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Commentary

A Brief Note on Chemiluminescent Enzyme Immunoassay Application in Pharmacoepidemology

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DESCRIPTION

The chemiluminescent enzyme immunoassay (CLEIA) uses a chemiluminescent substrate that reacts with a variety of enzymes used as labels. Enzymatic chemiluminescence reactions produce light similar to bioluminescence using natural substrates such as luciferin-adenosine triphosphate. Over the last two decades, much attention has been paid to the application of chemiluminescence in immunoassays, and various systems of substrates and enzymes have been developed. Current systems using luminol derivatives of peroxidase or dioxetane derivatives of alkaline phosphatase, including enhancers, enable highly sensitive immunoassays. These assays are powerful tools in practical diagnostics. The enzymatic oxidation reaction of luminol analogs has long been used in CLEIA. Using peroxidase with H_2O_2 is a common method compatible with alternative coupling enzymes that produce H_2O_2, such as glucose oxidase and uricase. The discovery of a luminol-based chemiluminescent enhancer by few researchers significantly increased the sensitivity of the assay. Enhancers include phenolic derivatives and aromatic compounds. For example, luminol peroxidase, which contains piodophenol as an enhancer for an optimized reaction mixture, achieves up to a 2-800 fold increase in luminescence. This assay can detect TSH at 0.04 μU/mL when serum is used as a sample. However, oxidative reactions such as luminol can be confused by several factors that cause an increase in non-specific background signals (noise).

Few scientists have developed chemiluminescent substrates for alkaline phosphatases that are very different from other compounds. This new substrate is known as the adamantyl dioxetane derivative AMPPD (3 (4 methylspiro [1,2'dioxetane 3,2'tricyclo [3.3.1.1] decane] 4 yl) phenylphosphate disodium). Unlike luminol, which requires an oxidizing compound from the outside of the luminol molecule, it does not require additional molecules for chemiluminescence. AMPPD is a new molecule that is a complete substrate because it is composed of an adamantyl group as a stabilizer for the entire molecule, a dioxetane bond as an energy source, a phosphoryl ester as a cleavage site for enzymes, and a phenyl group for chemiluminescence. Cleavage of AMPPD phosphodiester bonds by alkaline phosphatase causes chemically initiated electron exchange luminescence by releasing electron-rich dioxetane. Depending on the substrate concentration, chemiluminescent signals with a maximum wavelength of 477 nm can be detected within minutes to hours. The sensitivity of alkaline phosphatase test systems using AMPPD is less than 10-20 mol.

Immunochemistry is an excellent method for demonstrating the presence of these cytokines in the hypothalamus, pituitary gland, or other areas of the central nervous system, and when combined with measurements of release *in vitro* or in push-pull cannula, cytokines you can decide whether it is actually produced and released or not within the tissue and its origin. In this regard, the IL1α and IL1β nervous systems of the hypothalamus have been described based on immunocytochemical studies. This should be combined with an attempt to detect cytokine messenger RNA reported for IL1β in the hypothalamus and its localization by ***in situ*** hybridization. Cytokine receptors must also be present in the brain and pituitary gland. These are the main methods that have been used to study the possible roles of various objects and cytokines in the hypothalamic-pituitary axis.

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