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SHORT-TERM TESTS, GENOTOXICITY AND CARCINOGENICITY IN LIGHT OF A MULTIVARIATE STATISTICAL EXPLORATION

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Summary. - *This is a brief overview on a series of studies performed in the Istituto Superiore di Sanità (Italian National Institute of Health), all focusing on the ability of mutagenicity short-term assays of identifying genotoxic agents and predicting carcinogenicity. The analytical tools of such studies were the multivariate data analysis statistical techniques. The overall picture points to three main classes of assays, on the basis of their responses to the chemical agents in the available data bases (mainly the comparative studies). The implications for practical chemical testing and battery design are discussed.*

Riassunto (Saggi a breve termine, genotossicità e cancerogenesi: uno studio statistico). - *Questa presentazione riassume i risultati ottenuti all' Istituto Superiore di Sanità nell' analisi delle prestazioni dei saggi a breve termine di mutagenesi, sia per quanto riguarda l' identificazione di agenti potenzialmente genotossici che la predizione della cancerogenesi. Come strumenti analitici sono state utilizzate le tecniche statistiche conosciute col nome di metodi multivariati di analisi dei dati. L' intera serie delle analisi ha condotto alla ripartizione dei saggi in tre gruppi principali, in base alle loro risposte sperimentali alle sostanze chimiche (come risulta dallo studio dei risultati delle più importanti basi di dati). In questa presentazione sono descritte le implicazioni pratiche per l' uso dei saggi sulle sostanze chimiche e per la costruzione di batterie di tali saggi.*

Introduction

The attention that has been put in the last 15 years on the mutagenicity short-term tests (STT) derives from the fact that they are tools for both scientific investigation and risk assessment. In the latter context, they can play two roles: a) identification of potentially genotoxic agents (this is their more natural role); b) prediction of the carcinogenic properties of chemicals. Point b) derives from two lines of evidence. The first is the somatic mutation theory of cancer, that mantains that mutational events are involved in the development of several kinds of cancer. The second is the empirical recognition that many carcinogens are also mutagens; in the 70's, when Salmonella assay was first used to screen chemicals, the claimed correlation between mutagenicity and carcinogenicity was more than 90%.

The scientific investigation in the area of STTs had to respond to the basic questions: what are the performances (i.e. experimental responses to the chemicals) of the various assays? How correlated are the performances of different genetic end-points, organisms and systems? How much, how well and which assays may be useful for assessing mutagenic and carcinogenic risk? In spite of the huge amount of experimental results produced in this area, such problems have proven to be much more complicated than it was thought 15 years ago.

In order to give an answer to such questions, the large comparative studies in which many systems are tested with the same range of chemicals are of paramount importance. At the Istituto Superiore di Sanità, we have re-evaluated the major genotoxicity data bases; the novelty of our approach is represented by the systematic introduction of multivariate data analysis methods in this field of research.

Methods

These statistical methods are basically different than the classical approaches. They are specifically designed for the description of large data sets, and re-organize the information in a more "readable" form. They enable the description of phenomena about which little is known, and are aimed at searching the typology underlying the data without the use of *a priori* hypotheses. Thus they help construct new theories. The ability to simultaneously treat a large number of variables (multivariate approach) generates global views of large amounts of data and affords

descriptions of complex patterns of relationships that may be lost with the traditional univariate statistical approaches [1].

Conclusions

With the use of such methods, we have re-evaluated the 4 major genotoxicity data bases: 1) International Program for the Evaluation of Short-Term Tests for Carcinogens (IPESTTC), in which 35 STTs (both *in vitro* and *in vivo*) were tested with 42 chemicals belonging to the most relevant chemical classes and including both carcinogens and non carcinogens [2]; 2) International Program for Chemical Safety (IPCS), where many *in vitro* eukaryotic STTs were tested with 8 carcinogens negative in Salmonella [3]; 3) Gene-Tox, which is a collection of peer-reviewed literature data [4]; 4) U.S. National Toxicology Program (NTP), in which the 4 most widely used *in vitro* assays (Salmonella, chromosomal aberrations and SCE in CHO cells, mutation in mouse lymphoma L5178Y cells) were tested with 73 chemicals whose animal carcinogenicity had been previously assessed in well controlled and standardized experiments [5].

Most of our results, together with their theoretical and practical implications, are summarized in [6, 7].

In our first study, we analyzed the IPESTTC data base. The resulting picture pointed to the presence of 3 main groups of assays in terms of homogeneity of response to the chemicals tested. Salmonella was in the central group, and performed in a way similar to that of the most widely used *in vitro* STTs (such as chromosomal aberrations and SCE in CHO cells, mutation assays in mouse lymphoma and V79 and CHO cells, UDS in human fibroblasts, etc.). A number of other *in vitro* assays formed a second group. This was characterized, with respect to the Salmonella group, by a higher sensitivity and lower specificity for the identification of carcinogens and non carcinogens. The third group was composed by the *in vivo* assays, with poorer sensitivity and higher specificity for carcinogens. On the basis of this structure, we demonstrated that a reliable battery of assays for predicting the rodent carcinogenicity should include 3 tests, one for each group. The specifity of *in vivo* assays, and the sensitivity of second group *in vitro* assays (e.g. *Saccharomyces cerevisiae* XV185-14C, Syrian hamster embryo cells transformation assays) were complementary to each other and to Salmonella. On the contrary, the most widely used *in vitro* assays were not complementary to Salmonella for discriminating between carcinogens and non carcinogens. These results, in their general trends, were confirmed by our re-analyses of IPCS and Gene-Tox.

The IPESTTC experimental results had two intrinsic problems. First, the number of chemicals studied was limited (42), and so was their representativity of the "universe" of chemical compounds. Second, the classification as non carcinogens of some IPESTTC chemicals was questionable and scarcely reliable. As a consequence, the conclusions on the relationship between carcinogenicity and mutagenicity are seriously hampered. However, it must be remarked that the picture of the relationships among assay performances is still valid.

In light of the above results, the NTP study (with its well validated protocols, standardized carcinogenicity data, and the vast range of chemical structures studied) can be considered as a powerful magnification lens on a group of assays (the 4 most widely used *in vitro* STTs) previously characterized as a homogeneous class. Our re-analysis of the NTP data has first re-confirmed the pattern of relationships among assays performances put in light in the IPESTTC. It has also confirmed that the 3 other *in vitro* assays (chromosomal aberrations and SCE in CHO cells, and L5178Y mutation) did not complement neither to Salmonella nor to each other for discriminating between carcinogens and non carcinogens; the best battery attained only 61% accuracy, which is a value very similar to the accuracy of Salmonella alone. It must be noted, however, that a positive result in one of these tests is highly predictive of the potential carcinogenicity of a chemical. For example, a chemical positive in Salmonella has 70 to 80% probability of being a carcinogen. The real problem is that a compound negative in Salmonella has about the same probability of being a carcinogen or a non carcinogen. It must be emphasized that even if the 4 *in vitro* STTs do not complement for predicting carcinogenicity, the NTP results pointed to the need of carrying out a range of assays to improve the Salmonella identification of genotoxicity.

As above stated, the NTP data concern 4 STTs that had turned out to be in the same group on the basis of the previous results (IPESTTC, IPCS, gene-tox). In other words, they showed rather homogeneous performances. As far as the two other groups of assays pointed out by IPESTTC are concerned, the cluster of the most sensitive *in vitro* assays has been neglected by the most recent research; as a consequence, the conclusions on them cannot be considered solid enough. The *in vivo* STTs are presently the object of much interest. In this moment, the NTP is testing a number of *in vivo* assays with the compounds previously used with the 4 *in vitro* STTs. These data, when available, will surely add important informations to our knowledge about mutagenicity STTs.

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