Mechanisms of caffeine on the central nervous system (cns)

Caffeine is a central nervous system (CNS) stimulant, known medically as trimethylxanthine with a chemical formula of C8H10N4O2 [1]. It is soluble 1 in 46 of water, has a dissociation constant pKa of 0. 6 [2], and a log P value of -0. 07 [1]. From the structure of caffeine (fig. 1), three amide groups and two aromatic amine groups could be observed. Although the amide groups of caffeine are neutral, the presence of the aromatic amines groups indicates that caffeine is a drug with weak basic properties.

Caffeine is found naturally in coffee, tea and chocolate, and sometimes is used as an added energy boost in energy drinks or soft drinks, hence making it the world’s most widely consumed psychoactive substance. Unlike many other psychoactive substances, caffeine is legal and has been listed as a “ multiple purpose generally recognized as safe food substance” by the Food and Drug Administration [3]. It is used both medically and recreationally to reduce physical fatigue, ward off drowsiness and restore mental alertness. Besides, it is sometimes used to increase urine production due to its mild diuretic properties [4]. Caffeine is also included in some common over-the-counter medications such as aspirin and appetite suppressants, for the purpose of enhancing the effect of the primary active ingredient or reducing the drowsiness side effect [5]. Apart from the oral formulation, caffeine is also available in other various formulations such as intramuscular injections and suppositories. The suppositories with the ingredients of ergotamine tartrate and caffeine are used to relief migraine [6] while others with the ingredients of chlorobutanol and caffeine are used to treat hyperemesis [7].

Mechanism of Action

Caffeine acts as a nonselective antagonist of adenosine receptors in the brain [5], therefore it readily crosses the blood-brain barrier to exhibit its mode of action. Once in the brain, caffeine acts as a competitive inhibitor and binds to the adenosine receptors on the surface of cells as it is structurally similar to adenosine (fig. 2).

The binding of adenosine to the receptor normally causes drowsiness by slowing down nerve cell activity. However, the binding of caffeine to the adenosine receptor is an antagonist mechanism of action. Instead of slowing down the cell's activity, caffeine causes the nerve cells to speed up, therefore having the effect of reducing drowsiness or increasing alertness. Besides, caffeine also causes the constriction of brain's blood vessels, helped in relieving headache or migraine [4]. Also, due to the increased neuron firing in the brain, the pituitary gland then releases hormones that stimulate the adrenal glands to secrete adrenaline [4]. Adrenaline is the " fight or flight" hormone which helps the body to adjust to sudden stress. It increases the rate of heartbeat, raises the blood pressure, as well as speeds up the conversion of glycogen into glucose to provide extra energy to the muscles. This is further explained why caffeine is widely used to ease tiredness and restore alertness.

Pharmacokinetics

In the discovery of new drugs, the ability of the drug to partition and arrive at its site of action is of great importance. Many drug candidate molecules are discarded due to poor pharmacokinetic properties involving physicochemical parameters of absorption, distribution, metabolism and

elimination (ADME). Avdeef et al [2] stated that poor solubility and permeability account for many pharmacokinetic failures. The pharmacokinetics of the drugs are dependent on their ability to dissolve in the body and then partition across various cellular membranes into the blood and subsequently into target tissues, by different transport mechanisms, to exhibit their mode of actions. However, the ability of drugs to partition across cellular membranes depends on a variety of factors such as the drug's molecular size, degree of ionisation, lipophilicity and etc. Therefore, the study and characterisation of the factors governing the pharmacokinetics of caffeine is very important as they play an important role in ADME.

Pharmacokinetics properties of caffeine show that the absorption of caffeine mainly takes place in the stomach and small intestine in the body. There is a study showing that caffeine from coffee or other beverages is absorbed within 45 minutes of ingestion [8]. The same study also stated that caffeine can be distributed throughout all the tissues in the body [8]. However, the metabolism of caffeine takes place in the liver and the three metabolites produced are Paraxanthine, Theobromine and Theophylline [9]. The elimination of caffeine is a first-order reaction [10].

Partitioning of Caffeine

Partitioning of drugs is defined as the movement or redistribution of drug molecules from one liquid to another. When an excess of drug compounds is added to a system of two immiscible liquids which approximates the cellular membranes, it will partition itself between the two liquids until each becomes saturated. However, if the amount of drug compounds added is not sufficient to saturate the liquids, it will distribute between the two immiscible

liquids according to a definite concentration ratio. Polar drugs usually favour the aqueous phase while non-polar drugs usually favour the organic phase. The partitioning of a drug is affected by various molecular properties, for example molecular size, lipophilicity and charge (degree of ionisation) of the drug [17].

Generally, the partitioning of a drug across the membrane increases with increasing lipophilicity [17], thus indicating the importance of the lipophilicity of a drug. The importance of the lipophilicity is further supported by the passage of drug into the central nervous system (CNS) by crossing the blood-brain barrier (BBB) [17]. As BBB is composed of many tight junctions in between endothelial cells, the amount of drug that is able to partition across BBB is reduced to a greater extent compared to the amount of drug that is able to partition across GIT. As a result, the lipophilicity of a drug plays a major role in the passage of drug across BBB. Caffeine is a CNS stimulant, so it must be able to cross the BBB to exert its stimulant effect in the brain. Therefore, the lipophilicity of caffeine should be investigated.

The lipophilicity of a drug is usually determined by the Partition Coefficient (P), which is defined as the ratio of concentrations of un-ionised compound between the two phases. Therefore, the lipophilicity of caffeine can be determined using the partition coefficient equation as follow:

Where Corg is the concentration of the drug in organic layer while Caq is the concentration of the drug in aqueous layer

As the partition coefficient of a drug provides a thermodynamic measure of its hydrophilicity-lipophilicity balance, it is widely used to predict the

absorption, distribution and elimination of drugs within the body. The partition coefficient of un-ionised drug is called the True Partition Coefficient (PTRUE). The drug is hydrophobic (or lipophilic) when PTRUE value is greater than one due to the higher concentration of drug in the organic phase compared to the aqueous phase. However, the drug is hydrophilic (or lipophobic) when PTRUE value is less than one as a result of a lower concentration in the organic phase compared to the aqueous phase.

Alternatively, Log P can be used to determine caffeine’s lipophilicity. It is defined as the logarithm of the ratio of the concentrations of the un-ionised compound in the two immiscible phases, which can be written as:

When log P value is greater than zero, the solubility of the drug compound in the organic phase is greater than in the aqueous phase. When log P value equals to zero, the drug compound has an equal solubility in organic and aqueous phases. When log P value is smaller than zero, the solubility of the drug compound in organic phase is less than in aqueous phase. As mentioned before, caffeine has a log P value of -0. 07, thus indicating that caffeine is hydrophilic and its solubility in the aqueous phase is greater than in the organic phase.

Apart from that, the lipophilicity of a drug can also be determined by Hansch-Fujita parameters, which help in identifying the functional groups that contribute to the lipophilicity of the drug. A predominance of hydrophobic groups (+Ï€) will lead to an increase in partition coefficient, thus making the drug more lipid soluble and able to pass through the lipid membranes more easily. However, a predominance of hydrophilic groups (-

Ï€) will lead to a decrease in partition coefficient, making the drug less lipid soluble and less likely to penetrate the lipid membranes.

Therefore, the lipophilicity of caffeine could also be examined by the use of Hansch-Fujita parameters. As can be seen from the structure (fig. 1), caffeine has few hydrophilic regions represented by amine and amide groups. These hydrophilic groups would reduce the absorption of caffeine, thus leading to a reduction of pharmacological effect exerted by caffeine. However, caffeine also contains few hydrophobic regions, which are represented by aromatic and aliphatic hydrocarbons. These hydrophobic groups would increase the lipophilicity of caffeine, thus allowing it to be absorbed into the body, especially across the BBB into the brain and exert its action.

However, if the drug is a weak acid or a weak base then ionisation will significantly alter the partitioning of drug as only the uncharged or un-ionised molecules will partition into the hydrophobic cellular membrane effectively by passive diffusion. Given that caffeine is a weak base, log P is therefore not an appropriate predictor to determine the lipophilicity for caffeine due to its ionisable properties and log P only describes the partition coefficient of neutral or un-ionised drug molecules.

The correct predictor for ionisable drugs which can be used is the Distribution Coefficient (Log D). It is defined as the ratio of the sum of the concentrations of all ionised and un-ionised forms of the drug in both phases, which can be written as:

Without taking into account the ionisation of the drug, log D of the drug at a given pH can also be estimated by knowing its log P and pKa values [16].

Caffeine is a basic drug with a log P value of -0. 07 and a pKa value of 0. 6, thus log D of caffeine at a given pH can be calculated, using the equation as follow:

Alternatively, the Apparent Partition Coefficient (PAPPARENT) can be used to predict caffeine’s behaviour at varying pH environments in the body, using the equation as defined below:

PAPPARENT = PTRUE Ã- funionised

Where funionised is the fraction of drug which is un-ionised at certain pH

As mentioned previously, the degree of ionisation of a drug is another important factor affecting the partitioning properties of a drug. Therefore, the degree of ionisation is also a useful parameter for predicting the solubility and the absorption of a drug. The degree of ionisation is dependent on both the pH of the solution and the pKa of the drug itself. For that reason, both pH and pKa have to be taken into consideration in determining the partitioning properties of a drug. The pKa (acid dissociation constant) of a drug is a physicochemical parameter, widely used to indicate the degree of ionisation of a drug with respect to the pH of the environment, thus predicting the amount of drug absorption at a particular site within the body. According to the pH-partition hypothesis, only the un-ionised non-polar drug permeates the cellular membranes [17], indicated that partitioning of a drug largely depends on the pH of the environments due to its effect on the degree of ionisation of the drug. In the body, the gastrointestinal tract (GIT) exhibits a significant pH gradient, from as low as 1. 5 in the stomach to as high as 8. 0 in the small intestine, thus the pH-partition hypothesis predicts

that the absorption of ionisable drugs might vary across the GIT due to the difference in degree of ionisation at different pH [2].

As caffeine is a weak base, it will become ionised in solution and the degree of ionisation will then alter its partitioning as only the un-ionised forms of molecules will penetrate the cellular membranes effectively by passive diffusion, as previously described. Knowing the pKa value of caffeine allows us to calculate its degree of ionisation at different sites in the body (fig. 3), hence indicating the site that caffeine is most likely to be absorbed. The degree of ionisation of caffeine at certain pH can be predicted using the equation as follow:

As can be observed from the table above (fig. 3), the percentage of ionisation of caffeine is relatively small at all pH conditions in the body, indicated that it is largely un-ionised in the body, hence showing that caffeine is readily absorbed as soon as it reaches the stomach.

Although lipophilicity and degree of ionisation are the key factors affecting the partitioning of caffeine across the cellular membranes, properties and characteristics of the membrane are also very important [17]. They are many different types of cellular membranes present in the body, for example gastro-intestinal tract (GIT) and blood-brain barriers (BBB). Besides, they are composed of different thicknesses or types of components, thereby influencing the interactions between drug molecules and membranes as well as affecting the partitioning of a drug across the membranes. As a result, apart from investigating the partitioning of caffeine between the organic and

aqueous phases, it is also important to study the interactions between caffeine and membrane components.

Cellular Membranes

It is important to know that in the body, there are many barriers which a drug has to cross before it becomes effective, for example, gastrointestinal tract (GIT) and blood-brain barriers (BBB). All the living cells in the body are surrounded by cell membrane, separating the inner part from the outside environment, as well as controlling the movement of substances in and out of cells [11]. Cellular membranes are composed primarily of a layer of amphipathic phospholipids, with the hydrocarbon tails being hydrophobic whereas the polar head groups being hydrophilic. When dispersed in aqueous solution, the phospholipids are arranged to form a lipid bilayer so that the water “ hating” hydrophobic tails are shielded by the water “ loving” hydrophilic head groups from the surrounding aqueous environment (fig. 4).

Phospholipids are the major class of lipids in cell membranes, composed of many different components such as phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylethanolamine (PE) and etc. PC is usually the most abundant phospholipid found in mammalian cells, particularly in the outer leaflet of the plasma membranes [12], whereas PS is only found less than 10% of the total phospholipids, mainly in myelin from brain tissue [13]. PC is a zwitterionic (neutral) phospholipid [12] while PS is an anionic (acidic) phospholipid [13] at physiological pH (fig. 5 and 6). These components are varied in structure, degree of saturation and charge state, thus affecting the ability of drugs to penetrate the membranes.

As a result, cellular membranes are the barriers that drugs (caffeine) have to cross to reach their biological targets and exhibit their actions. In order for caffeine to be absorbed, it must pass from the aqueous solution of the extracellular fluid (outside of cell) to the aqueous solution of the intracellular fluid (inside of cell) by crossing the lipid membrane. For efficient transport, caffeine must be sufficiently soluble in both aqueous and the lipid phases to partition to its site of action (i. e. caffeine must be hydrophobic enough to partition into the lipid membrane, but not too hydrophobic until it is unable to partition out into target tissues) [14]. Besides, the characteristics of membranes should be taken into consideration as well while examining the partitioning behaviour of caffeine as they are composed of different components, as previously described. Therefore, the investigations of properties and characteristics of both caffeine and membrane are of great importance as they contribute various factors to affect the absorption and partitioning of caffeine in the body.

In vitro Models

In the development of new drug compounds, the prediction of in vivo drug absorption is a big concern. Therefore, various in vitro models were developed to predict the absorption of drugs from the partitioning behaviour in biphasic systems [15]. These models usually consist of two immiscible phases, one of which is composed of water or aqueous buffer and the other is composed of organic solvent. The commonly used organic solvents are n-octanol, cyclohexane, chloroform or heptane. The movement (partitioning) of drugs from one phase to another is then assessed. There is a variety of systems being developed to examine the lipophilicity and partitioning of

drugs, for example octanol-water system, cyclohexane-water system, liposomal systems, chromatography systems and etc [15].

For many decades, the n-octanol-water model is the method of choice in predicting drug absorption [15]. The use of water-saturated octanol or “ wet octanol” as the organic phase is thought to be the best as the water-saturated octanol shows a more complex structure compared to the pure octanol, giving it a more alike structure to that of the phospholipid bilayer [2]. About 25 mol% of water is thought to dissolve in the water-saturated octanol, forming water clusters that are surrounded by octanol [2] (fig. 7). The polar hydroxyl groups of octanol molecules are hydrogen-bonded to the water molecules while the aliphatic tails of octanol molecules form a hydrophobic region similar to the hydrocarbon core of phospholipid bilayer [2].

In addition, the hydrogen bonding characteristics possessed by the water-saturated octanol are thought to be similar to those of cellular membranes, thus suggesting that the distribution of drugs into the octanol (hydrophobic organic phase) simulates, to a certain extent, their ability to diffuse passively across cellular membranes.

However, there are few issues being raised with the use of octanol-water model. The octanol-water model approximates the transport of drugs through the cellular membranes taking into account only the passive transcellular diffusion mechanisms. In reality, the passage of drugs across the cellular membranes might be occurred through different transport mechanisms, for example channel-mediated or carrier-mediated passive

transport and active transport (fig. 8). The passive transport involves the movement of drug molecules down a concentration gradient while the active transport involves the movement of drug molecules against its concentration gradient with the use of energy.

Besides, the structure of cellular membrane in the body is far more complex compared to the simple two-phase octanol-water model as in addition to phospholipids, cellular membrane also contains cholesterol, glycolipids and proteins which might affect the partitioning of drugs.

Despite these observed differences, the octanol-water model is still being used in this project to determine the partitioning behaviour of caffeine due to its simplicity and properties that simulates the cellular membranes in the body.

PAMPA involved using a membrane filter which contains different phospholipids to predict in vivo activity while Caco-2 cells mimic the intestinal epithelial cells to determine drug transportation/ partition across intestinal cells. [18][19]

Sources

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Pages 259-278