Neural-Derived Extracellular Vesicles in Clinical Trials
Message in a Bottle

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The release of extracellular vesicles (EVs) from cells has been a recent area of extensive study in basic biology.[1,2] Extracellular vesicles are considered to be a vital means of communication and molecular signaling used by many cell types, and EVs accomplish these tasks by delivery of cargoes carrying nucleic acids (eg, noncoding RNAs), proteins, and metabolites. The most prominent established role of EVs in neurology thus far is in biomarker development.[3] Recent studies[4,5] have found that many serum or cerebrospinal fluid biomarkers, such as neurofilament light, tau, and others (historically measured directly from unfractionated fluids) actually reside in EVs, implying that these insoluble cytoplasmic proteins are probably generated from sick or injured cells and then released within EVs as cargo.

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Within the brain, EVs are widely involved in shuttling signals between neurons and a variety of nonneuronal cell types.[6] Extracellular vesicles originating in the brain can travel beyond it and cross the blood-brain barrier; once in the circulation, they may either exchange information with the body or be metabolized as waste. Extracellular vesicles originally extrude from a parent cell and carry some of its cellular cargo. Per current convention, these membrane-bound structures are classified by size and origin into either microvesicles (100 nm-1 µm in diameter) that bud off cells or exosomes (30-100 nm in diameter) secreted from cells.[2] Because they are currently not easily distinguished biochemically, we use the encompassing term *EVs* when referring to EV subtypes, including exosomes. Our increasing realization that healthy cells in the central nervous system (CNS) produce EVs present in the systemic circulation allows the exciting possibility that EVs will enable monitoring of processes that reflect CNS physiologic mechanisms. In this context, the study in this issue of *JAMA Neurology* by Athauda et al[7] provides important insights into the potential advantages and current limitations in this approach of using serum EVs as messages from the brain.

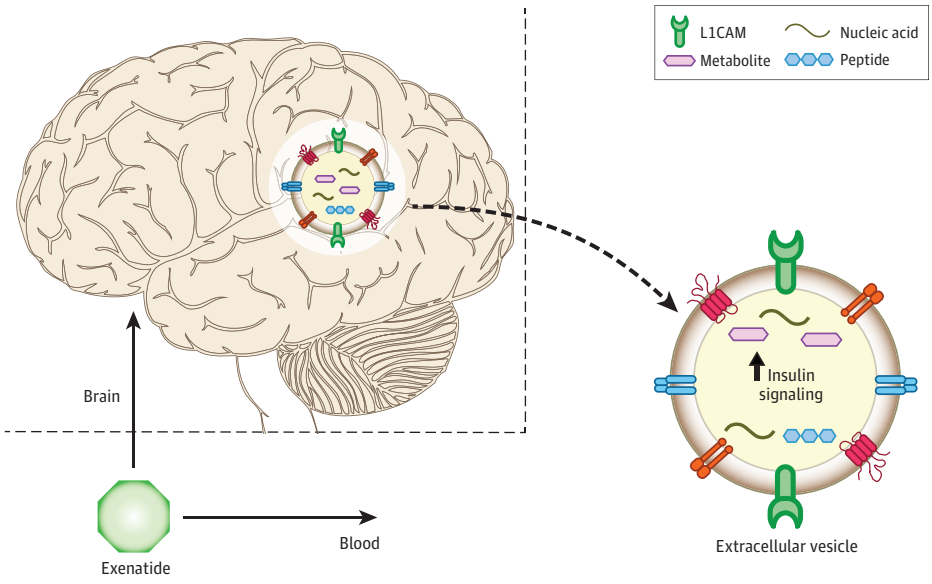
Athauda et al[7] probed whether serum EVs could measure brain target engagement in a clinical trial for Parkinson disease (PD) (**Figure**). In the original randomized, placebo-controlled trial, patients received subcutaneous injections of exenatide, a glucagon receptor agonist used in type 2 diabetes, once weekly for 48 weeks, followed by a 12-week washout period. The rationale for testing this drug was based on robust preclinical studies that found beneficial effects on several mechanisms, including impaired insulin signaling, relevant to PD. Exenatide improved motor scores in patients with moderate PD,[8] and this effect persisted beyond the period of drug exposure. In the current study, the authors isolated neural-derived EVs from peripheral blood sampled from participating patients at 48 and 60 weeks, during the time of significant motor improvement. They found that within EVs, exenatide treatment had augmented molecular measures of brain insulin signaling. Specifically, the drug increased tyrosine phosphorylation of the insulin receptor and its downstream targets, including protein kinase B and mechanistic target of rapamycin. Some of these molecular changes correlated with treatment-induced improvement in the motor scores of patients with PD.

In short, neural-derived EVs from blood samples of patients with PD had augmented insulin signaling—a molecular change anticipated by treatment with a glucagon receptor agonist that crosses the blood brain barrier. This is an important study because it highlights the potential use of EVs for assessing a drug's target engagement with the brain, a high-value measure in clinical trials. A simple blood test that yields a reliable biomarker of drug activity is far preferable for practitioners and patients compared with invasive cerebral spinal fluid measures; imaging, such as positron emission tomography, which requires brief radiation and radioactive tracer injection; or even magnetic resonance imaging measures. One of the chief values of this type of inexpensive, noninvasive, and rapid assay of serum samples is that it can be performed virtually at any interval and may be performed as frequently as warranted to establish drug efficacy in future clinical trials. It could also allow important personalized medicine approaches to discriminate between individuals who are responders and those who are nonresponders to drug therapy or perhaps allow individualized dose-finding studies in clinical practice.

However, the current study raises several questions about the use of blood-derived EVs for clinical trials in neurology. Although the simplicity of a blood test to gauge the brain is compelling, one wonders whether these EVs are truly neural derived. For example, the results of this study rely on purifying CNS-derived EVs using an immunopurification approach reliant on the presence of a neural L1 cell adhesion molecule (L1CAM) found in these EVs. However, L1CAM is not exclusively expressed in the CNS, and use of this as the marker of neural EVs likely results in isolation of an impure population. Other tissues that express L1CAM include skeletal muscle and fat, which are likely to be affected by exenatide. Thus, although the authors provide compelling evidence that EVs purified using L1CAM have alterations consistent with pharmacologic effects of exenatide, it is difficult to be sure that these reflect changes specifically in the CNS and not in other peripheral tissues that express L1CAM.

In addition, the very nature of EVs, carriers of cargo that signal to remote structures, suggests that these EVs may undergo changes in enzyme activity based on other influences encountered during transit from their original parent cell to the ultimate target. Thus, contents of neural-derived

Figure. Exenatide-Induced Changes in Neural-Derived Extracellular Vesicles (EVs) Harvested From Blood Samples



This diagram models neural-derived EVs, marked by the L1 cell adhesion molecule (L1CAM) peptide, crossing from the brain into the blood, where they can be harvested from serum.

exosomes reflect a potentially dynamic communication of signals within its changing biologic context and could be altered by drugs or conditions encountered in the peripheral circulation, possibilities that remain to be examined.

However, it may not matter whether isolated EVs are purely derived from the brain or whether they undergo some physiologic drift, if they still reliably report a robust biochemical measure (proteins, metabolites, or nucleic acids) that correlates with a meaningful neurologic outcome, as this study shows. It is possible that the neural EVs studied by Athauda et al[8] accurately reflect effects of exenatide in the CNS or that exenatide might augment insulin signaling in the body, which transmits a key signal to the brain that protects against symptoms of PD. It is also possible that exenatide acts on alternate pathways important in PD that are parallel to or converge on insulin signaling. Thus, we should not overinterpret associations in the Exenatide-PD trial as proof that the identified EV alterations causally mediate mechanisms of motor protection. Nonetheless, this study provides reason for optimism because of the evidence that a drug targets physiologically relevant biochemical signaling measured over time in individual patients in an easy-to-study way using EVs isolated from serum samples.

In addition to their use in clinical trials, there are many reasons to be enthusiastic about the potential for EVs to markedly affect the way we detect, monitor, and treat neurologic disease. Several small studies of neural-derived EVs harvested from peripheral blood have found alterations of EV content in the context of disease: higher Aβ and tau in traumatic brain injury[4] and Down syndrome,[9] decreased synaptic proteins in Alzheimer disease and frontotemporal dementia,[10] increased neurofilament light in sports-related concussions,[5] and altered levels of autolysosomal proteins in preclinical Alzheimer disease.[11] These studies[4,5,9-11] suggest that neural-derived EVs measured from the blood could serve as biomarkers—or even early omens—of neurologic disease. Equally exciting are the proposed functions for EVs, ranging from cellular transmission of neurodegenerative disease[12-15] to nanovessel drug delivery.[16] Collectively, emerging basic and translational studies are expanding our understanding of diverse EV signals within the brain, between the brain and the rest of the body, and in both health and disease. Applying our increasing knowledge of EVs to drug targets in clinical trials and biomarkers for disease could ultimately alter clinical care.

ARTICLE INFORMATION

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