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Commentary

Time to think small: Using extracellular vesicles to assess the effects of long-term opioid use

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The United States is currently in the midst of an opioid crisis, an epidemic that has worsened since the start of the Covid-19 pandemic [1]. In addition to the well-known risk of fatality resulting from opioid overdose, in the past two decades several studies have demonstrated the potential neurodegenerative effects of long-term opioid abuse [2–4]. On this note, there is a burgeoning awareness of the potential for utilizing extracellular vesicles (EVs) as biomarkers of neurodegeneration. Typically, cells secrete a diverse array of EVs that include exosomes, microvesicles and apoptotic bodies. During their biogenesis, EVs encapsulate bioactive cargo from their parental cell, including proteins and lipids, as well as genetic information in the form of miRNAs, mRNAs, and siRNAs [5].

The cell-to-cell transfer of diverse cellular cargo *via* EVs is a widespread process. EVs are transported through the body and fuse with specific cell types to deliver their bioactive cargo. Considering that EVs are able to cross the blood-brain barrier, and are present in multiple body fluids such as blood, urine and/or cerebrospinal fluid, EVs may serve as useful biomarkers for a variety of human diseases including neurological disorders. The biophysical properties of EVs, as well as their cargo, reflect the microenvironment and physiological conditions that trigger their formation. An improved understanding of EV dynamics and their contents following acute and chronic opioid use, could help improve our understanding of disease pathophysiology, including neurodegeneration outcomes.

Evidence suggests a role of EV-associated cargo, specifically non-coding regulatory miRNAs, in mediating the body's response to a variety of addictive substances, including cocaine, cannabinoids, nicotine, alcohol and opioids (reviewed in [6]). A recent study investigated the impact of *in utero* and postnatal oxycodone exposure on the miRNA signatures of brain-EVs and their association with neurodevelopment [7]. They found that oxycodone exposure modulates the microRNA cargo of brain-EVs, and that synaptodendritic damage to primary neurons is associated with impaired brain development. The role EVs play in oxycodone abuse is unclear, and to date, no study has characterized brain cell derived-EVs to understand the potential neurodegenerative effects of long-term oxycodone self-administration. There is a significant gap in knowledge regarding the role of EVs and their cargo in addiction pathology and neurodegeneration. Given the current opioid crisis, the need for novel blood-based tools to help decipher the dynamic molecular changes in the brain that are associated with opioid abuse is of paramount importance. Furthermore, understanding dynamic changes that occur at different stages of opioid abuse may aid the development of novel treatment interventions.

In a carefully executed study recently published in *EBioMedicine*, Kumar and coworkers [8] first used MRI to demonstrate that gray matter volume is lower in non-human primate subjects self-administering oxycodone compared to controls in brain regions associated with cognition and reward such as the frontal cortex, putamen and parietal cortex. Further, Kumar et al., demonstrated the presence of neurodegeneration-associated proteins and miRNAs in EVs isolated from plasma of subjects that self-administered oxycodone for approximately 3 years. The combination of these two minimally invasive methods offers a particular advantage to translating these findings to clinical populations. To our knowledge, the body of work by Kumar and colleagues is the first to provide evidence that the neurodegenerative effects caused by long-term oxycodone exposure could be assessed by studying the composition of subpopulations of brain cell-derived exosomes isolated from plasma. In turn, paving the way for the potential use of exosomes as a source of biomarkers to better predict the possible neurodegenerative and pro-inflammatory effects of opioid abuse.

Interestingly, the study also provided *in vitro* evidence to suggest that differential EV content is associated with altered functionality. Specifically, neuronal-derived EVs induce glucocorticoid receptor translocation to the nucleus and promote a pro-inflammatory response in monocytes *in vitro*. Taken together, EVs are likely just one component in a complex cascade of molecular biology that leads to opioid-associated neurotoxicity. By improving our

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understanding of EV biology and the role of EVs in opioid-associated neurodegeneration, in addition to providing prognostic/diagnostic value, EVs could emerge as a useful *in vivo* therapeutic target to inhibit disease propagation.

Further studies are required to understand the potential of EVs as biomarkers for opioid-associated neurodegeneration. When conducting future experiments, researchers should consider the following: How specific are the markers chosen to identify the EV source – are they unique to the parental cells, or absent in a subset of EVs? Are differences in EV cargo detectable at the serum level, thus mitigating the need for EV purification? A simplistic approach, that avoids substantial pre-analytical manipulation is more attractive, especially for clinical translation. What is the biodistribution of brain cell-derived EVs? What are the target cells of specific brain cell-derived EV subpopulations? How does acute *versus* chronic oxycodone use impact brain cell-derived EV release? Considering the vast heterogeneity in circulating EV populations, the pursuit for EV-associated protein profiles and miRNA signatures that are associated with neurodegeneration is challenging. Thus, carefully designed high-throughput EV characterization studies coupled with functional screening is likely required. Of particular note, in order to aid interstudy comparison and interpretation, it is imperative that, as the authors did in this study, researchers abide by the minimal information criteria for extracellular vesicle research (MISEV) which is set forth by the International Society of Extracellular Vesicles (ISEV) [9]. A diligent and careful approach should be taken when defining EV 'cargo'. Often, common EV isolation methods readily co-isolate non-EV material.

In conclusion, Kumar and colleagues present a thought-provoking approach to better understand the molecular and biological effects of oxycodone, and these findings may be applicable to other drugs of abuse pending further assessment. Brain cell-derived EVs following oxycodone self-administration may serve as a surrogate to predict the pathophysiological status of the host brain cells.

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