MEDICAL-PHARMACEUTICAL BIOTECHNOLOGY

TOXICOLOGY MODULE

4 CFU + 1 CFU Laboratory

Prof. Ernesto Fedele (fedele@difar.unige.it; 010-3532659)

Prof. Massimo Grilli (grilli@difar.unige.it; 010-3532021)

INTRODUCTION TO TOXICOLOGY

Toxicology: branch of science that investigates the nature, structure, and effects of harmful chemicals (xenobiotics) on living beings. It is also concerned with toxic effects caused by endogenous molecules.

Toxicology identifies the cellular, molecular and biochemical mechanisms of action responsible for toxic effects and assesses the probability of their occurrence following exposure to xenobiotics.

Xenobiotic: any natural or synthetic chemical entity alien to organisms (e.g. drugs, industrial chemicals, pesticides, heavy metals, natural toxins, etc.).
Among different xenobiotics, we can distinguish:
Toxin: a toxic substance that is produced by a biological system (animals, plants, fungi, bacteria).
Toxicant/Toxic agent: a toxic substance that is produced by or is a by-product of anthropogenic activities.

We can identify general and specialized areas of Toxicology.

GENERAL AREAS OF TOXICOLOGY

Descriptive Toxicology
This area deals with the development of appropriate toxicity tests to provide information for safety evaluation of xenobiotics and for their regulation.
Tests are performed on laboratory animals and are designed to provide predictive information on risks to humans and environment.

Mechanistic Toxicology (Molecular)
It concerns the identification of cellular, molecular and biochemical mechanisms by which xenobiotics exert their toxic effects.

This area plays a key role for:
a) development of appropriate toxicological tests with translational value for risk assessment. As a matter of fact, the knowledge of the different mechanims of toxicity is instrumental for setting up appropriate tests that can evaluate the toxicity of a given xenobiotic.
b) development of effective therapies for intoxications. The same is true in this case. If you do not known the mechanism by which a xenobiotic mediates toxic effects, you will not be able to effectively treat an intoxicated person.
c) design of alternative chemical entities with a safer toxicological profile. In many cases, the knowledge of the structure-activity relationships can help to synthesize other molecules with a more favorable safety profile.
d) increasing knowledge in physiology, biochemistry, cell biology, pharmacology, etc.

Indeed, in many cases we have discovered the molecular actors of different cell functions by studying the mechanisms of toxicity of xenobiotics. For example, we now know how exocytosis of neurotransmitters works due to the studies on the molecular mechanisms of toxicity by clostridium tetani and botulinum that are responsible of tetanus and botulism, respectively.

Regulatory Toxicology

On the basis of data provided by descriptive and mechanistic toxicology, this branch decides whether or not a xenobiotic poses a sufficiently low risk to be marketed.
In EU, the main regulatory agencies are ECHA (European Chemicals Agency) and EMA (European Medicine Agency). In USA, FDA (Food and Drug Administration), EPA (Environmental Protection Agency). In Italy, Ministero della Sanità, Istituto Superiore di Sanità, Agenzia Italiana del Farmaco, Ministero dell'Ambiente.

Toxicogenomics

This field of science combines toxicology with genomic technologies to integrate specific alterations in gene and protein expression with functional responses of particular cells and tissues.

Nanotoxicology

This is a rather new field of toxicology that deals with the study of the toxicity of nanoparticles (< 100 nm), given their increasing use in many fields of science.

SPECIALIZED AREAS OF TOXICOLOGY

Foresinc Toxicology

It is a hybrid of analytic chemistry and toxicology, it deals with the medico-legal aspects of the toxic effects and it is essential in establishing the cause of death in post-mortem investigations.

Clinical Toxicology

It mainly concerns the diagnosis of pathologies caused by or associated with the exposure to a toxic agent, and the development of appropriate therapeutic strategies to treat poisonings.

Environmental Toxicology

It focuses on the impact of chemical pollution of the environment on human and non human organisms.

GENERAL PRICIPLES OF TOXICOLOGY

A poison can be defined as any chemical (as it is or as a product of biotransformation) capable of producing a toxic response in a biological entity by damaging its functions or by producing death.

Such a definition is not useful because every known chemical has the potential to cause damage or death if present in sufficient amount in the organism.

In the XVI century, it was Paracelsus, considered the father of modern toxicology, the scientist who enunciated the first principle of toxicology:

"Omnia venenum sunt: nec sine veneno quicquam existit. Dosis sola facit, ut venenum non fit (All things are poison: nothing exists that is not poison. Solely the dose determines that it is not a poison)

Philippus Aureolus Theophrastus Bombastus von Hohenheim
Paracelsus (1493-1541)

Therefore, the dosage is the first determinant for toxicity.

ABSORBED DOSE

In general, the toxic effect is dose-dependent. Under the same conditions, the dose determines the residence time of a chemical in the organism, its duration of action and the development of toxicity.

However, it is not the only factor that influences toxicity. In fact, we have also to consider:

A) Routes of exposure
B) Duration of exposure
C) Frequency of exposure
D) Elimination rate

ROUTES OF EXPOSURE

The major routes by which xenobiotics enter the body are:
a) Gastrointestinal tract (ingestion)
b) Lungs (inhalation)
c) Skin (percutaneous)

However, there are other possibile routes of exposure to toxicants/toxins such as subcutaneous, intradermal, intramuscular, intravenous (e.g. animal bites/scratches, drug administration).

Exposure can be classified as:
1) Accidental (toxic agents in food chain, water, air, etc.)
2) Occupational (during the performance of a job)
3) Voluntary (drug addiction, suicide)

In general, the route of exposure influences the magnitude and the rapidity of the toxic effect.
Descending order of effectiveness:
Intravenous > inhalation > subcutanous > intramuscular > intradermal > oral > dermal.

One important parameter to take into account is the detoxication function of the liver. Therefore, if a toxicant is detoxified by the liver, it is expected to be less toxic following oral exposure (first-pass effect) than by any other route.

N.B. The liver has also toxication functions.

DURATION AND FREQUENCY OF EXPOSURE

In the case of human exposure to toxic agents, duration and frequency of exposure cannot be rigorously defined. In this case, exposure is classified as:

Acute: a single episode related to the exposure to a xenobiotic

Subchronic: repeated exposure over several weeks or some months

Chronic: repeated exposure for many months or years

In the case of toxicity tests on laboratory animals, these parameters are rigorously defined by the regulatory authorities.
Duration and frequency of exposure is classified as:

- *Acute:* single administration of a toxicant. In some cases, there can be repeated administrations but within a period of max. 24 hours.
- *Subacute:* repeated exposure for max. 1 month.
- *Subchronic:* repeated exposure for 1 to 3 months.
- *Chronic:* repeated exposure for more than 3 months.

Different routes of exposure are tested.

Another factor to consider together with exposure frequency is the *elimination rate*.

<u>Toxicokinetic considerations</u>

A chemical producing severe effects with a single dose may have no effects if the same total dose is given with a frequency lower than its elimination rate (half life).
On the contrary, a chemical that does not produce toxic effects after a single dose may produce toxic effects if the exposure frequency is higher than its elimination rate, thus favouring accumulation in the body.
Moreover, there are also some physiopathological considerations to be made. In fact, the repair mechanisms of the target tissue/organ are also important for toxicity in relation with exposure frequency. In fact, if the interval between exposures is sufficient for a complete repair of tissue damage, then it can be hypothesised that it will not produce any long-term toxicity, though acute toxicity may occur.

The picture is even more complex since an acute exposure can cause immediate but also delayed toxic effects that can be similar or different from those caused by chronic exposure.

Similarly, chronic exposure can also produce toxic effect after each exposure, in addition to long-term effects.

To further complicate this field of science, toxic effects are also influenced by:

A) Species differences
B) Ethnic differences
C) Individual differences

And can have different characteristics:
- ❖ Immediate and delayed toxicity
- ❖ Reversible and irreversible toxicity
- ❖ Local and systemic toxicity

Finally, we have also to consider that in many cases toxicity can be induced not by a single agent but by the interactions of different chemicals.