survival the parasite can only switch sugar source to fructose. This proves that PfHT is vital for the parasite with regards to survival as it can only switch sugar source to survive rather than bring in energy via another transporter or another mechanism. Further, confirming that PfHT is genuine target for the next generation of anti malarial drugs. In terms of a targeting approach of the PfHT transporter I believe in this current time, the chemical approach looks very promising. The genetic approach did in fact genetically validate PfHT as a drug target in which it also showed loss of function of PfHT, but it is no more than a validation technique rather than a targeting method. Whereas the chemical approach in terms of compound 3361 has shown to work in vitro and also in vivo within a rodent. Within the rodent it killed P. berghei and but is also effective in killing other species of Plasmodium. Although working in a vivo environment within rodents, this inhibitor has yet to be tested in humans. Research proves it can work as a potential anti malarial drug, but no drugs have been made with compound 3361 at the heart of them. Currently, researchers are a stage where they have discovered and tested this compound, and have also published results of their findings. To develop this as part of a real drug, it must work in vivo within humans which would require clinical trials. But there has been no further mention on the development of compound 3361 as a new anti malarial drug presently. I believe that the study has got to a stage where they have only published their findings and that is it, as there is no further indication of progress. Also another consideration for this study is

mechanism of action of PfHT needs to be known. This would be beneficial for research and drug development purposes.

Conclusion

Overall the viability of targeting sugar transporters has proven effective as they are the main energy transport mediums for sugars into the parasite organism. Once they are targeted they kill off the organism which has been proven. The aim of this is to kill the parasite that causes malaria. The success of identifying an inhibitor has made targeting sugar transporters even more viable. With reports of resistance to the current range of anti malarial drugs this is welcomed news. The importance in being able to target sugar transporters in P. falciparum has been emphasized by resistance to current drugs and the lack of any alternatives. This is why I would say it is welcomed news because a possible inhibitor molecule has been identified and in the future it could be used in drugs. With the significance of malaria as a worldwide disease that kills millions of people each year this could be the new way to fight malaria. Currently there are other anti malarial drugs that are undergoing clinical trials and are expected to be used as early as 2020.