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Björn Ekwall, an outstanding Swedish cell toxicologist

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ABSTRACT

Dr. Björn Ekwall (1940–2000) was an outstanding Swedish cell toxicologist who made pioneering contributions to the field of *in vitro* toxicology. In particular, he formulated the so called "basal cytotoxicity concept" (1983) which provided a conceptual basis for the estimation of acute systemic toxicity of chemicals in humans by the use of *in vitro* tests. Björn Ekwall formulated, initiated and, together with a group of dedicated Scandinavian toxicologists, guided the MEIC project (Multicentre Evaluation of *In Vitro* Cytotoxicity Programme, 1989–1999), in which 50 reference chemicals were voluntary tested in 100 laboratories worldwide by 61 different *in vitro* assays. This project was unique because human sub-lethal and lethal blood concentrations were used for a first time as a reference system for the evaluation of predictability of *in vitro* tests for human acute systemic toxicity. The results of MEIC project have shown good correlation between human LC_{50} values (50% lethal concentrations) and IC_{50} values (50% inhibitory concentrations from basal cytotoxicity tests), by the use a battery of three 24-h basal cytotoxicity tests ($R^2 = 0.77$). The MEIC project paved the way to the present validation projects, under EU 6th Framework programme, such as ACuteTox, Sens-it-iv, and ReProTect.

1. Basal cytotoxicity concept

Björn Ekwall was born on June 13, 1940 in Uppsala, Sweden. He had studied medicine at the Uppsala University Medical School, and became medical doctor 1969. In 1980 he defended his PhD thesis at the Department of Anatomy at Uppsala University, under the title: "Combined drug toxicity to HeLa cells in the MIT-24 test system and its relevance to human drug toxicity". His studies included the testing of several chemicals in simple cultured cell test system; and he made a first effort to evaluate the relevance of *in vitro* toxicity for human acute systemic toxicity.

Already during his PhD studies Ekwall got the idea to try to predict human acute systemic toxicity of chemicals by the use of cell-culture tests, instead of the use animal LD_{50}-determinations (Ekwall, 1980a,b). The screening of toxic compounds in mammalian cultured cells led Ekwall to the conclusion, that "Most agents (80%) had a similar toxicity *in vitro* and *in vivo*, suggesting a lethal interference in man with basal functions common to all specialized human tissues as well as cultured cells, i.e., basal cytotoxicity." (Ekwall and Ekwall, 1983). This pioneering hypothesis, designated as the "basal cytotoxicity concept", claimed: "Most human cells have two aspects... One aspect is the structure and functions common to all of them, which may be called the basal cell functions. Another aspect consists of structures and functions typical to each cell, tissue, or organ, which may be called the organ-specific functions. The basal cell functions always support the specific cell functions." (Ekwall and Ekwall, 1983). Further, Ekwall also classified the toxic effect of chemicals in living cells to three categories: (1) basal cytotoxicity (average cell line toxicity) resulting from interference with structures and/or properties essential for cell survival, proliferation and/or functions. These effects can involve integrity of cell membrane and cytoskeleton, metabolism, cell division, synthesis and degradation of cellular components, etc. (2) organ-specific cytotoxicity, which affects organ-specific functions and structures, (3) extracellular toxicity or, in other words, toxicity at the organizational level (Ekwall and Ekwall, 1983; Bernson et al., 1986).

A second hypothesis of Ekwall claimed that "... basal cytotoxicity can be tested with undifferentiated cell lines ...by the use of simple and inexpensive cell-culture tests". He described in several papers that many aspects of acute systemic toxicity in man can be predicted by undifferentiated cells, "...including both continuous and finite cell lines..." (Ekwall and Ekwall, 1983, 1988).

2. MEIC and EDIT programmes (1989–1999)

In 1983 and 1984 Ekwall called two meetings in Uppsala. Cell toxicologists from the Nordic countries were summoned to form the Scandinavian Society for Cell Toxicology (SSCT). Not only did Ekwall take initiative to form the first scientific society in the field

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of *in vitro* toxicology but he also expressed the mission of this society: (1) to arrange annual meetings for toxicologists to discuss and promote the study of effects of chemicals in cellular models; (2) to take on responsibility for performing a large international study based on his hypotheses. Ekwall realized very early the importance of validation of *in vitro* tests, to demonstrate their relevance for the acute systemic toxicity evaluation in humans. He also realized that one single laboratory cannot provide all necessary data; only cooperation of the scientists from several laboratories may help to select the best *in vitro* assays and may give a guarantee of reproducibility of such assays.

A full presentation and aims of the MEIC programme were published 1989 in the MEIC Newsletter by Walum, Chairman of the MEIC committee and Director of the Program, and Ekwall, Editor of the Newsletter and Managing Director of Validation. In this issue, the list of 50 reference chemicals was published for a first time, as well as the aims and rules of the project were described (Walum and Ekwall, 1989). The MEIC Newsletter then appeared four times per year and provided communication between the MEIC committee and laboratories involved in the project.

One of the fundamental ideas of the MEIC programme was that laboratories should volunteer for participation in the study and test the 50 substances in their respective cellular system without any economic compensation and then submit their results to the MEIC committee.

In comparison with other validation studies initiated in 1980s, the MEIC project had some unique features. Evaluation of the relevance of *in vitro* cytotoxicity tests for acute human systemic toxicity was based on correlation of IC_{50} concentration from cytotoxicity tests with the human toxicity data. Ekwall had designed a sub-project, called MEMO, aiming to collect human blood concentrations (in serum and/or plasma), together with the description of the concrete clinical and forensic cases of poisoning. As data sources, poison centers in several countries were used, supplemented with information from textbooks, international journals and databases. To avoid a biased selection, the set of 50 reference chemicals was chosen by the experts of Swedish Poison Information Center. The chemicals with known lethal human doses, sublethal and lethal blood concentrations from clinical and forensic medicine data, time between ingestion and death, target organ/organs, toxic mechanisms of poisoning, as well as LD_{50} values for rodents were chosen. Later, the human data were summarized in a series of 50 MEIC monographs, available at internet (<www.cctoxconsulting.a.se>).

The MEIC project also was aimed to evaluate the relevance of rodent LD_{50} values for human acute lethal dosage, and to compare the predictability of *in vitro* tests with that of animal tests. Another important goal of the project was to build up a battery of *in vitro* tests with the best prediction of human acute toxicity (Bondesson et al., 1989; Ekwall et al., 1991).

The results of the MEIC project have been published in ATLA, between 1996 and 2000, in eight papers (Parts I–VIII). In Part I the methodology of 68 *in vitro* toxicity assays used in different laboratories to test the first 30 reference chemicals was described (Clemedson et al., 1996a), whereas Part II contained results of these 68 *in vitro* assays (Clemedson et al., 1996b). Further, Part III presented the results of additional 16 *in vitro* assays used for testing of the first 30 reference chemicals (Clemedson et al., 1998a); and Part IV presented results from the testing of additional 20 chemicals in 67 assays (Clemedson et al., 1998b). Part V contains a database of human data for all 50 reference chemicals, i.e., oral acute single lethal doses, clinically measured acute lethal serum concentrations and post-mortem serum concentrations, human kinetic data (absorption, time to peak after ingestion of chemical, kinetics, elimination half-time, distribution volume, passage across blood–brain barrier, etc.) (Ekwall et al., 1998a).

Part V also contains human peak blood concentrations (LC_{50}, 50% lethality) at an acute poisoning, estimated from the LC_{50} curves drawn as a geometrical mean of the time-related curves for sub-lethal and lethal poisoning (Ekwall et al., 1998a). This new approach, proposed by Ekwall and approved by MEIC committee, allowed to assess a relevance of *in vitro* assays by comparison of IC_{50} values with human LC_{50} values.

In Parts VI and VII (Ekwall et al., 1998b; Clemedson et al., 2000), a comparison of rat and mouse oral LD_{50} values with human acute lethal doses was done, aiming an evaluation of predictability of human lethal dosage by LD_{50} values. A comparison between IC_{50} values from 61 *in vitro* assays used for testing of 50 reference chemicals, and human lethal peak concentrations demonstrated that both human cell line and primary culture assays gave the best average results (R^2 = 0.64–0.68), whereas mammalian and fish cell lines demonstrated a worse correlation (R^2 = 0.52–0.58). The statistical evaluation of the results has been done by use of linear regression analysis (Parts VI and VII) and by a multivariate partial least square (PLS) modeling with latent variables analysis (Part VIII, Ekwall et al., 2000). The most predictive test battery for estimation of basal toxicity was chosen, containing four endpoints and two exposure times: protein content (24 h), ATP content (24 h), inhibition of elongation of cells (24 h), and pH change (7 days).

Although the MEIC project has shown that a good correlation (around 70%) can be obtained between *in vitro* basal cytotoxicity data and human lethal blood concentrations, some of the reference chemicals (about 14%) were misclassified and represented outliers (Ekwall et al., 1998b). Therefore, after finalizing of MEIC programme, Ekwall started in 1999 a new international multicentre programme called EDIT (Evaluation-guided Development of New *In Vitro* Tests), under the guidance of the Cytotoxicology Laboratory (CTLU) which was founded by Ekwall in 1983, in Uppsala. The purpose of the EDIT programme was to expand the battery of assays obtained in MEIC project by supplementary toxicity assays, e.g. organ-specific assays and toxicokinetic-based assays (Ekwall et al., 1999). Unfortunately, the EDIT project could not be completed because of Björn Ekwall death.

However, both MEIC and EDIT projects contributed to the presently ongoing development of cell based toxicology tests. During last decade new international projects, under EU 6th Framework programme, e.g. ACuteTox, Sens-it-iv and ReProTect, were initiated aiming to develop simple and robust testing strategies for replacement of animal toxicity tests used for regulatory purposes. One such project, ACuteTox (2005–2010) aims to identify factors that can optimize the *in vitro–in vivo* correlation for predicting acute systemic toxicity (www.acutetox.org; Sjöström et al., 2008).

3. Conclusions

The pioneering hypotheses of Björn Ekwall on basal cytotoxicity were important for the rapid progress of *in vitro* toxicology seen today. He gave us a scientific rational for extrapolation of *in vitro* data to the *in vivo* situation: the concept of basal cytotoxicity and the idea that basal cytotoxicity is a very common phenomenon. He provided experimental evidence, both from his own laboratory and through the MEIC results, to substantiate the validity of these hypotheses. He gave us a set of 50 (!) reference chemicals with standardized, well-documented human toxicity data.

The striving of Björn Ekwall to optimize a test strategy for prediction human acute toxicity may be fulfilled by the today's international projects.

To honor the memory of Björn Ekwall and to reward scientists making important contributions in the field of *in vitro* toxicology, by performing *in vitro* test development, mechanistic studies, or validation activities, the SSCT founded 2001 the Björn Ekwall Memorial Foundation (BEMF).

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