Seminar Outline

- Background information
  - What are antibodies?
  - What are catalytic antibodies?
- Catalytic antibodies used in therapeutics
  - Transition-state analogue approach
    - Antibody catalyzed cocaine degradation
  - Hapten-substrate approach
    - Oxidative degradation of nicotine using catalytic antibodies
  - Reactive immunization
    - Using catalytic antibodies to activate prodrug
- Conclusion
Important Terms

- **Antibody**: An immunoglobulin protein produced by the immune system in response to the invasion of a foreign substance.

- **Hapten**: A small molecule that reacts with a specific antibody but cannot induce the formation of antibodies unless it is bound to a carrier protein.

- **Antigen**: Any substance that elicits an immune response when introduced into an animal.

- **IgG**: The most common immunoglobulin, which is distributed between the blood and extravascular fluid. The IgG is used in the formation of catalytic antibodies.

Structure of Antibodies

Antibodies vs. Enzymes

- High affinity and specific binding
- Stabilization of transition state vs. ground state


http://bio.winona.edu/berg/308s04/Lec-note/chap6.htm
Catalytic Antibodies

- Catalytic antibodies utilize
  - Antibody specificity
  - Enzyme’s catalytic power
Methods of Forming Catalytic Antibodies

Transition-state Analogue Approach

Hapten-Substrate Approach

Reactive Immunization Approach

Catalytic Antibodies
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What is Cocaine?

- An alkaloid found in the leaves of *Erythroxylon coca*

- Blocks removal of dopamine from a synapse in the reward pathway of the central nervous system

Previous Method to Treat Cocaine Addiction

- This method depletes and inactivates available antibodies

Degradation of Cocaine

- The hydrolysis of cocaine’s benzoyl ester yields two biologically inactive products.
- This transformation is an attractive approach to destroy cocaine prior to its absorption into the brain.

\[ (-)-\text{Cocaine} \rightarrow \text{Ecgonine Methyl Ester} + \text{Benzoic Acid} \]

Transition-State Analogue Approach to the Formation of Catalytic Antibodies

Transition-state Analogue Approach

Basic transformation with a predictable transition state

Hapten must be stable and mimic structural and electronic properties

Epitope must be large enough to bind well with antibodies

The hapten elicits antibodies which stabilize the transition-state of the desired reaction

Catalytic Antibodies
Transition-State Analogue Approach

- Previous work indicated transition-state analogue using the phosphonate monoester, yielded artificial esterases with the greatest activity.

\[
\begin{align*}
\text{(-)-Cocaine} & \quad \text{Transition State} \quad \text{Ecgonine Methyl Ester} + \\
\text{Benzoic Acid} & \\
\text{Phosphonate Mono-Ester} & \quad \text{Transition State Analog}
\end{align*}
\]

Importance of Using a Linker

- Allows for exposure of the epitope to antibody
- Stimulate immune response
- Linker variations
  - Tether site
  - Length
Formation of Transition State Analogue

Coupling Transition-State Analogue to a Carrier Protein

Other Synthesized Transition-State Analogues

Transition State Analogue 1

Transition State Analogue 2

Transition State Analogue 3

How Catalytic Antibodies are Synthesized

http://www.scripps.edu/~yunfeng/personal/researchweb.html
Formation of Monoclonal Antibodies

- The half-life of regular IgG antibodies could not withstand testing-immortalize and culture

Monoclonal Antibody Production

http://biology.kenyon.edu/courses/biol114/Chap08/Chapter_08b.html
Formation of Active Cocaine Degrading Antibodies

Results of Cocaine Degradation Using 15A10

- LD90 (16 mg/kg) of cocaine was administered to rats previously injected with catalytic antibody 15A10.

Results of Cocaine Degradation Using 15A10

Conclusions for Cocaine Degrading Antibody 15A10

- Efficacy
  - $K_m = 220 \mu M$
  - $K_{cat} = 2.3 \text{ min}^{-1}$

- Improvements
  - Linker length
  - Butyrylcholinesterase studies
  - Site-directed mutagenesis
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Facts about Nicotine

- Nicotine is the most widely addictive drug in the world
  - Using nicotine gum and nicotine patches to quit smoking afforded less than 20% success rates

- Nicotine binds to nicotinic cholinergic receptors in the mesolimbic dopamine system


Previous Work on Nicotine Addiction

- Nicotine gum/patches
- Nicotine vaccine

Oxidative Degradation of Nicotine

- Six main metabolites of nicotine, many are formed from P-450 oxidation

Hapten-Substrate Approach

Does not adhere to transition-state stabilization approach

Commonly used when there is an external source of energy

Used to investigate interaction of energy with a substrate within the confines of an antibody

The hapten mimics the substrate to elicit antibodies

Catalytic Antibodies
Hapten-Substrate Approach for Nicotine Degradation

Inject mouse with antigen which mimics Nicotine

Antibody-nicotine mimic hapten complex

New elicited antibodies

New elicited antibody

+ light, oxygen

Riboflavin

Nicotine degradation products

= Nicotine mimic hapten bound to a carrier protein

= Nicotine

Requirements for Nicotine Hapten

- (S)-configuration stereochemistry
- Similar structural properties
- Sufficient alkyl linker length, \(~12 \text{ Å}\), to expose all structural features

Nicotine

NIC hapten 3

Synthesis of Hapten 3

Coupling Hapten 3 to a Carrier Protein

Hapten 3

Carrier protein
KLH or BSA
Coupling ratio:
19:1 BSA

The elicited monoclonal antibodies were screened against nicotine and irradiated with UV light in the presence of riboflavin.

Preliminary Results

- No catalysis was observed

Conclusion
- Antibodies might be binding too strongly to substrate
- Design hapten that binds weaker

Synthesis and Reasoning Behind Hapten 4

- Substrate binding by antibodies to hapten 4 would not be as tight as hapten 3

Antibodies vs. Ozonolysis

- The two most proficient monoclonal antibodies, TD1-36H10 and TD1-10E8 were tested vs. ozonolysis

Results of Nicotine Degradation

- After 6 hr of white light irradiation in the presence of either antibody, no detectable nicotine remained.

Further Results of Nicotine Degradation

- There was a 10-fold rate enhancement vs. uncatalyzed reaction

Conclusions on Nicotine-Degradation Using Catalytic Antibodies

- Antibodies with modest affinity may be more efficient
- Efficacy
  - Requires 20 µM antibody for 200 µM of nicotine
- Practical applications
  - Nicotine mimic metabolites
  - Biologically active products
  - In vivo systems
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- **Conclusion**
Prodrug Activation

- Administration of a drug in its non-toxic form (prodrug), which is enzymatically converted into its active form

Catalytic Antibodies and Their Use in Prodrug Activation

- Catalytic antibodies can access reactions that are not catalyzed by endogenous enzymes

Reactive Immunization Approach

Immunization with a chemical reaction rather than an inert chemical

The antibody interacts with the substrate that shares chemical reactivity with the antigen used to induce it

This approach allows for the use of more diverse substrates

A reactive hapten can be covalently bound in the binding pocket of a specific antibody

Catalytic Antibodies
Selecting a Hapten for Aldolase Antibody

- Appropriate reactivity
- Requires a mechanism-based “trap” in the active site of the antibody
- Ability to form the enamine
- Provide appropriate binding sites for intermolecular reaction

\[
\begin{align*}
\text{Hapten 1} & \\
\end{align*}
\]
Formation of Hapten-Antibody Complex-Trapping Lysine

Formation of Catalytic Aldolase Antibody

Inject mouse with hapten 1 coupled to a carrier protein

Immune response

Elicited Antibodies

Monoclonal Antibodies

38C2 or 33F12

Antibodies are screened for the formation of the enaminone intermediate

\[ \lambda = 316 \text{ nm} \]

Mechanism Using Catalytic Aldolase Antibody

Diversity of the 38C2 Catalytic Antibody

- More than 100 different aldol additions or condensations have been formed by a single catalyst

Prodrug Activation by 38C2 via a Tandem Retro-Aldol-Retro-Michael Reaction

Etoposide

- Etoposide is an antitumor drug used in the treatment of the early stages of pediatric neuroblastoma
- Current survival rates of stage 4 neuroblastoma are quite poor

Activation of Prodrug 6 to Etoposide

Prodrug 6

Ab38C2

Etoposide

Prodrug 6 Activation Results

- Comparison in growth inhibition activity of prodrug 6 in the absence and presence of 38C2

Prodrug 6 Activation Results

- The effect of 38C2-mediated prodrug 6 activation

Activation of Doxorubicin and Camptothecin Prodrugs by 38C2

A New Type of Prodrug Activation—Enediynes

- Biologically active enediynes cleave DNA
- Benzenoid diradical abstracts hydrogen atoms from the DNA backbone

Dynemicin A

Neocarzinostatin chromophore

Activation of Endiyne Prodrugs Under Basic Conditions

Pathway of Activation of Prodrugs of Dynemicin Analogs

Results Using 38C2 to Activate Dynemicin

Other Approaches for Prodrug Delivery

- ADEPT-Antibody-directed enzyme prodrug therapy

Other Approaches to Activate Prodrugs

- **ADAPT**: Antibody-directed abzyme prodrug therapy
  - Use of catalytic antibody instead of enzyme in drug activation
    - Transformations which cannot be performed by enzymes
    - Activity of catalytic antibodies is lower
Conclusions on Aldolase Prodrug Activation

- Catalyze transformations not available to enzymes
- Efficacy
  - $K_m = 54 \, \mu M$
  - $K_{cat} = 4.4 \times 10^{-3} \, \text{min}^{-1}$
- Consequences
  - Substrate selectivity
  - Localization
Final Thoughts

- Different approaches can be employed to synthesize catalytic antibodies
  - Basic transformations-Transition-state analogue approach
  - External energy source required-Hapten-substrate approach
  - Diversity in substrates-Reactive immunization

- Improvements need to be made before catalytic antibodies can be used in a clinical setting
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