Preclinical Toxicology and Pharmacokinetics of Anti-Infective Agents

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‘Tis now the very witching time of night, when churchyards yawn and hell itself breathes out contagion to this world.

--William Shakespeare, *Hamlet*
Worldwide Major Causes of Death

Challenges to Battling Infectious Agents

- Viruses are frequently "hypermutable," i.e., they frequently mutate into variant strains, rendering vaccines useless.
- Bacteria and parasites develop resistance to antibiotics faster than we can develop new ones.
- Many anti-infectives have significant toxic side effects.
- There are many cultural, religious, educational and political issues that get in the way.
- Ability to do genetic modification of pathogens (e.g., biowarfare weapons).
- The war against infectious disease will never be won; we can only hope to win individual battles.
The biggest challenge... Lack of Funds

- Malaria, Chagas disease and TB don’t affect western nations (“diseases of poor people”)
- Sexual transmission of HIV is largely a problem in sub-Saharan Africa
- Significant perception that HIV, hepatitis, and STDs are “lifestyle choice” diseases
- Liability to companies developing vaccines is substantial
- Biodefense therapeutics will (hopefully) never be sold or used
- Recent moves by developing nations to invalidate US drug patents
- Profit margin is minimal, cost of development is great
The New Anti-Infective Paradigm

✧ **National Institutes of Health**
  • >$4B/year on anti-infective research, most from Natl. Institute of Allergy & Infectious Diseases
  • Numerous drug discovery grant programs
  • Large CRO infrastructure available for support

✧ **Military/Homeland Security**
  • Bioshield: clinical research, drug stockpiling

✧ **The World Health Organization**

✧ **Private Foundations**
  • Global Alliance for TB Drugs
  • Gates Foundation
  • International Partnership for Microbicides
  • Other private donors
Growth in NIAID vs NCI

Funding ($B)

1999 2000 2001 2002 2003

NIAID
NCI
Bioterrorism Funding at NIAID

- 2001: $0.00
- 2002: $400.00
- 2003: $2,000.00

Funding (M)

$0.00 $400.00 $800.00 $1,200.00 $1,600.00 $2,000.00

2001 2002 2003
Major Categories of Infectious Diseases of Interest to NIAID

- **Tropical Diseases**
  - Malaria, tuberculosis, Chagas disease

- **Sexually Transmitted Diseases**
  - HIV, Hepatitis B, Herpes, human papilloma virus (HPV)
  - Chlamydia, Trichomoniasis, syphilis, others

- **Other Viral Diseases**
  - HIV, Ebola, Hepatitis A-E, Hanta virus, West Nile, SARS

- **Biowarfare Agents**
  - Anthrax, plague, Tularemia, smallpox, monkey pox, etc.

- *The Big Five*:
  - HIV, malaria, TB, STDs, bioagents
NIAID Support for Therapeutic Development

- **Screening contracts**
  - Submit your drugs; get them tested for free against broad range of pathogens

- **Synthesis contract**
  - Custom synthesis and bulk GMP synthesis

- **Clinical Manufacturing**

- **Analytical Chemistry**

- **Preclinical Safety and Pharmacokinetics (SRI)**
  - Pilot studies (*in vitro* and *in vivo*)
  - IND-directed tox
  - Pharmacokinetics
  - Infected animal models
Typical IND Requirements of Anti-Infective Therapeutics

- **Toxicity in one rodent and one non-rodent species**
  - Route/formulation same as in proposed clinical trials
  - Duration at least as long as proposed clinical exposure

- **Pharmacokinetics, two species**
  - Pharmacokinetics (time course after dosing)
  - Oral bioavailability (if an oral drug)
  - Tissue distribution (if systemic absorption of non-systemic drug)

- **Genotoxicity**
  - Ames test, mouse lymphoma mutagenesis, mouse bone marrow micronucleus
IND Requirements (Optional)

- **Local irritation** (if appropriate)
  - Vaginal/rectal/dermal irritation
  - Local Lymph Node Assay for skin sensitization

- **Metabolite identification** (if metabolites found)

- **Protein binding** (if binding is suspected)

- **Comparative metabolism**
  - human vs. rodent hepatocytes/microsomes
  - need determined case-by-case

- **Drug-drug interactions** (if administered with other drugs)

- **Immunotoxicology**
  - Required for drugs given to immune-compromised patients or where evidence of immune suppression seen in tox studies
  - Required for *all* drugs in Europe
IND Requirements (cont.)

- All safety studies conducted under Good Laboratory Practice regulations (GLP)
- Pilot and dose-range finding studies not required to be done under GLP
- Pharmacokinetics/metabolism studies not required to be done under GLP

- This does not include CMC section, clinical manufacturing, IND preparation, clinical investigator brochure, etc.
Malaria

- Infection by *Plasmodium falciparum*, a parasite that infects red blood cells
- ~500M new infections a year; ~2-3M people a year die of it
- 90% of deaths in children under age of 5
- Chloroquine, mefloquine, halofantrine currently are primary drugs for treatment
- Most strains are becoming drug resistant; death rate is expected to climb dramatically
- Malaria is a disease of poor people in underdeveloped countries only
Requirements of New Malaria Drugs

- Effective against drug-resistant strains of malaria
- Need to maintain relatively high blood levels
- Must be administered orally
- Must be stable in warm climates
- Must be inexpensive to produce
- May be used for prophylaxis; therefore, toxicity must be minimal
Degeneration of Cardiac Muscle in Rats by Chloroquine

Chloroquine Diphosphate

$$\text{Cl} \quad \text{N} \quad \text{N(CH}_2\text{CH}_3)_2 \quad \text{CH}_3$$
AQ-13

- AQ-13 is an analog of chloroquine synthesized at Tulane University.
- Effective against drug-resistant strains of malaria because they can’t pump it out.
- Preclinical data developed by SRI:
  - single- and repeat-dose toxicity studies (rats and mice; primates done elsewhere)
  - pharmacokinetics (blood levels of drug)
  - genetic toxicology studies
  - stability of drug in capsules
- No significant cardiac toxicity or mutagenicity
Rats were administered AQ-13 for 7 days using the same treatment regimen (loading dose + daily maintenance doses) that will be used in human patients. Blood samples were collected at selected time points and blood levels of the drug were determined using HPLC.
Use of Laboratory Animals

- Use of laboratory animals requires Animal Care and Use Committee (ACUC) approval for all experiments
- Animal use regulated by USDA, OLAW, AAALAC
- There are tighter rules for treatment of animals than for treatment of human staff
- 416 rats, 108 mice and 40 Cynomolgus macaques died bringing AQ-13 to the clinic
- Expected number of lives saved over the next 10 years: 30M; 27M children under the age of 5
"The single biggest threat to man's continued dominance on the planet is the virus."

--Joshua Lederberg
Some Viral Diseases

♦ **Hepatitis C**
  - 4M Americans infected; 30,000 new cases/yr
  - over 85% of cases develop into chronic hepatitis

♦ **Filoviruses: Ebola/Marburg**
  - produce hemorrhagic fever; survival <10%
  - currently rare, and limited to regions of Africa and Asia
  - potential as a bioterrorist weapon

♦ **AIDS**
  - 42M people currently infected; 5M new cases in 2002
  - 25M people have died; 3.1M deaths in 2002
  - 70% of cases are in sub-Saharan Africa
  - 85% of patients in clinical trials have developed resistance to at least one drug in multi-drug regimens
I would never stoop to using \text{SEX} to get your attention
“Love is a sickness full of woe, all remedies refusing”
---Samuel Daniel [1615]

“If you love, you will suffer”
---Agatha Christie [1977]
New STD Infections Each Year in the U.S.

HIV Sex Myths Debunked

♦ Myth: Most HIV infections are the result of homosexual activity and IV drug abuse
  • Reality: Over 70% of worldwide HIV infections are considered to be due to heterosexual activity

♦ Myth: 50% of all prostitutes are HIV positive and they are the major source of HIV infections worldwide
  • Reality: Except for southern Africa, HIV rate among prostitutes is about the same as the population at large (<2%)

♦ Myth: Use of spermicides during intercourse helps kill pathogens such as HIV
  • Reality: New evidence suggests that spermicides such as nonoxynol-9 actually increase the risk of HIV infection due to increased vaginal irritation
Vaginal Microbicides

♦ Gels, foams or creams that women can apply before intercourse to prevent transmission of STDs
♦ “Woman-controlled prevention”
♦ Requirements of a good vaginal microbicide
  - Must kill all pathogens
  - Must not harm normal vaginal bacteria (“Lactobacillus sparing”)
  - Must not produce vaginal irritation
  - Must not be spermicidal
  - Must be inexpensive to manufacture
  - Must be easy to use, colorless, smell nice, look pleasing, feel comfortable, taste good, and have no oral toxicity
Studies on Vaginal Microbicides

♦ **Vaginal Irritation**
  - usually conducted in rabbit
  - daily administration (1 ml) for 10 days
  - microscopic evaluation of vaginal epithelium
Erosion of Vaginal Epithelium by Nonoxynol-9
Studies on Vaginal Microbicides

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  • microscopic evaluation of vaginal epithelium

♦ Sperm Motility
  • used to confirm that material is non-spermicidal
Rat sperm in culture
Effect of Various Drugs on Sperm Motility

![Graph showing the effect of various drugs on sperm motility. The x-axis represents concentration (µg/mL) ranging from 0.1 to 5000, and the y-axis represents motility (% control) ranging from 0 to 180. Different drugs are represented by different lines: Nonoxynol-9 (orange), Boric Acid (red), Benzalkonium Chloride (light green), Miconazole (yellow), and AZT (coral). The graph illustrates how each drug affects sperm motility at various concentrations.]
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♦ Vaginal Absorption
  • systemic absorption from the vagina can be significant
  • distribution of microbicide formulation within the vagina should be uniform (i.e., coat the entire inside of vagina)
Rabbit Vagina 10 min after administration of KY Jelly with Methylene Blue dye
Fluorescein-labeled KY Jelly in rabbit vagina (2 hr post-treatment)

Urinary bladder of the same animal
### Tissue Distribution of Lamivudine (LAM) and Ganciclovir (GCY) in Rat and Rabbit After Vaginal Administration

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Rat LAM</th>
<th>Rat GCY</th>
<th>Rabbit LAM</th>
<th>Rabbit GCY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vagina</td>
<td>10.1%</td>
<td>8.4%</td>
<td>0.1%</td>
<td>0.1%</td>
</tr>
<tr>
<td>Lrg Intest.</td>
<td>11.2%</td>
<td>32.2%</td>
<td>9.5%</td>
<td>10.2%</td>
</tr>
<tr>
<td>Sml Intest</td>
<td>15.6%</td>
<td>27.4%</td>
<td>0.5%</td>
<td>0.5%</td>
</tr>
<tr>
<td>Bladder</td>
<td>0%</td>
<td>0%</td>
<td>19.0%</td>
<td>12.6%</td>
</tr>
<tr>
<td>Blood</td>
<td>0.5%</td>
<td>0.2%</td>
<td>0.06%</td>
<td>0.02%</td>
</tr>
</tbody>
</table>
Treatment of Candidate Biowarfare Pathogens

♦ Old Drugs for Old Diseases
  • Primary issue is stockpiling adequate quantities
    – e.g., smallpox vaccines, Cipro for anthrax

♦ Old Drugs for New Diseases
  • Drugs already approved, but not for new indications
    – e.g., Cipro is only approved drug for anthrax, but we are pretty sure others work (doxycycline, levofloxacin)
    – Impossible to do clinical trials (where would the patients come from?)
    – FDA is accepting labeling based solely on animal efficacy data
    – Many old drugs (e.g., gentamicin) have been around since long before GLP toxicology studies required
Biowarfare Pathogens (cont.)

- **New Drugs for New Diseases**
  - Large efforts (grants, private, other) to identify new chemical entities
  - Small molecule, human polyclonal antibodies, antimicrobial peptides, etc.
  - Absolutely no way to make money doing this, so effort is exclusively funded by federal government
  - Accelerated screening programs have recently been put in place through NIAID for *in vitro* and *in vivo* models
Conclusions

♦ There will be no “cure” for many infectious diseases, only temporary victories.
♦ Bioterrorism concerns have led to accelerated development of therapeutics
♦ The product development cycle must accelerate if we are to keep up with drug resistance
♦ The only funding will come from govt and non-profits
♦ Though not discussed, everything I said today about therapeutics also applies to vaccines