The effect of various iso-osmotic solutions on hemolysis of erythrocytes in *Oryctolagus cuniculus* (Leporidae)

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Abstract

The blood of animals is heterogeneous and contains a variety of dissolved molecules, in addition to the red blood cells. Depending on their chemical structure, these substances vary in their ability to move across the plasma membrane of a cell. However, the plasma membrane is much more permeable to water than it is to most solutes, due to the presence of aquaporins. Accordingly, the cell may experience an osmotic effect if concentrations of the solute differ on either side of the membrane. The degree of osmosis depends primarily on the ability of the solute to cross the plasma membrane.

To investigate the physical properties that determine how a solute diffuses across the plasma membrane, three groups of related chemicals were tested for their ability to cause lysis of red blood cells from the European rabbit, *Oryctolagus cuniculus.* The time required for the cells to undergo hemolysis was used as an indicator of how readily the solute was able to cross the membrane. For two of the three groups, there was a correlation between the molecular weight of the molecule and the degree of membrane diffusion. The higher the molecular weight of a substance, the more difficulty it had in crossing the membrane. A low lipid-water partition coefficient was also correlated with the solutes inability to permeate the membrane. Relating the physical properties of molecules to their ability to cross the lipid bilayer allows us to develop a theoretical basis for predicting which chemicals would readily diffuse into the cell, an important consideration in drug design.

Introduction

In the bodies of animals, the kidneys help regulate the concentration of solutes and water in the blood, thereby maintaining the osmolarity of the blood plasma. It is crucial that the body maintains the concentration of solutes in the blood plasma at such a level that it is isotonic to the interior of red blood cells. If the concentration of solutes in the blood increases or decreases from its normal level, this could lead to the damage of the red blood cells. An excess of solutes in the blood would lead to shrinking (crenation) of the red blood cell, while an excess of water in the blood would cause the cell to gain an influx of water and potentially lyse (Coldman 1969).

However, not all solutes are equal in their ability to cause lysis of the red blood cell. Various properties of the molecule, such as its hydrophobicity and its size, affect its ability to move across the plasma membrane (Hammarlund 1961). Since plasma membrane is much more permeable to water than it is to most solutes, the ability of a solute to cross the plasma membrane determines whether the cell is likely to experience an osmotic effect from a difference in solute concentration. In the case that the molecules are not able to cross the plasma membrane at all, osmosis will occur as water moves from the area of low solute concentration to high concentration. If the membrane is somewhat permeable to the solute, the effect of osmosis is temporary, and will last until enough solute crosses the membrane and restores the system to equilibrium.

The lipid-water partition (LWP) coefficient is a measurement of how hydrophilic or hydrophobic a substance is, and this helps us to predict whether the molecule will be able to diffuse across the plasma membrane (Ramsden 1993). Molecules with a high LWP coefficient are hydrophobic, and distributed to the lipid bilayers of cells, while molecules with a low LWP coefficient are found in aqueous solutions such as blood plasma or the cytoplasm. In relation to the topic of osmosis in cells, the LWP of a molecule indicates whether it is likely to pass through the plasma membrane, and the speed at which it diffuses across the membrane (van Meer 2008).

We can use this property of various molecules to predict how a sample of blood would interact with a solution of the substance. In a solution with a high LWP coefficient, we would expect the substance to be hydrophobic and distribute itself in the plasma membrane (Kubinyi 1979). If the molecule is only slightly hydrophobic, it may be possible for it to enter the cell by partitioning into the lipid bilayer on the non-cytosolic side of the membrane, and then disassociating from the membrane on the cytosolic side. However, very hydrophobic molecules are unlikely to leave the lipid bilayer and enter the cell (Paloncyova 2013). In a solution with a low LWP coefficient, the molecule is readily soluble in aqueous solution, so it seems more likely to cause hemolysis by entering the cytosol of the cell. The speed at which the molecule crosses the plasma membrane is also dependent on other factors, such as whether transporters for that molecule exist in the membrane.

Depending on the nature of the molecule, solutions of equal concentration can have different effects on the tonicity of the red blood cell. This experiment was conducted to examine the behaviour of red blood cells in solutions of different molecules. Twelve substances were examined, that were grouped into three main categories (group A, B, and C) based on their physical and chemical properties.

The molecules in Group A include ethanol, ethylene glycol, urea, and thiourea. Transporter proteins for urea exist on the cell membrane, and facilitate the diffusion of urea into the cell (Goodman 2002). For this reason, we hypothesize that a red blood cell in a solution of urea would undergo hemolysis. Due to its transporters, we predict that urea would very quickly move across the plasma membrane, and by moving from an area of high to low concentration, cause the cell to burst (Wurster 1963). Based on the intermediate LWP coefficients of the other three solutions (ethanol, ethylene glycol, thiourea), we may also expect them to be able to diffuse across the plasma membrane.

Group B contains solutions of monosaccharides or disaccharides. All of the solutions have a very low lipid-water partition coefficient of 0.00003, and a relatively high molecular weight between 150 – 342 g/mol. Due to the low LWP coefficient of the sugars, it is unlikely they would be able to permeate the plasma membrane without the help of transporters (Tian 2013). Glucose transporters are present in the cell membrane and allow it to pass into the cytosol (Navale 2016). However, based on the high molecular weight of the sugar molecules, we hypothesize that blood will not mix rapidly with the solution, and therefore will not undergo hemolysis.

The solutions in Group C contain glycerol and glycerol acetates. Glycerol in animal sources is produced when the body uses stored fat as a source of energy, and triglycerides are released into the bloodstream as glycerol. The cell membrane is itself composed of glycerophospholipids, which are formed from a backbone of glycerol molecule. As glycerol and its acetates are structurally similar to the lipids that form the cell membrane, we hypothesize that the molecules in solution will be able to permeate the plasma membrane. However, the molecules in Group B have differing levels of hydrophobicity, as evidenced by their different LWP coefficients. We therefore predict that molecules with a very high LWP (with a very hydrophobic nature) would tend to remain in the plasma membrane and not pass into the cytosol, while molecules with a relatively lower LWP could enter the cytosol and may cause lysis of the cell.

Materials and Methods

The experiment was conducted according to the procedures outlined for “Laboratory 1: Properties of Membranes” in the Laboratory Manual for BIOL 3060 at York University.

Results

Our observations suggest that there is a correlation between molecular weight and time to hemolysis. Data from Groups A and B show a positive linear relationship between molecular weight and time to hemolysis (Figure 1). The greater the molecular weight of the substance, the longer it took for red blood cells to lyse in solution. This relationship does not exist for the chemicals in Group C; the data points for this group do not show a pattern.

An inverse relationship also exists between the lipid-water partition coefficient of a molecule, and the time required for red blood cells to lyse in solution. This is observed to be the case for chemicals in Group A and B (Figure 2). As with the previous figure, data from Group C does not show the same pattern as the other groups. There is also an extreme value in Group C which has a very high lipid-water partition coefficient.

Figure 1: Molecular weight of a substance compared to time elapsed before hemolysis of red blood cells. Five drops of rabbit blood (*Oryctolagus cuniculus*) were added to 5 mL of solution at a concentration of 0.2 M and timed until the mixture visibly changed from opaque to clear.

Figure 2: Lipid-water partition coefficient of a substance compared to time elapsed before hemolysis of red blood cells. Five drops of rabbit blood (*Oryctolagus cuniculus*) were added to 5 mL of solution at a concentration of 0.2 M and timed until the mixture visibly changed from opaque to clear.

**Table 1:** Physical properties of solutes and the length of time required for red blood cells to lyse in a 0.2 M solution. Five drops of rabbit blood (*Oryctolagus cuniculus*) were added to 5 mL of solution at a concentration of 0.2 M and timed until the mixture visibly changed from opaque to clear.

Discussion

After examining the behaviour of red blood cells in solution with several molecules, some groups of chemicals do show a trend in their data. That is, there seems to be a relationship between the lipid-water partition coefficient of a molecule, and the length of time required for the blood cells to lyse in solution. It seems that molecules with a LWP coefficient that is extremely high or low have difficulty passing through the membrane. In contrast, those substances with an intermediate nature (neither strongly hydrophilic, nor strongly hydrophobic) would be better suited to permeate the plasma membrane. This ambivalent character of the molecule would allow it to integrate into the plasma membrane, and also dissociate from the membrane once inside the cell (Jambeck 2013).

The substances in Group A were ethanol, ethylene glycol, urea, and thiourea. These molecules have a relatively low LWP coefficient and molecular weight, compared to the chemicals in Groups B and C. The data in figure 1 show a linear trend between the molecular weight of the molecule and time to hemolysis. As molecular weight increases, the time to hemolysis also increases. Figure 2 shows a different relationship, that between the LWP coefficient and time to hemolysis. The molecules with a lower LWP coefficient have a longer time to hemolysis than chemicals with a higher LWP coefficient, suggesting that an extremely hydrophobic molecule will have difficulty crossing the plasma membrane.

For substances with a very low lipid-water partition coefficient and a high molecular weight (Group B, mono- and disaccharides), we observed that the blood did not mix easily when added to the solution, and formed a layer above the solution instead of falling through. This is consistent with the fact that all of the sugars have an extremely low LWP coefficient, at ~0.00003 (Figure 2), and a high molecular weight (Figure 1). The strongly hydrophilic nature of the molecule would not readily pass through the cell membrane, other than by moving through glucose transporters. The large size of the sugar molecules also creates a physical impediment, making it more difficult for the blood to sink into the solution.

For Group C, glycerol and its acetates, we had predicted that the low lipid-water partition coefficient of glycerol suggests that the red blood cells would move slowly through the solution. The experimental results confirm this to be the case. In solutions of glycerol, the blood does not move through the solution nor does hemolysis occur. The red blood cells collect in a layer on top of the solution of glycerol, and have difficulty flowing through the solution. Unlike in glycerol, red blood cells added to the glycerol acetates (monoacetin, diacetin, triacetin) are observed to lyse after some time. The times to hemolysis vary between monoacetin, diacetin, and triacetin, and the data does not suggest a clear relationship between either LWP coefficient or molecular weight and time to hemolysis for this group. For this group, there is no linear trend in the data presented in Figure 1 and Figure 2. However, our results do align with our prediction that either a molecule which is either strongly hydrophilic or strongly hydrophobic (corresponding to extremely low or extremely high LWP coefficient values). This is illustrated by the fact that glycerol (low LWP) and triacetin (high LWP) both have long times to hemolysis.

These results support our hypothesis that chemicals with an intermediate hydrophilic nature are better able to permeate the plasma membrane than substances with a strongly hydrophilic or hydrophobic character. This property is relevant to the field of pharmacology and drug design. When trying to predict the bioavailability and metabolism of a potential drug, the ability of the drug to permeate the lipid bilayer gives an indication of how readily it will be able to enter the cell (Paloncyova 2013). The molecule's hydrophilic or hydrophobic nature also indicates whether it is likely to accumulate in the membrane.

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