Identification of Active Components in Tobacco Smoke
Scientific Abstract

It is estimated that more than 10,000 compounds are present in tobacco smoke. The majority of these compounds have not been structurally identified nor have their pharmacological activities been determined. The current approach to the determination of the pharmacologically active components of tobacco smoke is tedious and complex and involves multiple, sequential fractionations guided by biological testing. We have developed an alternative approach based upon on-line liquid chromatography affinity screens using columns containing immobilized receptors and drug transporters [1] and demonstrated that a column containing immobilized nicotinic acetylcholine receptors (nAChRs) can be used to screen tobacco smoke condensates, to identify known and unknown compounds that bind to individual nAChR subtypes, and to produce a chromatographic fingerprint that can be used to establish active component patterns (ACPs) [2]. The initial aim of this project is to screen tobacco smoke condensates obtained from standard, light and flavored cigarette brands for compounds that bind to the nAChRs and to establish ACPs. In the proposed study, the nAChR affinity column will be used to fractionate the condensates into high, medium and low affinity fractions and reverse phase chromatography will be used to establish the corresponding ACPs. The ACPs will then be used to guide preparative scale chromatographic in order to obtain sufficient quantities of the components for biological testing. The second aim is to determine the binding affinity and functional activity of individual compounds contained in the ACP, at several nAChR subtypes [3]. The third aim will be to repeat the study using extracts from the same cigarettes used to obtain the tobacco smoke condensates in order to compare the ACPs and to identify the source of active components in the tobacco smoke condensates.

The goals of the project are to optimize and validate the determination of ACPs, to establish the role of this technique in the regulation of tobacco products and to determine the effect of additives on the products produced by the combustion of tobacco leaves. Our on-line affinity chromatography is the best method for isolation and purification of tobacco leaf components and tobacco smoke products that activate nAChRs. This technique will identify tobacco components that might be either additive or synergistic with nicotine and lead to increased dependence on cigarettes or potentially increased withdrawal, which promotes relapse. Together, these aims will identify ACPs that can aid in the regulation of tobacco products, and concurrently identify additional active components present subsequent to combustion of tobacco leaves.

[1] Moaddel R, Wainer IW, Nature Protocols, 4: 197-205. 2009
[2] Maciuk A, et al. J. Pharm. Biomed. Anal. 48: 238-246, 2008
[3] Toll L, et al. Neuropsychopharmacology, e-pub ahead of print.