Abstracts

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Abstract: A survey of QWBA, using current methodologies was given followed by a discussion of possible future advances in using this technique.

Keywords: Tissue Distribution; QWBA; ADME

QWBA — CURRENT METHODOLOGIES AND FUTURE ADVANCEMENTS POSITIONING OF IMAGE

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Abstract: Pharmaceutical companies conduct tissue distribution studies to provide development project teams with preclinical and clinical drug safety data supporting IND/NDA submissions. Tissue distribution is performed in accordance with ICH Guidelines as well as pharmacological evaluations of a new chemical entity, including Absorption, Distribution, Metabolism and Excretion (ADME) in the intact animal. The accurate identification of tissue on the images is critical for the conduct of these studies by QWBA. The conventional method of QWBA has some factors that may inhibit the analysis of derived radioactivity in tissues, such as the capability of viewing and analyzing tissues with low concentrations of radioactivity on the autoradioluminograms. Recent advancements have included overlay techniques and 3D modeling which allows the most accurate analysis available.

Keywords: Tissue Distribution; 3D Model; Overlay; Quantitative Whole Body Autoradiography; Histology; ADME; Imaging

Introduction: Tissue distribution falls under the second of the four pharmacological evaluation categories of ADME (Absorption, Distribution, Metabolism, and Excretion). In accordance with ICH and GLP guidelines, this research provides drug safety data of a new chemical entity using radiolabeled test articles in intact animals. These data are primarily collected for looking at exposure levels and the kinetics of drug exposure to tissues.

There are two general methods of tissue distribution: Quantitative Tissue Dissection (QTD) and Quantitative Whole Body Autoradiography. QTD is the combustion or solubilization of tissues followed by liquid scintillation counting for determination of concentration. QWBA is the acquisition of autoradioluminograms from thin sections for the determination of radioconcentration and localization of radioactivity within a discrete region of tissue. One advantage of the QWBA method is the prevention of contamination from surrounding tissue or matrices as seen with QTD.

Current QWBA methods of analysis, on the other hand, have their own set of challenges. The conventional QWBA method of analyzing tissues is to select the tissues of interest on a 2D autoradioluminogram. However, the analysis may be difficult if the tissues of interest have radioactivity concentrations that are similar to adjacent tissues or the levels of radioactivity concentrations are very low (i.e. similar to background). This has led to inconsistencies within the data because many tissues were either selected incorrectly or the concentrations of the tissues were determined to be below limits of quantitation (BLQ) or not detected (ND), when in fact, they have a discernable radioactivity concentration value.

The confidence in tissue distribution data has increased with new advancements to the procedures and technology of QWBA. New methodologies include overlay techniques and 3D model visual aids. These innovational methods have pushed the envelope of the QWBA world and increased the potential of this relatively new science to influence areas beyond the boundaries of the ADME arena.

Methodology: The method of overlaying has eliminated the guesswork from the analysis of the autoradioluminograms (i.e., image displaying radioactivity). Using appropriate analysis software, the autoradioluminogram is overlaid with a corresponding optical image of the block surface or acquired section. The optical image can be registered with the autoradioluminogram by aligning the two images together using registration markers and adjusting the images appropriately to match exactly. Tools within the software can be used to transition between the two images. Optical images can be used to identify tissues on the autoradioluminograms in which the lower levels of radioactivity concentrations are indistinguishable from the background or surrounding tissue. The autoradioluminogram can be manipulated within the software to enhance the boundaries of the radioactivity. This will further demonstrate the precision of the overlay and the actual radioactivity concentrations of each tissue.

The autoradioluminogram can also be overlaid with histological images. Within the appropriate analysis software, the same procedure can be used to overlay the histological image with the autoradioluminogram. Transitioning between the two images can provide histological verification of site specific radioactivity (HVSSR) in tissues down to near cellular level.

While the overlay method provides an area of interest with the most accurate measures of radioactivity concentration, the data are still only being viewed in a 2D plane. The new method of 3D modeling gives a much closer look at the details of tissues, as well as the distribution of radioactivity to discrete regions within the tissues. Appropriate software compiles the surface block images. The tools within the software are used to segment and characterize the different areas of interest. After all the images are segmented, the software can then be used to render the 3D reconstruction model of the images.

Once the 3D model of the surface block images is created, autoradioluminograms, which have been previously overlaid with their corresponding surface block images, can be compiled along with all of the surface block images. The autoradioluminograms will replace the corresponding surface block images. The same procedure used to segment the areas of interest on the surface block images is applied to the autoradioluminograms, but the areas of interest are selected based on the various concentrations of radioactivity.

New advancements are currently under development to extrapolate the radioactivity throughout the 3D model which will lead to the reconstruction of just the radioactivity in a 3D model.

Results: It can be concluded that the data collected using the overlay method is more accurate than the conventional method of autoradiography. The information provided is much more detailed with regards to the tissue structures and location in order to determine the concentrations of radioactivity. The overlay method additionally provides information about the quality of the tissue. The optical images provide useful information for a toxicologist or pathologist to determine the condition of a tissue (i.e. necrotic tissue) by examining the actual color and structure of each tissue.

The 3D model, together with the autoradioluminograms, shows uniformity and homogeneity of the radioactivity. It has helped to mark out different concentrations at different depths of tissue. In 3D, the tissue of interest can be analyzed on the outside and inside; making is possible to pinpoint the localization of radioactivity in every tissue, not just within the perimeter of the tissue, but also in specific sites within the tissue. Tools within the analysis software have been used to obtain exact dimensions of tissues, which assist with mapping out discrete regions in an organ and measuring exposure of derived radioactivity to smaller structures.

Discussion: The new methodology of overlaying and 3D modeling band together to advance the data that exceeds the limits of conventional QWBA methods. The data can help to support other areas of research such as toxicology, neurology, and oncology.

Having the surface block images alone gives more detailed information about the actual tissue characteristics. Since the surface block images are of the actual sample and not a digitized representation, the optical image captures the actual color of the tissue which makes them a visible reference. This helps to identify tissues that may be deformed or show signs of disease. The optical image can confirm or deny that there is an issue with a particular tissue when the autoradioluminogram is unclear. The surface block images and the radioactive images together further add to the wealth of information available. For example, there could be a question as to whether the drug affects the fetal brain. From looking at the radioactivity alone, it can be seen that there is minimal activity around the area of the fetus; however the exact locations of the various levels of radioactivity are hard to distinguish. Having the surface block image overlaid with the radioactive image can clearly demonstrate, without a doubt, that the radioactivity did not get into the fetal brain.

The 3D model is not a digitized representation of tissue like an MRI or CAT scan image would produce. The 3D model is a reconstruction of the actual tissue using optical images which is a much more detailed and more accurate method for acquiring the necessary data. These advancements produce better data and information which in turn produce higher quality drugs and safer treatments for humans.