IgE-mediated anaphylaxis to drugs: true and fake antigens

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Conflict of interest statement
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Understanding IgE mediated anaphylaxis to drugs:

50yr old man;
Knee operation;
Anaphylaxis to i.v. cefuroxim within minutes; pos. SPT and BAT

32 yr old female;
Acute generalized urticaria & bronchospasm (min) after i.v. Sulfamethoxazole /Trimethoprim (Bactrim™); pos. SPT and BAT to Bactrim™
Immune system and small molecules: it`s all about drug binding to proteins: how and where

• **Hapten**: Haptens are relatively small molecules that can bind by covalent means to a large carrier, which makes them able to elicit an immune response

• **Carrier**: mostly a protein, e.g. human serum albumin (HSA)

• **Covalent bonds**: A covalent bond, also called a molecular bond, is a chemical bond that involves the sharing of electron pairs between atoms; very stable (-)

• **Non-covalent (=molecular) interactions** rely on electrostatic interactions, ion pairs, hydrogen pairs ... Even though they are weak individually, cumulatively the energies of molecular interactions are significant (Kd: $10^{-5}$ to $10^{-6}$ M)
Drugs bind to proteins.......

- Drugs interact («bind») to proteins constantly: **non-covalent bindings**
  - Labile
  - Reversible
  - fast
  - **Ignored** by the immune system

- All drugs, including hapten-drugs as well

- Hapten-drugs are drugs able to bind to proteins by **covalent bonds** («adducts»)
  - Stable
  - Not-reversible
  - slow
  - **New antigen** for immune system

- Few drugs (e.g. beta-lactams)
Non-covalent binding is the dominant type of inter-molecular force in supra-molecular chemistry. These non-covalent interactions include - ionic bonds, - hydrophobic interactions, - hydrogen bonds, - van der Waals forces and dipole–dipole bonds. Drugs – also those able to bind by covalent bonds – bind first via non covalent bonds to proteins.
STEP 1  
(fast)  
non-covalent binding

STEP 2  
(slow)  
covalent bond ANTIGEN

Amoxicillin  

AA lysine residue 190 of HSA:  
LDELRDEGKASSAK

Amoxicilloyl  

lysine with open beta-lactam ring

M I N U T E S

L I D E N T I C A L

H O U R S
Beta-lactams (penicillins, cephalosporins, piperacillin...) are chemically reactive molecules (haptens), which conjugate spontaneously to the carrier molecule (hapten-carrier complex).

**E.g.: First**, Beta-lactam antibiotics (as amoxicillin ①) bind via noncovalent interactions to certain regions of the protein (e.g. albumin) ②. This initial and fast interaction positions the drug favorably to facilitate binding to available lysines: **Then** ③, by opening the beta-lactam ring (amoxicilloyl), the beta-lactam binds covalently to lysins (e.g. pos 190, 432, 525, 541, ...)

Non-covalent interactions occur in high amounts & precedes covalent interaction and are very fast!

Meng Ji, 2017
The formation of covalent bonds at pH 7.4 between beta-lactams and carrier protein is SLOW. It takes a few hours (mass-spectrometry).

The role of haptenation in penicillin and cephalosporin allergy, Sherin Vareeckal-Joseph, PhD thesis, Univ of SA, Adelaide, Australia
Hapten-Dogma:

Drugs are too small to elicit immune reactions per se.

Only if the drug binds by **covalent bonds** to a larger protein, they represent an own antigen (drug-protein «adduct»).

**Non-covalent drug protein bindings** occur all the time; these complexes are **ignored** by the immune system as they are labile, reversible and not forming a stable, own antigen.
Anaphylaxis to a beta-lactam (cefuroxim)

Anaphylaxis (cardiac arrest) to i.v. cefuroxim (perioperative, knee operation) within minutes;
pos. SPT
pos. BAT

\[ \text{IgE} \quad \]

How is it possible that clinic (anaphylaxis), skin tests, BAT occur so rapidly (3-15min), if formation of covalent bonds, presumably required for reaction, last hours????
IgE mediated reactions (e.g. anaphylaxis) to **inert** drugs (not haptens)

Acute generalized urticaria & brochospasm (min) after i.v. **Sulfamethoxazole** /Trimethoprim (Bactrim\textsuperscript{R}); pos. SPT and direct BAT to Bactrim\textsuperscript{R}
The transformation of SMX to it`s reactive compound requires metabolism and oxidation (SMX-NO)!

**LIVER**

Cyp2C9, CYP2C8 → sulfamethoxazole hydroxylamine

**periphery**

R→NH SO NH OH → nitroso sulfamethoxazole

> 10hrs to transform SMX to a hapten

SMX needs **metabolism** (liver) to generate SMX-NHOH **>10hrs**!
Pat K.-Sch. 1977: urticaria & bronchospasm after Bactrim monotherapy (Sulfamethoxazole /Trimetoprim)

If one detects a positive skin test after 15min – *it must be due to SMX itself*
Hapten-Dogma:

Drugs are too small to elicit immune reactions per se

Only if the drug binds by covalent bonds to a larger protein, they represent an own antigen (drug-protein «adduct»)

Non-covalent drug protein bindings occurs all the time;
these transient complexes are ignored by the immune system as they are labile, reversible and not forming a stable, own antigen
Hypothesis:

non-covalent drug protein complexes can elicit effector functions like MC-degranulation in already IgE-sensitized individuals.
**COVALENT (-) drug-protein adduct** after hours

**TRUE:** Stable, needed for initiation of IgE

**TRUE:** Albumin

**drug**

**NON-COVALENT (··) drug protein binding** immediate

**FAKE:** Mimics the covalent drug protein complex; **No** induction of IgE

**FAKE:** Albumin

**drug**

Some non-covalent drug protein complexes can be stable enough to interact and cross-link surface bound (FcεRI) IgE. These «fake antigens» are formed immediately and in high concentrations: anaphylaxis.
IgE-mediated Hypersensitivity

**Sensitization**

- **days**
- **no symptoms**

**Re-exposure**

- **minutes**
- Urticaria, anaphylaxis, etc.

**1.** Covalent hapten-protein adduct

**2.** Th2 cell

**3.** Mast cell unresponsive

**4.** IgE cross-linking by non-covalent drug-carrier complex (fake antigen) and massive MC degranulation

**Dendritic cell**

**Naive T cell**

**B cell**

**Plasma cell**

**Interleukin-4**

**IgE antibodies**
Acute reactions due to «fake antigen»

These fake antigens (immediately formed, high amounts) explain:

1. **Anaphylaxis** to iv (or oral) drugs, which can occur <5min after injection, first signs start <1min....

2. **Positive skin prick tests** (within 15min) to drug-carrier complexes (amoxicillin, cefuroxim, RCM, ........) *before* stable drug-carrier complexes can be formed

3. **Positive SPT/i.d. to drugs** (15min) like SMX, which need **metabolism** to generate reactive metabolites (SMX-NHOH/SMX-NO, >10hrs). The IgE was induced to the metabolite SMX-NO and is cross-reacting with SMX

4. The positive **in vitro BAT** to drugs, which occur fast, without in vitro metabolism (cefuroxim, SMX, PPI...)
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Anaphylaxis to drugs: Overcoming mast cell unresponsiveness by fake antigens

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Abstract
Our understanding of IgE-mediated drug allergy relies on the hapten concept, which is well established in inducing adaptive reactions of the immune system to small molecules like drugs. The role of hapten-carrier adducts in re-challenge reactions leading to clinically relevant drug allergy is less clear. Reduced clinical sensitivity could...

Why is drug induced anaphylaxis so severe and fast?

1. Because the drug binds by covalent bonds to the everywhere available carrier proteins to form a «true» antigen
2. Because the drug binds by non-covalent bindings to the carrier proteins, which happen to imitate a true antigen (drug-protein adduct) and is able to cross-link drug specific IgE
3. Because a minute amount of drug itself is sufficient to stimulate and cross-link IgE (without carrier)
Why is drug induced anaphylaxis so severe and fast?

1. Because the drug binds by covalent bonds fast to the everywhere available carrier proteins to form a «true» antigen
   - false, It is not so fast....

2. Because the drug binds by non-covalent bindings immediately to the many carrier proteins, which happen to imitate a true antigen (drug-protein adduct) and are able to cross-link drug specific IgE
   - correct, It is fast, and some are affine and sufficiently stable

3. Because a minute amount of drug itself is sufficient to stimulate and cross-link IgE (without carrier)
   - false, You need a protein to cross-link....

4. Drug metabolism is very fast and reactive metabolites are immediately available
   - false, metabolism needs hours...
Novel pathway of IgE-mediated drug allergy

- Covalent hapten-protein adduct
- Non-covalent drug-carrier complex

Naive T cell → Th2 → B cell

Days

MC unresponsiveness

Minutes

Fake antigen causes MC degranulation

No symptoms

IgE-mediated drug allergy

Symptoms