SECTION

APPLICATION OF PATHOMORPHOLOGICAL METHODS IN TOXICITY STUDIES IN ANIMALS

by
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The primary objectives of toxicological testing are to determine the effects of chemicals on biological systems and to obtain data on the dose-response characteristics of the chemicals. Selection of the most appropriate test procedures, coupled with strict adherence to acceptable experimental practice and astute observation, is of paramount importance in experimental toxicology.

Several types of toxicity testing procedures have been developed for acute, subacute and chronic studies. From the viewpoint of pathology, the variety of toxicity tests leads to many different approaches, and skill and flexibility in working procedure seem to be far more important than is a strict schedule.

Pathomorphological examination is often the cornerstone of experimental toxicology, with decisions regarding the safety of a compound based on this evidence. This fact charges the pathologist with the special responsibility to carry out the studies in the way most likely to produce optimum results (1-3).

The basic ideas of morphological methods as applied in toxicology are the following:

- provide information on the cause of animal death in the course of experiment;
- estimate the degree of irritating effects of chemicals depending on the routes of administration (injury of the alimentary canal, skin, peritoneum or respiratory tract and lungs);
- define the degree of development and morphological characteristic of injuries in organs affected to distinguish reversible functional and morphological changes from severe irreversible injury; and
- define the dose-response relationship of morphological changes.

The pathomorphological investigation is based on post-mortem examination, microscopic examination and ultrastructural studies.

Gross Pathology

The important stages of pathomorphological methods are the post-mortem examination, selection of tissues and the methods of tissue preservation. In the majority of laboratories, gross pathology is usually performed by experienced technicians or biologists under the guidance of a pathologist. Personnel should follow a certain scheme of necropsy technique and should be able to recognize abnormalities. Abnormalities found and weight of organs required must be recorded on a necropsy card. Dead animals should be examined as soon as possible, unless precluded by cannibalism or deep autolysis. An inadequate post-mortem macroscopic examination cannot be replaced by microscopic study. On the other hand, a well-performed post-mortem examination may provide optimum information for microscopic study and may, in certain cases, facilitate more selective microscopic examination.

Microscopic Examination

The selection of organs and tissues to be collected for fixation is determined by the type of toxicity experiment and by the clinical and biochemical data. Sufficient material should be collected to prevent the necessity of having to repeat a certain experiment, but strict rules for selection of tissues are generally impossible to provide.

Acute toxicity study

Data from the literature and from different laboratories, as well as our personal experience, point out the necessity for performing pathomorphological investigation in acute toxicity tests, even when the compound tested is of very low toxicity based on the value of LD_{50} (or LC_{50}) alone. High nonlethal doses of some compounds of relatively low toxicity may provoke significant and probably irreversible tissue injury.

In acute toxicity studies, a routine morphological procedure is followed at the Institute of Occupational Medicine in Lodz. Animals are killed after 2 or 14 days of single exposure on at least two dose (or concentration) levels: high, in the range 0.5 - 1 LD_{50} (or LC_{50}) and low, in the range 0.1-0.2 - LD_{50} (or LC_{50}). For histological examination, the following organs are selected: brain, heart, lungs, spleen, kidneys, adrenals, liver, pancreas, stomach, duodenum, colon, urinary bladder and gonads (4). Routine histopathology requires microscopic examination of the lungs, liver, kidneys, testes and alimentary canal (or skin and trachea, depending on the routes of administration).

Subacute and chronic toxicity studies

When subacute or chronic studies are performed, the number of organs and tissues examined is usually increased. In addition to the organs listed above, other organs and tissues which must be examined are the entire alimentary and respiratory tracts, all endocrinal glands, spinal cord, peripheral nerves, skeletal muscles, aorta, lymph nodes, femur including the joint, skin, bone marrow, eyes, exorbital lacrimal gland and all other tissues with abnormalities found during post-mortem examination.

The selection of tissues in sensitization or irritation studies depends on the method of exposure. In studies of eye irritation, gross examination of the eye and conjunctivae seems adequate, while in dermal irritation or sensitization studies, histological examination of the skin is advisable. Comparison of macroscopic and microscopic findings should be done in these cases. In our point of view, gross pathology and microscopic examination of inner organs are not required in routine sensitization and irritation studies.

Fixation of tissues

Proper fixation of tissues seems to be very important in the laboratory procedure; sometimes it may even be the crucial point of proper tissue elaboration. The application of an inappropriate preservation technique or an unsuitable fixative solution may destroy the experiment or may provide misleading findings. On the other hand, application of the "universal" fixative may provide the

possibility of introducing special histological techniques as well as the use of histochemistry or electron microscopy. The most common "universal" fixing solutions are 10% neutral Baker's formalin (formol-calcium) and 10% formalin in a phosphorus buffer. In some circumstances, tissues stored in buffered formalin solution may be used for electron microscope examination (5).

The three techniques used in tissue preservation are immersion, inflation and perfusion. Immersion is most commonly used in routine histological investigation, but in some circumstances may not result in satisfactory preservation. Inflation is most often applied to fixation of the lungs in order to prevent the common artefact resembling pulmonary collapse. The best preservation of tissues is obtained by whole body perfusion. The use of perfusion fixation cannot be avoided when tissues of the central or peripheral nervous system are to be examined. Fixation of the brain by immersion leads to a number of artifacts which are indistinguishable from degenerative changes. The whole body perfusion or perfusion of isolated organs, such as the liver or kidneys, should be performed when election microscopy studies are undertaken.

Preparation of tissues

Tissue elaboration should be performed by well-trained staff. In particular, the trimming of tissues should be carried out by personnel with a basic knowledge of pathology. In some laboratories, this important step is done by technicians, but at the Institute of Occupational Medicine in Lodz, pathologists or biologists trained in pathology are responsible for this stage of tissue elaboration. For certain organs, special trimming procedures are needed.

Tissue processing (dehydration and embedding in paraffin wax) may be done by hand or, in the case of a great number of specimens, by using automatic tissue processing machines (Histokinette). Tissue sectioning can be performed at a thickness of 4-5 µm, and sections can be routinely stained with hematoxylin, eosin or a comparable routine stain.

Strict rules of tissue elaboration are impossible to give, but in our experience, a standardization of laboratory work should be kept at all stages of processing.

Electron Microscopy

Ultrastructural studies, if introduced to toxicological investigation, are usually limited to a selected field and are secondary to the use of light microscopy on the selected tissue or organ. In our laboratory at the Institute of Occupational Medicine in Lodz they have been introduced most often to discover signs of injuries which are below the diagnostic means of light microscopy due to low exposure level or to other experimental circumstances. The development of analytical electron microscopy using principles of X-ray analysis makes the obtaining of qualitative and even quantitative chemical information on selected morphological structures possible.

Quantitative Analysis

Quantitative analysis of morphological changes seems important in toxicological investigations, especially when the compound is administered to animals in several different dose or concentration levels. In ordinary practice, however, semi-quantitative methods are used more often, some of which develop in the course of an individual study (6-8). A new tool in quantitative morphology studies is a system based on the analysis of TV image (Quantimet 720 - Imanco, Micro-Videomat - Opton). This system provides measurements of area, perimeter, length, density or volume size in selected images formed by optical, transmission electron and scanning electron microscopes.

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