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Binding of synthetic cannabinoids to human serum albumin: site characterization and recognition mechanisms

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Abstract

Background: The use of new psychoactive substances (NPS) has been increasing since the beginning of the 2000s, with synthetic cannabinoids being one of the most reported groups. Consumers of these drugs typically seek their known psychoactive effects, such as relaxation and euphoria. Numerous reports of morbidity and mortality have been associated with the consumption of these substances [1,2]. Therefore, toxicokinetic and toxicodynamic studies are needed to better understand the behaviour of these NPS. **Objective:** This study aims to evaluate the binding affinity of a series of synthetic cannabinoids to human serum albumin (HSA), obtain insights into the binding sites and better understand the recognition mechanisms. **Methods:** Zonal elution chromatography was used to assess the binding affinity of five synthetic cannabinoids using a HSA-based column, CHIRALPAK® HSA. Mixtures of potassium phosphate buffer (67 mM, pH 7.0) and acetonitrile were used as mobile phases. Displacement experiments with concentrations ranging from 0-20 µM of well-known site-specific probes, including warfarin, (*S*)-ibuprofen and L-tryptophan were carried out. Both studies were performed using high-performance affinity chromatography (HPAC), which is a widely used and effective technique for evaluating intermolecular interactions between HSA and drugs [3]. **Results:** The binding percentages (*%b*) ranged from 98.7% to 99.9%. FUBIMINA showed the highest binding affinity, with a *%b* of 99.9%. Competition for site I was observed between warfarin and four synthetic cannabinoids. Molecular docking studies supported experimental findings, allowing to elucidate the recognition mechanisms, identify binding sites, and characterize their interactions with the protein. Surprisingly, the binding affinity of all synthetic cannabinoids increased in the presence of (*S*)-ibuprofen. **Conclusions:** The synthetic cannabinoids bound to HSA with high affinity. Extrapolating this situation to real-world scenarios, particularly when synthetic cannabinoids are consumed, especially in uncontrolled high doses, alongside other drugs with high affinity to site I of HSA, a serious issue may arise. Additionally, all compounds showed increased retention to HSA in the presence of (*S*)-ibuprofen, resulting in a lower free fraction of these compounds. Consequently, (*S*)-ibuprofen may serve as a promising candidate for exploring potential strategies to mitigate synthetic cannabinoids' harmful effects.

Keywords: synthetic cannabinoids; high-performance affinity chromatography; displacement studies.

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