Drug Assay Using Antibody Mimics Made by Molecular Imprinting

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CONDENSATION OF THE RESEARCH

PURPOSE OF THE STUDY

To explore synthetic polymers as an alternative to antibodies in drug assays

RESEARCHERS' APPROACH

Organic monomers were polymerized in the presence of a ligand. The resulting polymer stores the ligand shape after the ligand is removed by solvent extraction. Radiolabelled ligand binding assays using this imprinted polymer were compared in performance to standard immunoassays.

OBSERVATIONS

Polymers were prepared using methacrylic acid as the functional monomer and ethylene glycol dimethacrylate for cross-linking. Theophylline (a bronchodilatator) or diazepam (a tranquilizer) were used for imprinting during polymerization. Scatchard plot analysis of binding data revealed multiple dissociation constants, suggesting a heterogenous population of binding sites. Cross-reactivity to major metabolites was found for 3-methylxanthine in the theophylline polymer and for several benzodiazepine derivatives in the diazepam primed polymer but in percentages not higher than known for typical antibodies.

The inhibition of radiolabeled (^{3}H) ligand binding to the polymer is inversely related to the concentration of drug present in the sample. This principle was utilized for a new drug assay. Measurements of known standard drug dilutions were reliable (variation coefficient $< 6.5\%$) and in good and linear agreement to the results of enzyme-multiplied immunoassays as a control (correlation coefficient of 0.98).

CHEMTRACTS—BIOCHEMISTRY AND MOLECULAR BIOLOGY 4:75–76 (1993)
 CCC 1045-2680/93/020075-02$04.00

COMMENTARY ON THE RESEARCH

Mosbach's group started to investigate the idea of molecular imprinting more than 10 years ago.[1] However, even in 1985 the method could only be applied for the separation of racemic mixtures (e.g., D-phenylalanine and L-phenylalanine ester, separation factor $\alpha = 1.3$ in acetonitrile).[2] One interesting application involved the resolution of racemic drugs like β-blockers.[3] Similar results were obtained by other groups.[4,5]

In the present work the principle of molecular imprinting has been put to work ingeniously as a measuring tool in a competitive binding assay. Not only is the shape of the ligand preserved in the polymer, but it is actually turned into a highly specific measuring device. Apart from some cross-reactivity, the specificity obtained is sufficient to distinguish between very similar drugs; for instance, xanthine and theophylline (1,3-dimethylxanthine). Furthermore, the concentration range of sensitivity is comparable to that of antibodies.

The new technique obviously offers a chemical alternative to antibodies. The higher stability of polymers is a potential advantage for this method, in particular if they are used for repeated measurements such as in clinical laboratory assays or in process control. A drawback is the requirement of suitable interaction points in the imprinting ligand. So far the imprints have only been successful for small molecules, though studies for proteins and DNA are underway.

A somewhat opposite strategy to molecular imprinting involves molecular container compounds where the incarcerand is actively chosen by the chemist to provide a suitable cavity for the guest molecule.[6] In this technique the guest molecule can only be protected against a hostile environment; for example, a drug can be placed in a hydrophobic cavity to pass body barriers. Nonetheless, release at a place of desired action remains a formidable task. Furthermore, quantitative measurements are not possible.

Even more significant than measurement applications is the discovery of an independent and completely new mode to recreate a recognition environment for ligands. The method is fast and cheap, and the imprinted polymers are sufficiently stable to be reused. The elimination of the use of antibody producing laboratory animals is attractive. A direct and exciting use could well involve studies of the interaction of drugs with artificial receptors created by molecular imprinting. The results from such facile experiments are likely to yield a better understanding of the specificity of actual molecular recognition phenomena. Current hypotheses and models can be tested quickly. The utility should be especially poignant when three-dimensional receptor structures are hard to come by.

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