

Relationship between protein concentration and bound drug concentration biology e...

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The protein concentration used in the experiment ranged between normal plasma serum concentrations to concentrations that demonstrated one to one protein to drug ratio. The binding ratio (B/F) ranged from 0.00 to 1.40 for BSA and 0.00 to 0.80 for AAG, with B representing the bound molar drug concentration, while F represented the free unbound molar drug concentration. The actual BSA and AAG concentrations used in the equilibrium dialysis and the B/F ratio are shown in table 6. The binding ratio (B/F), were plotted against the corresponding concentrations of AAG and BSA in figure 17 and figure 18 respectively.

B/F ratio

BSA concentration (uM)

AAG concentration (uM)

6.4

19.2

303

606

3.66

6.4

18.3

19.2

Drug concentration (uM)

6.4

0.000.000.391.020.120.080.450.65

19.2

0. 000. 060. 761. 290. 170. 140. 350. 53

Table 6: Binding ratio of quinidine under different concentrations of AAG and BSA used in equilibrium dialysis

Figure 17: Relation of concentrations of BSA to the binding ratios (Bound/Free) of Quinidine Sulfate determined by equilibrium dialysis. The higher and lower dashed lines represent the best-fit line for high and low drug respectively. The solid linear line is the best fit line for all data points (high and low drug concentration); determined via least squares regression analysis ($r^2 = 0.95$, $P < 0.001$). Gradient of best-fit line represents the nK value. Error bars represent standard error.

Figure 18: Relation of concentrations of AAG to the binding ratios (Bound/Free) of Quinidine Sulfate determined by equilibrium dialysis. The higher and lower dashed lines represent the best-fit line for high and low drug respectively. The solid linear line represents the best-fit line for all data points (high and low drug concentration); determined via least squares regression analysis ($r^2 = 0.817$, $P < 0.01$). Gradient of best-fit line represents the nK value. Error bars represent standard error.

R²-value

P value

nK value (uM⁻¹)

Standard error

Lower 95%

Upper 95%

Albumin (BSA)

0. 9500. 000040. 001920. 00180. 001480. 00236

α 1-acid Glycoprotein (AAG)

0. 8170. 002100. 026620. 00520. 014040. 03919

Table 7: The linear regression and correlation values of figure 17 and figure 18. Refer to Appendix III. The linear correlation of bound-to-free drug ratio and BSA concentrations is strongly significant ($R^2 = 0.95$, $P < 0.001$); the linear regression line is shown in figure 17. AAG seems to bind quinidine extensively and the bound-to-free drug ratio and AAG concentration is also significantly ($R^2 = 0.82$, $P < 0.01$) correlated to the concentration of protein. Table 7 shows that there is a strong linear regression and correlation between protein concentration and B/F ratio, hence demonstrating that with increasing protein concentrations, there would be an increase in bound drug concentration. Based on the experimental results, there is a strong linear correlation between the drug binding ratio and albumin, however, it was reported by Nilson (1977) that there was no significant correlation observed between them.[9] This discrepancy in results could be explained by the

difference in albumin concentration used. The presence of a significant correlation could be due to the large albumin concentration range used in this study; whereby very low (6.4 μM) and high (606 μM) concentrations of albumin were used. Whereas, in Nilson's study, only physiological serum concentrations of albumin had been used, which was a rather narrow range of concentrations which did not vary much.[9] With reference to the results, AAG had a weaker linear correlation ($R^2 = 0.82$, $P < 0.01$) as compared to BSA probably due to the influence of a narrow AAG concentration range which had been used in this study. From the gradient of the two curves, the binding affinity, nK , of Quinidine Sulfate to BSA is $1.9 \times 10^{-3} \text{ uM}^{-1}$, while the binding affinity of AAG is $2.66 \times 10^{-2} \text{ uM}^{-1}$. This again supports the hypothesis that the affinity of quinidine with AAG is greater than that of BSA. This is probably due to the basic nature of the drug; as a number of studies have shown that basic drugs tend to have higher affinity to AAG and is less affected by albumin variations. [11, 41] Therefore, due to quinidine's higher affinity with AAG, this may lead to the assumption that: patients with increased AAG plasma concentrations due to an acute myocardial infarction [42, 45], would have a significant increase in drug-protein binding. The effect of protein concentration variation on drug binding and its affinity will be further explained in section 5.7.

5.7 Variation of Protein concentration and its implication on the pharmacokinetics of Quinidine Sulfate

The drug-protein binding is characterised by the parameter, fraction unbound (f_u) or fraction bound (f_b). A simplified mathematical model was used to understand the results obtained, and to investigate how bound drug

fraction varies with protein concentration, and subsequently understand how these protein variations might affect the pharmacokinetics of quinidine. As mentioned, this model is a simplified model, therefore this model is used under the assumption that the kinetics of binding and dissociation are rapid; meaning that this model simulates an equilibrium reaction at all times. The following graphs are plotted based on the nK value and the four different protein concentrations of BSA and AAG obtained from figure 17 and 18 respectively. Figure 19: Plot of bound drug fraction as a function of BSA concentration (protein-drug binding, relative to Quinidine concentration) ratio using a simplified mathematical model. Affinity is obtained from figure 17. Lines represent drug-binding affinities (product of binding strength and BSA concentration). Shaded area indicates the position within the graph in which physiological BSA to quinidine ratio fall in; ratio 10 to 100. Figure 20: Plot of bound drug fraction as a function of AAG concentration (protein-drug binding, relative to Quinidine concentration) ratio using a simplified mathematical model. Affinity is obtained from figure 18. Lines represent drug-binding affinities (product of binding strength and AAG concentration). Shaded area indicates the position within the graph in which physiological BSA to quinidine ratio fall in; ratio 0.1 to 1.

Based on figures 19 and figure 20, it noticeably shows that with increasing protein-to-drug ratio, there is a significant increase in drug bound fraction. It also shows that with increasing protein-to-drug ratio, there is an increase in binding affinity (refer to appendix 3 for the mathematical model data and macros).

At physiological albumin concentrations and under therapeutic concentrations of quinidine (6.4 μ M), the protein-to-drug ratio would be within the range of 10 to 100. This would indicate that the protein to drug ratio would have been near the plateau region. As for AAG, the protein-to-drug ratio would fall within the 0.1 to 1.0 range; hence quinidine would be found within the steepest region of the curve. This meant that a slight change in AAG concentration or protein-to-drug ratio would result in a significant change in drug binding. Therefore, this would suggest that clinically significant changes in quinidine binding are more likely due to changes in AAG, as patients who have an increased AAG plasma concentration due to an acute myocardial infarction, would have a substantial increase in protein-drug binding; resulting in reduced quinidine interaction at the site of action.[11] It seems that changes in plasma albumin concentration is less likely to cause any extensive changes in drug binding due to several reasons: firstly, HSA has lesser variation in plasma concentration as compared to AAG [11]; secondly, at therapeutic quinidine concentrations the drug falls within the plateau region of the estimated mathematical model, demonstrating that any changes in the protein concentration would not drastically affect drug-protein binding. Using the mean binding parameters for BSA and the mathematical model, it can be

predicted that at least a 50% decrease in albumin concentration would be necessary to obtain about 17% increase in quinidine free fraction. This predicted value was very similar to a study conducted by Edwards et al (1983), reporting a 20% increase in quinidine free fraction following a 50% albumin concentration decrease.[11] According to Edwards et al (1983), the mean AAG plasma concentration in patients with trauma or acute myocardial infarction is usually increased to around 1.97mg/ml, which is $\sim 47\mu\text{M}$. He reported that quinidine free fraction was reduced by $> 40\%$.[11] However, based on the mathematical model shown in figure 21, it was predicted that an increase from $18.3\mu\text{M}$ (normal AAG plasma concentration) to $47\mu\text{M}$ of protein would result in a 28% decrease in unbound fraction. This discrepancy in results could be due to the inaccuracy of the AAG nK parameter used in the mathematical model, as the AAG concentrations used in this study was rather narrow (range between $3.7\mu\text{M}$ to $19.2\mu\text{M}$), hence the protein-to-drug ratio currently falls within the steepest slope of the graph. Therefore, protein concentration should be increased so that sufficient protein is available for saturation to occur, subsequently allowing a more accurate prediction of protein binding using the protein-drug binding model. Figure 21: Plot of bound drug fraction as a function of AAG concentration (protein-drug binding, relative to Quinidine concentration) ratio using a simplified mathematical model. Comparing two AAG concentrations; one at $47\mu\text{M}$ (elevated plasma concentration due to myocardial infarction) and the other at $18\mu\text{M}$ (normal AAG plasma range). Therefore, even though albumin is the most abundant protein in the plasma, it appears that $\alpha 1$ -acid glycoprotein is a major protein in the binding of quinidine, agreeing with other studies.[9,

11, 42] Hence, this could possibly mean that removal of albumin might not affect quinidine binding if the concentration of AAG is large enough, but AAG removal could have affected quinidine binding significantly. Since AAG in the plasma is subjected to such great variation, protein binding of quinidine suggests conceivable pharmacodynamics and pharmacokinetics variations in quinidine treated patient.[42] According to Fremstad et al. (1979), he demonstrated a significant positive correlation between the free fraction of quinidine and both volume of distribution and total body clearance,[11, 54] and that increase in bound drug could lead to less drug available for metabolism, prolonged drug clearance and half-life. It seems that in patients with acute myocardial infarction, quinidine might be less effective in reaching the target site, therefore this could be compensated with titrating the dose. However, giving a patient too high a dose could potentially be harmful to the patient as clearance of the drug is extended because protein bound drugs cannot be renally filtrated, hence the drug would accumulate in the body and consequently lead to toxicity. Also, an increase in protein binding can significantly affect quinidine clearance from the body especially when quinidine is cleared mainly via hepatic metabolism, as high protein binding is associated with lowered drug elimination.

5. 8 Limitations

This study had created a baseline understanding of quinidine sulphate with the two different proteins, AAG and albumin. Unfortunately, apart from the time constraints, there were several other limitations that hindered a comprehensive understanding of protein binding of quinidine. Firstly, in spite

of studies claiming that the degree of protein binding amongst BSA and HSA is often very similar, hence having used BSA in replacement of HSA to understand drug-protein binding;[3] animal proteins do differ from human serum. Such differences include: protein structural variations at the binding sites of the two albumins and quantitative differences with respect to the extent and affinity of drug binding. It has also been reported that significantly lower protein binding has been found in commercially available BSA than that found in HSA. This is probably due to the fact that HSA contains only one tryptophan residue rather than two in BSA.[44] Unfortunately, in this experiment, due to the limited amount of HSA available, BSA was used in replacement. Secondly, in-vitro measurements and predictions of protein binding by just adding physiological concentrations of albumin into a test medium might not be the best representative and model of protein-binding as compared to using pure native serum[44]. This is due to the fact that plasma contains many other proteins, and under physiological conditions, proteins such as lipoproteins might interact with albumin and affect the binding affinity of quinidine to albumin. There might even be competitive binding between the HSA, AAG and other proteins. Thirdly, the mathematical model used in this study was a simplified model that took into account the assumption that the experiment kinetics of binding and dissociation are rapid, though there might be exceptions. Lastly, the results reported in this study were under normal room conditions and not under physiological conditions. Hence apart from the protein concentration, drug concentration and pH of the experiment being kept under physiological conditions; the temperature and the presence of

electrolytes were not kept under physiological conditions, which could have potentially affected binding.

5. 9 Further improvement studies and work development

As mentioned above, there are many limitations to this project and more can be done to improve the experiment and subsequently, allow better understanding of the interactions of quinidine to the two different plasma proteins. The following recommendations can be proposed, which are based on the current understanding of drug-protein binding and also a build up on this study.

- (i) While studies in animal serum are valuable, the experiment would be more accurate and relevant if HSA was used instead of BSA, or if the results were supported or compared with findings from HSA.
- (ii) When drug-protein binding in humans is being investigated, the use of standardised and well-characterised pooled human serum and albumin is preferred.
- (iii) More variation in protein concentrations should be used, especially for AAG, to allow a complete graph to be plotted from protein to drug concentration ratio 0. 1 to 100. This would provide a better estimate and understanding of the drug-protein binding.
- (iv) Quantitative measurements and calculation of the bound fraction when variations of both albumin and AAG were added together. This would study the effects of AAG and albumin drug binding in the presence of one another.
- (v) Further research can be done on varying other factors such as pH, temperature and drug concentration so that a holistic study on the protein binding of quinidine can be done.
- (vi) Binding of drug to equipment (eg. vials) should be

investigated when appropriate to ensure accuracy of the measurement of unbound or bound fraction.

6. CONCLUSION

The spectrofluorometry was selected to measure the concentration of free drug because it is a more accurate and sensitive instrument; therefore it is more appropriate as compared to UV-spectrometry for measuring minute amounts of quinidine at physiological concentrations. The time taken for quinidine sulphate to equilibrate in equilibrium dialysis was 4 hours in the absence of protein, and around 5 hours in the presence of protein. Therefore, 5 hours was sufficient to ensure that equilibrium dialysis had occurred. Upon investigating the relationship between protein concentration and the bound-to-free drug ratio, the nK values obtained indicate that AAG has a high binding affinity to quinidine as compared to albumin. It also shows that with increasing protein concentration, there would be an increase in bound drug concentration. With the use of a simplified mathematical model, the protein concentration of both AAG and albumin was varied and the results showed that at physiological concentrations of quinidine, a change in albumin concentration would not have much effect on the bound drug fraction, however, a slight change in AAG plasma concentration would have a significant effect on the bound-to-free drug concentration ratio. Therefore, when there is an increase in AAG plasma concentration due to a myocardial infarction, there would be a significant increase in protein binding of quinidine, which suggests conceivable pharmacodynamics and pharmacokinetic variations; as an increase in bound drug could lead to less

free drug available for metabolism, and therefore prolonging drug clearance and half-life. Hence, in patients with acute myocardial infarction, quinidine might be less effective and could be compensated with titrating the dose slightly higher, however at too high a concentration, it could potentially be harmful to the patient.