The Role of the Study Director in Nonclinical Studies

Pharmaceuticals, Chemicals, Medical Devices,and Pesticides

EDITOR
William J. Brock

ASSOCIATE EDITORS
Barbara Mounho
Lijie Fu

WILEY

Table of Contents

List of Tables

List of Illustrations

added to the appropriate wells. (d) The cells are exposed to the test material for 1 hour. Then, the plates designated for phototoxicity end point are exposed to 5-J/cm^2 UVA light for 50 ± 2 minutes at room temperature. The plates designated for cytotoxicity end point are kept in the dark at room temperature for 50 ± 2 minutes. (e) The plates are decanted and the cells are washed once with 125 µL of prewarmed HBSS or EBSS. Culture medium (100 µL) is added to all wells and the cells are incubated in standard culture conditions for 24 ± 1 hours. (f) The culture media is decanted and 100 µL of the neutral red-containing media are added to each cell-containing well. The plates are incubated in standard culture conditions for 3 ± 0.1 hours. (g) The media is decanted and each well is rinsed once with 250 µL of HBSS or EBSS. Neutral red solvent (100 µL) is added to each well for a minimum of 20 minutes incubation at room temperature with shaking. (h) The absorbance of the neutral red at 540 or 550 nm is measured with a plate reader. EBSS, Earle's balanced salt solution; HBSS, Hank's balanced salt solution; NRU, neutral red uptake; UVA, ultraviolet A. See color insert.

FIGURE 10.3. General Outline of the BCOP Assay. (a) Corneal excision: Upon receipt, defective eyes are discarded. (b) Mounting of corneas and incubation (32 ± 1°C for 1 hour): Chambers are filled with complete MEM (1% FBS and 2 mM L-glutamine). (c) Refeeding and initial opacity reading: Chambers are filled with fresh, complete MEM without phenol red; a baseline opacity is recorded. (d) Control and test material treatment (10 minutes [liquid], 4 hours [solid]): The medium is removed from the anterior chamber and 750 µL of the test or control material is applied to the epithelial surface (four to five corneas per treatment). (e) Rinsing control and test materials: The corneas are

rinsed thrice with complete MEM with phenol red and once with complete MEM without phenol red. (f) Final opacity reading and addition of fluorescein (1.5 hours): 1 mL of a 4 mg/mL fluorescein solution for liquids (top) and 1 mL of a 5 mg/mL fluorescein solution for solids (bottom). (g) Permeability quantification (optical density at 490 nm). BCOP, bovine corneal and opacity permeability; FBS, fetal bovine serum; MEM, minimum essential medium. See color insert.

FIGURE 10.4. General Outline of the SIT Assay and Its Specifications Based on Tissue Model. (a) Tissue receipt: Upon receipt, tissues are incubated first for 1 hour and then overnight (with media change) in standard culture conditions (37 + 1°C in a humidified atmosphere of 5 + 1% CO_2 in air). (b) Tissue treatment: Triplicate tissues are treated topically with control and test materials as specified in the table at the bottom of the figure. (c) Tissue rinsing and (d) posttreatment expression incubation: After exposure, tissues are rinsed and then placed in the incubator at standard culture conditions for an initial posttreatment incubation of 24 ± 1 hours. After the initial posttreatment expression incubation, the tissues are transferred in fresh medium and placed back in the incubator for the remainder of the 42 ± 2 hours posttreatment incubation. (e) MTT reduction: The MTT assay is performed as specified in the table at the bottom of the figure. Individual tissues are placed into wells containing unreduced MTT solution and incubated at standard culture conditions for 3 hours. (f) Isopropanol extraction: The tissues are placed in isopropanol at room temperature for 2 hours to extract the reduced MTT. Extracted MTT is thoroughly mixed and transferred to a 96-well plate. (g) Spectrophotometric quantification: Optical density at 550 nm (OD_{550}) or 570 nm (OD_{570}) is determined using a

article-related or not would require one to know what doses (or exposure) were used in each group. See color insert.

FIGURE 16.4. Bone Marrow, Rat, H&E. These images demonstrate changes in the cellularity of the rat bone marrow at different doses when dosed for 14 days. Compared with the control tissues (0 mg/kg/day), all other groups show reduced or hypocellular bone marrow elements. Additionally, the stroma of the bone marrow changes in the 150 mg/kg/day image from adipose tissue to fibrous connective tissue. *The 150 mg/kg/day dose group was sacrificed after 8 days of dosing due to clinical signs of toxicity. See color insert.

FIGURE 16.5. Submanidublar Lymph Nodes, Rat, H&E. These images are taken at the same magnification of lymphoid tissue collected from rats in a 3-month oral gavage toxicity study. Sporadic variation in this tissue was observed in all dose groups. There were no other test article-related findings in the immune system. Therefore, this finding was considered spontaneous normal variation and a background finding. See color insert.

FIGURE 16.6. Kidney, Rat, H&E. These images are taken from rats in a chronic gavage toxicity study in the kidney near the corticomedullary junction. The control kidney is generally unremarkable. The high-dose kidney has findings of degeneration (dilated tubules filled with proteinaceous fluid) and inflammation (mononuclear cell infiltration) that could be lumped under the finding "chronic progressive nephropathy." See color insert.

FIGURE 16.7. Liver, Rat, H&E. These images are from the liver of two dosed rats in two toxicity studies of varying lengths. The liver in the shorter study has a small number of mononuclear inflammatory cells

accumulating within the parenchyma. The liver in the longer study has a large number of mononuclear inflammatory cells coalescing and additionally forming small granulomas. If these findings were observed at different dosing intervals with the same test article, then alignment of terminology is warranted to indicate that the finding on the left is a more incipient version of the one on the right. See color insert.

FIGURE 16.8. Lung Diagrams. These diagrams provide an example of the importance of macroscopic data to the microscopic examination. In these drawings, necropsy findings are represented by dark areas that have various distributions. The horizontal bar represents the location of the protocol tissue that would be routinely examined in the microscopic examination. The lungs with diffuse and multifocal findings would have these findings end up on the protocol tissue; the lung with the regional finding would require the macroscopic data to ensure that the necropsy finding was examined microscopically.

FIGURE 16.9. Rat Tissue Blocking. Each image in this collection represents a single tissue cassette with a collection of similar fixed and trimmed tissues covering all protocol tissues from a single animal in a toxicity study. The histotechnologist and the pathologist both need to verify that all tissues are accounted for and subsequently examined during the microscopic portion of the study. Note that the bone sections will require decalcification post fixation. Mand., mandibular; LN, lymph node. See color insert.

FIGURE 16.10. Kidney, Rat, Periodic Acid–Schiff (PAS) stain. These images were taken from a toxicity study in which the kidney was identified as a target organ after examination of the H&E slides. The PAS special stain was subsequently added to the examination to better

characterize changes in the tubular epithelium basement membrane. The arrows in the high-dose image points to thickened basement membranes. See color insert.

FIGURE 16.11. Spleen, Rat, Immunohistochemistry. These images demonstrate common chromagen-based immunohistochemistry. The immunoreactive target stains brown after which the nonreactive tissue is counterstained with hematoxylin. The spleen on the left is stained with an anti-CD79a antibody to demonstrate the B cell-rich follicles, while the spleen on the right is stained with an anti-CD68 antibody, which shows a macrophage-rich red pulp. See color insert.

FIGURE 16.12. Liver, Rat, H&E. The liver image from the high-dose group individual has a number of morphological changes to the tissue that warrant description in the microscopic data. One prominent finding in the high-dose liver are the clear spaces of vacuoles in the cytoplasm of the hepatocytes. The preferred descriptive (objective) term for this is hepatocellular vacuolation. A morphological diagnosis that connotes the pathological process might be lipidosis, an etiological diagnosis that connotes the cause might be toxic hepatopathy, and a disease diagnosis used to describe a clinical condition might be drug-induced liver injury. Only the descriptive term of hepatocellular vacuolation should be used for the microscopic data in a toxicology study. See color insert.

FIGURE 16.13. Determining the Reversibility of a Test Article-Related Finding. The primary evidence of reversibility for a test article-related finding is a reduction in the severity/incidence of the finding after an appropriate wash-out period. The diagram indicates