Epidémiologie animale et épidémiologie humaine : le cas du chlorure de vinyle monomère

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sous la présidence du Professeur Raymond LATARJET

Final report on VCM studies by Professor Cesare MALTONI

PUBLICATIONS ESSENTIELLES

SOMMAIRE

Vinyl chloride carcinogenicity bioassays (BT project) as an experimental model for risk identification and assessment in environmental and occupational carcinogenesis

Final results of the effects of long-term exposure to a range of 14 doses by inhalation (from 30,000 to 1 ppm) and of 6 doses by ingestion (from 50 mg to 0.03 mg/kg b.w.), on Sprague-Dawley rats (1).

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Part I : GENERAL REMARKS

1) Introduction

First of all we wish to thank Professor DAUDEL and Professor LATARJET for the kind invitation to present an up-to-date report of our studies on Vinyl Chloride (VC) and also to express our pleasure to be once again at the Institut Curie.

A few years ago, we already presented here an up-dating report of the results of our experimental project on the long-term effects of VC.

This project was carried out in our Laboratory of Experimental Carcinogenesis in Bentivoglio (BT), near Bologna (2). It encompasses 17 different experiments, started in sequence from 1971 to 1975, and lasted nearly seven years. Historically, the early results of the first experiments represented the start of a new approach to the problem of environmental and occupational carcinogenesis as they clearly proved the value of experimental bioassays in identifying and assessing the carcinogenic risk for humans.

The present report deals with the final data of the seven basic experiments of the BT project, which were planned to study the effects of long-term treatment with the monomer at different dose levels, both when given by inhalation (five experiments) and ingestion (two experiments).

This is the first official presentation of part of the final outcome of many years of work devoted to a study.

Moreover, what is obviously more important, we are aware of the meaning of the data presented here in deciding the strategies for the control of the effects of VC and in defining the most proper measures of prevention.

Furthermore this occasion provides the opportunity of presenting the VC carcinogenicity project as an experimental model not only for identifying an environmental and occupational oncogenic agent, but also for assessing the level of the risk it represents in relation to doses and in comparison with other agents. The latter goal is, in our view, the future task of the scientific community involved in environmental and occupational carcinogenesis.

2) The strategy for identification and assessment of environmental and occupational risk

It is well known that the onset of cancer in humans largely depends on exposure to environmental and occupational carcinogenic agents.

It is also known that part of these agents are natural and part man-made. The latter may increase in the human environment in number, since the growing progress in synthetic chemistry may lead to the production of new ones.

It is accepted that the major tool in cancer control is the control of environmental cancerogenic potential.

To carry out this strategy the first step is to detect the carcinogenic risks and then to protect mankind.

In general terms, the scientific strategy for detecting carcinogenic risks is different for newly synthetized compounds which, while available in laboratories, are not yet produced and diffused on a large scale, and for agents already widespread in the environment.

In the former early indication of carcinogenicity may discourage their production, if such production no human exposure.

The measures of protection for already diffused agents, whatever their nature (acts, rules, technological improvements, etc...), are the result, not only of the identification of the carcinogens, but also of other factors of a socio-economic and political nature. The final outcome will be the product of the value of risk, benefit, and the possibility and feasibility of measures of technical prevention. The major task of the scientific community in this case will be to provide the bodies involved with all the data which may contribute to the characterization of risk in qualitative and quantitative terms. The responsibility for this task parallels the level of socio-economic impact of the agents studied. Consequently, the scientific approach -both experimental and epidemiological- should grow in level of sophistication and accuracy.

The protocol for characterizing the carcinogenic risk of an agent as it may be visualized nowadays, both in theoretical and feasible terms, relies upon a sequence of different steps through which risk assessment for humans could suitably be reached (Table 1).

3) VC carcinogenicity as a milestone in environmental and occupational carcinogenesis : its history and contributions

The history of VC carcinogenicity (Table 2) is not only important in relation to the compound itself (its production, diffusion and uses), and for the consequent program of prevention, but in our own view, still more important are its contributions, particularly on methodological and scientific grounds, to the general problem of environmental and occupational carcinogenesis, with particular reference to the industrial field.

The direct consequence of the discovery of VC carcinogenicity was probably the greatest effort ever made for controlling the exposure to an industrial carcinogen in the workplace.

Furthermore, all of us are aware how drastically and suddenly the orientation of Public Health in the control of environmental and occupational carcinogenesis has changed since then and how much the work, the efforts and the regulations have since expanded in the field.

However, in a wider perspective, the major outcome of VC carcinogenicity is having provided crucial information for the scientific protocols and methodologies of risk identification and assessment (Table 3), and the basis for future scientific developments and orientations (Table 4).

Part II : BT PROJECT : THE PROTOCOL

1) Planning and experimental factors

The experiments were planned to study the effects of VC administered via different routes, at different concentrations, for various periods of times, by continuous or intermittent treatment, on animals of different species, strain, sex and age.

The compound was always supplied by the same source, and it contained a very low amount impurities (Table 5).

It was administered by four different routes (Table 6).

Fourteen concentrations were studied by inhalation, several of which with different schedules of treatment (Table 7).

Six concentrations were studied by ingestion, and four doses by intraperitoneal injection (Table 8).

Four types of animals were used (Table 9). The animals (apart from the hamsters) were breeds routinely employed in our Laboratory for many years. It should be pointed out that, whatever their use, all the animals of our colony

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undergo periodic examination and complete autopsy, giving us extensive information concerning their pathology.

In the VC project as in any other long-term bioassay, the animals are kept alive until spontaneous death. The plan of the experiments is shown in tables 10-16.

2) Conduct

For the experiments on VC, as well as for any other long-term experimental bioassays performed in our Laboratory, the procedure has been always the same highly standardized and controlled one. In particular, we wish to emphasize the following points in our laboratory standard procedures.

A) Compound

All the supplies of the compound used were examined in order to determine wether they meet the required standards.

B) Concentrations

The concentrations, particularly when the compound was given by inhalation, were controlled by continuous gas chromatographic monitoring.

C) Modalities of treatment

Treatment was always performed by the same people. This is particularly important for gavage, since the animals become accustomed to the same operator.

D) Control of the animals

The control of animal status was performed at least 3 times daily. Every 2 weeks, the animals were submitted to an examination for the detection of any gross change.

E) Weight of the animals

The animals were weighed every 2 weeks during and every 8 weeks after the end of treatment.

F) Autopsy

Full autopsy was performed on each animal. All the different parts of the body were explored, encompassing the central nervous system. Specimens for histology included the brain, Zymbal glands, interscapular brown fat, salivary glands, tongue, lungs, liver, kidneys, adrenals, spleen, pancreas, stomach, intestine, bladder, uterus, gonads, and any other organ with pathological lesions.

G) Histology

Specimens were trimmed in the standard way. Sections were routinely stained with Haematoxylin-Eosin and, when necessary, with special techniques.

H) Histopathological examination

All slides were screened by a junior pathologist and then reviewed by a senior pathologist. The same classification of the lesions was used by all pathologists.

3) Classification of data

All the gross and microscopic observations were classified and coded following our laboratory code (Table 17).

For each animal, an individual final card was finally prepared, which included data on experimental factors, survival, weight at 6, 12, 18 and 24 months, and any gross and microscopic lesion. An example is given in Table 18.

4) Presentation of pathological data

The results of the VC experiments, as well as the ones of any other experiment performed in our laboratory, are always presented with the same types of tables, in the same sequence.

This type of presentation has been made possible by the knowledge of the basic pathology of the animal used which enabled us to make an approximate census of the expected lesions.

Such a procedure permits a quick comparison among the results of different experiments of the same project and the results of projects studying different compounds.

5) Interpretation of the data

The data were submitted to statistical analysis. Although statistical analysis provides an extremely important tool for interpreting the meaning of the results of long-term bioassays, it should be stressed that there may be smaller differences between exposed and control groups which do not reach statistical significance, while these differences could still have a meaning from an oncological point of view (particularly in the case of tumours which are infrequent in the animal colony).

Therefore, the most important data should be commented both in the light of the statistical analysis performed and with a biological approach.

The methodological protocol adopted meets the requirements of the recent Good Laboratory Practices Act.

Its application to all the different projects performed in our laboratory in the last decade provides us with a set of results which may constitute the groundwork for risk assessment on experimental basis.

Part III : BT PROJECT : RESULTS

1) Past results

Past results (Maltoni 1977) may be summarized as follows :

a) VC, when given by inhalation, ingestion and possibly injection, produces tumours at different sites and of different types ;

b) VC correlatec tumours have been found in all the types of animals studied ;

c) the type of tumour response varies, depending on the species, the strain and the sex of the animals used. For some tumours the correlation with VC is established, for others it lacks definite evidence (Table 19) ;

d) the degree of tumour response is affected by age, and is greater in newborn animals ;

e). a clear-cut dose-response is an extremely important factor. In reducing the lenght of treatment there is a sharp fall in tumour onset.

2) Final results of the seven basic experiments

All seven experiments were terminated by the end of 1977. The last two years were devoted to processing and examining all the histological material (which amounted for nearly 100,000 slides), preparing the individual cards for the animals of the study and computing and statistically analysing the data.

The biological results are given in the following tables (Tables 20-103). In Table 104, the incidence and the latency time of liver angiosarcomas, in relation to route and doses, are summarized. The neoplastic lesions, whose relations with VC must, in our opinion, be considered, are listed in Table 105.

They are identified on the basis of one or more of the following parameters :

a) sharply enhanced incidence ;

b) rare or exceptional occurence in the colony of the animal used ;

c) clear correlation with VC treatment in other experiments of the project ;

d) dose-response relationship ;

e) association of precursor lesions.

Attention should be given not only to the tumours singularly considered, but also to their aggregation.

The following data have been statistically analyzed in relation to the treatment in each exposure group and sex :

a) the survival rate ;

b) the weight at 6-month intervals ,

c) the number of tumour-bearing rats ;

d) the incidence of different benign and/or malignant tumours.

A) Survival rate

VC has a clear-cut life shortening effect, which is dose related. A preliminary analysis, performed on experiment BT1, has shown that life shortening is independant of tumour increase. This points to a systematic toxicity and to a possible correlation between toxicity and carcinogenesis.

B) Weight

No effect of dose was observed except at 30,000 ppm by inhalation, when there is a decrease in weight gain.

C) Non-oncological pathological lesions

The incidence of inflammatory and histopathological regressive changes does not seem, in general, to depend on VC treatment. Only in experiments BT9 and BT15 do there seem to be a VC dependent dose-related enhancement of liver regressive changes.

Nor do there seem to be relationship with other changes, such as respiratory epithelium hyperplasia and adenomatous hyperplasia of the pituitary gland.

D) Tumours

Following statistical analysis using the Fisher exact probability test ($p \leq 0,05$) :

a) six of the eight tumours considered appear significantly in excess, at least at one dose level at least in one sex (Table 106) ;

b) total cancer-bearing animals per group were in excess at the doses indicated in Table 107 ;

c) no excess was observed in the number of rats bearing benign tumours.

The relation of liver angiosarcoma, Zymbal gland carcinoma, nephroblastoma and neuroblastoma and forestomach papilloma with VC is also shown by the correspondance analysis.

The Fisher exact probability test at 95 % confidence is, in relation to the above, not «sensitive» enough in our experimental conditions.

Biologically in our opinion the following results, although not statistically significant according to the test used, should be given proper attention :

Extra-hepatic angiosarcomas of different sites : these tumours are observed at a very low incidence dose in untreated Sprague-Dawley rats of our colony. Results of experiments BT1 and particularly BT9, however, strongly suggest a relationship between these tumours and VC exposure. This relationship is supported by the excessive incidence of extra-hepatic vascular tumours in mice treated with VC (BT4).

Hepatomas : few cases of hepatomas have been observed in treated groups, particularly in BT1. This tumour is exceptionnaly rare in our colony of animals, and none have been observed in the control groups of the seven experiments. Moreover the relationship with treatment is supported by the fact that a high incidence on hepatomas has been observed in Sprague-Dawley rats, following neonatal exposure to a high dose for a short period (BT14). In view of their rareness or non-observation in the colony of animals used, for the following tumours it should be stressed that attention should be paid at their onset, also at doses below the ones with significant results according to the statistical test used.

Zymbal gland carcinomas : an excess of these tumours is observed down-to 50 and 25 ppm.

Liver angiosarcomas : this tumour is extremely rare in the colony used (4 cases over several thousand untreated animals). Therefore, one must consider the onset of these tumours as important even at doses not shown by statistical analysis, and particularly below 50 ppm (5 liver angiosarcomas out of 120 animals at 25 ppm, and 1 liver angiosarcoma out of 120 animals at 10 ppm), and at 1 mg/kg b.w. (3 liver angiosarcomas out of 150 animals), and at 0.3 mg/kg b.w. (1 liver angiosarcoma out of 150 animals).

Nephroblastomas : the onset of a few of these tumours, observed after inhalation treatment at concentrations below 100 ppm and in groups treated by ingestion with 50 and 16.65 mg/kg b.w., is not casual in our opinion, given the extreme rarity of these tumours in rats.

Neuroblastomas : such a tumour has never been observed by us, up to present, in the Sprague-Dawley rats used in our Laboratory as control or otherwise treated. Therefore we consider as dependent on treatment the onset of these tumours, even at doses below 10,000 ppm, i.e. 6,000 and 2,500 ppm.

As far as the total malignant tumours are concerned, the excess in treated animals must also be evaluated on the basis of total malignant tumours per group. Such an approach shows a correlation with VC exposure, even at lower doses.

The meaning in oncological terms of the results at the lowest doses may be better evaluated in considering, not singly, but together, the tumours found to be VC dependent (Table 108).

None (or no increase) of these specifically VC related tumours has been observed at doses below 10 ppm and 0.3 mg/kg b.w.

Part IV : CONCLUSIONS

The experimental bioassays are an important tool in risk identification and assessment. However it is not easy to obtain adequate experimental data, and their interpretation may be difficult. Moreover a great deal remains to be done in the experimental field along this line, particularly in looking for animal models with optimum relevance to human extrapolation.

The final goal in fact is still human extrapolation, which can be improved only through available models in which animal and human data can be compared.

To return to VC, epidemiological investigations on occupationally exposed population groups have been made and are being carried out in different parts of the world, particularly in Western Europe and in the USA, with reference to general pathology and neoplasias.

These data are important in order to obtain indications on the effect of VC in humans and to control the extent of

parallelism between animal and human pathology. Furthermore, if these epidemiological investigations provide precise figures on the whole group considered, include figures on the level and lenght of exposure (so as to define homogeneous exposed groups), and collect all possible available data on pathology, this will provide an opportunity, unique at present, to compare animal and human data, both in qualitative and quantitative terms, and to help to find a possible key for extrapolating from animals to humans.

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