K18 A Spectroscopic Investigation of the Binding of Benzodiazepines to Human Serum Albumin

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After attending this presentation, attendees will understand how fluorescence spectrophotometry can be used to study the interactions of different benzodiazepine derivatives with human serum albumin. Attendees will become aware of the varying binding affinities of different benzodiazepines for human serum albumin and some important parameters used to characterize the binding.

This presentation will impact the forensic science community by providing further pharmacological and toxicological information on benzodiazepines, a class of drug that is commonly used therapeutically and is increasingly being abused in social settings.

Benzodiazepines are commonly prescribed central nervous system depressants which are found in a wide variety of different medications from sedatives, hypnotics, to amnesiatics, and anticonvulsants. Benzodiazepines are increasingly being used as recreational drugs often in combination with other drugs such as opiates and alcohol. Human serum albumin is the most abundant plasma protein in humans. Many drugs, including benzodiazepines, bind reversibly to albumin with albumin then acting as a carrier for the drug. This binding can increase the apparent solubility of the drug in the plasma and can influence the distribution, metabolism, and excretion of the drugs. Quenching of albumin fluorescence can be used to study the interactions of these drugs with albumin and was the method utilized in the current research to study the interaction of different benzodiazepines with human serum albumin. The quenching of albumin fluorescence by nine benzodiazepines were analyzed at three different temperatures, 24°C, 30°C, and 37°C. The nine benzodiazepines used in this study were alprazolam, bromazepam, diazepam, flunitrazepam, flurazepam, lorazepam, oxazepam, temazepam, and triazolam. Varying concentrations of each benzodiazepine were incubated with a 2.42μM solution of human serum albumin. The albumin solution was prepared in a 0.05M Tris buffer at a pH of 7.4. Benzodiazepines were tested at each temperature at concentrations ranging from 19.82 μM to 198.2 μM. Each concentration was analyzed five times. A fluorescence spectrophotometer with thermostated cell holder was used to measure fluorescence. The excitation wavelength was set at 290nm and the emission wavelength range was set at 300-500nm.

Quenching mechanisms associated with the binding between a quencher and a macromolecule can be static or dynamic. Stern-Volmer analysis of the data was used to characterize the quenching mechanism. Comparison of the nine benzodiazepine derivatives established that all derivatives exhibited a quenching rate constant of >> 2x1010L mol-1 s-1, indicating that static quenching was present. Static quenching as opposed to dynamic quenching signifies that a relatively stable complex is being formed between the benzodiazepines and the human serum albumin. Analysis of the fluorescence quenching using double log plots allowed the determination of the number of binding sites (n) and binding constants (Kb). The benzodiazepines that showed significant variation in binding affinity were diazepam and flurazepam. Diazepam, for example had a binding constant of 114.1 at 37°C whereas flurazepam had a binding constant of 1.808x106 at the same temperature. Diazepam had an "n" value close to one whereas flurazepam had an n value closer to two, possibly indicating the existence of more than one binding site. Van't Hoff analysis of the data was also used to calculate the thermodynamic parameters of binding and provided evidence of spontaneous binding and information on the involvement of hydrogen bonding and hydrophobic interactions in the binding of these drugs to albumin. The eventual goal is to use this information to determine if the affinity of these drugs for albumin can influence the analysis of these drugs in blood/plasma using current methods of sample preparation and analysis.

Forensic Science, Benzodiazepines, Fluorescence Spectrophotometry

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