

Quantitative Whole-Body Autoradiography

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INTRODUCTION

Distribution studies with new radiolabeled test compounds in animals form an important part in the preclinical drug development programs of many companies. The data obtained from these studies often form the basis for the assessment of human organ and tissue exposure to the test compound and the removal of residues/metabolites from those organs and tissues after drug administration. The data may also provide pharmacokinetic information with regard to any specific binding to, or selective affinity for, a tissue or organ by the test compound or its metabolites. The data may provide some indication as to the potential toxicological and pharmacological sites of action. In addition, much of the data derived from these studies are submitted to various regulatory authorities as part of the process of providing new safe drugs. This regulatory process has changed over the years and this will be discussed later.

The techniques most commonly used for these distribution studies are quantitative whole-body autoradiography (QWBA) and quantitative tissue distribution [QTD—by dissection and liquid scintillation counting (LSC)]. In this paper it is my intention to very briefly review quantitative whole-body autoradiography and to provide a background against which the other papers presented in this issue may be viewed.

QWBA

Whole-body autoradiography in its present form has developed from Ullberg's original method (1954) and in the 46 years since then, many scientists have defined and refined the materials and methods that we use.

The autoradiographic phenomenon was first noted by Niepce de St. Victor (1867) and subsequently reevaluated by Becquerel (1896a,b,c,d). Subsequent experiments using the autoradiographic process by London (1904a,b,c), Lacassagne and Lattes (1929), Lomholt (1930), and Leblond (1943) led Ullberg (1954) to publish his elegant method for the study of *soluble* xenobiotics *in vivo*. It was realized, at that time, that all the potential data present in the autoradiograms were not being used. Ullberg himself, in association with Berlin (1963), made many of the early attempts to quantify

the concentration of radioactivity detected. Because of the materials in use (X-ray film) only semiquantitative results could be achieved and QTD remained the major method of obtaining quantitative data. Cross *et al.* (1974) and Longshaw and Fowler (1977, 1978) defined some of the parameters affecting the accuracy of autoradiographic data by whole-body autoradiography, and Cross *et al.* (1974) produced a practical densitometer and calibration source for quantifying autoradiograms. Other scientists were assessing the sensitivity of X-ray film to β radiation (Coe, 1982; Franklin, 1980, 1983, 1985) while Geary *et al.* (1985) suggested a different isotope standards scale for use in QWBA albeit with the emphasis on use in microautoradiography and receptor binding studies. Alternative methods were proposed to overcome some of the pitfalls of the Cross method, for example, the stoichiometric analysis of the exposed X-ray film's silver content (Stevens, 1980). Section thickness was also thought to be an important factor in the process of quantifying autoradiograms, and Williams (1987) established the infinite thickness of a section containing carbon-14. Schweitzer *et al.* (1987) proposed an elegant but simple quantitation method using a calibration scale made from blood samples "spiked" with radioactivity.

The continuing interest in quantifying autoradiograms led to the commercial development of a number of software programs that could be used to analyze images captured or derived from film-based autoradiograms (Seescan, UK; Loates Associates, U.S.A.; Imaging Research Inc., Canada), while other scientists devised their own (Goochee *et al.*, 1983; D'Argy *et al.*, 1990). Subject to the standardization of certain experimental parameters close correlation between data obtained from QWBA and QTD could be achieved (Coe and Attwood, 1995; Zane *et al.*, 1996; Steinke, 1997; Lordi *et al.*, 1999). During the late 1980s and early 1990s the phosphor storage imaging plate became available together with its scanner and it quickly became apparent that this was a significant advance. This detection medium was shown to be more linear, to be more sensitive, and to have a wider dynamic range than any previous detector for analyzing the radioactive content of whole-body sections. Early work by

Sonoda *et al.* (1983), Miyahara (1989), Shigematsu (1992), Mori and Hamaoka (1994), Motoji *et al.* (1995), and Poitchoiba *et al.* (1995) quickly established some of the parameters that needed to be standardized or investigated. A collaborative study performed by over 20 Japanese companies (Forum, 1993; Tanaka, 1994) indicated that QWBA could usefully replace many of the more traditional methods of obtaining the data for ADME studies, especially the quantitative analysis of metabolites by TLC. At about the same time other forms of direct nuclear counting of the radioactivity in whole-body cryosections were becoming commercially available (Biospace, Canberra Packard) and there has been a review of these by Schweitzer (1995).

REGULATORY AFFAIRS

Over a similar period of time, there was a perception among scientists that each of the many regulatory authorities throughout the world had different experimental requirements as to the data to be submitted. The World Health Organization (WHO) started an investigation into the costs of supplying such different sets of data and eventually began a review of the process. Internationally recognized experts reviewed the methods, guidelines, and data required for acceptance of the data by the regulatory authorities. These were published in a series of globally agreed guidelines covering all aspects of the data required for submissions and to date these have gone from International Committee for Harmonisation (ICH) 1 to ICH 4. Of most interest to autoradiographers is ICH 4 signed in Tokyo in 1998, as this is the document that defines how our absorption, distribution, metabolism, and excretion studies may be performed. It is interesting to note that there are no designated experimental methods for any given data collection. Any method may be used, *provided that it has been validated*. The Japanese guidelines are quite clear and I quote “. . . 6. Newer methods, such as quantitative whole-body autoradiography, can be used to estimate the whole-body distribution of test substances, if they are properly validated. Such methods can also be used to determine the concentration of test substances in organs/tissues. It is recommended to elucidate the chemical forms derived from the test substance in those organs and tissues in which high concentrations or accumulations are recognized and in those which may be a target of the toxic or pharmacological effects of the test substance. . .” (my emphasis).

The “Guidelines for Non-Clinical Pharmacokinetic Studies” were published by the Ministry of Health and Welfare (MHW), Japan, on the July 29, 1998, and presented at the 5th ISSX Meeting by Dr. Ohno. Slide number 23 of Dr. Ohno’s presentation contained the following information and I quote:

“Figure on the correlation of the tissue distribution data by quantitative whole-body autoradiography and those by classical method using liquid scintillation counting.

We will accept the use of new technology when the validity of the new methods was indicated. Quantitative whole-body autoradiography was one of them. Use of quantitative whole-body autoradiography:

MHW will accept radioluminography for quantitative tissue distribution studies as an alternative to classical method using liquid scintillation counting, if the method is properly validated” (my emphasis).

There is growing evidence, both factual and anecdotal, that the regulatory authorities are accepting data derived from QWBA in ADME and human densitometry submissions in place of the more traditionally derived data (McCracken *et al.*, 1999; Ellender, 1999).

As noted earlier, an intercompany collaborative study had been conducted in Japan and the resultant data were accepted by the regulatory authorities as being comparable to that derived from more traditional methods. A similar study was performed in Europe, starting in 1993 with the proposal to do the study, the selection of the laboratories taking part, and the experimental parameters to be investigated. In 1994 there were discussions as to how the results would be published, followed in 1995 by a selective poster presentation of some of the results, at the First European Autoradiography Meeting in Edinburgh. At this stage agreement was also reached with the Japanese validation group (via Dr. Shigematsu). Other selective data have been presented at the Society for Whole-Body Autoradiography Meeting in Ann Arbor, Michigan, and at the Second European Autoradiography Meeting in Heidelberg in 1998.

The papers that follow are the in-depth results of that collaborative study and cover such topics as the technical validation of the system, standardization of the WBA method, sensitivity of the phosphor plate and its detection limits, self-absorption, method cross-validation (QWBA vs LSC) use with isotopes other than carbon-14, precision of measurement of tissue concentration (intercompany cross-validation), and a new study design for QWBA. The authors are to be congratulated on their efforts to elucidate the parameters of this relatively new technology and the need for its standardization to ensure that the systems currently in use may be said to be fully validated and acceptable to the regulatory authorities worldwide.

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