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THE METHODOLOGY OF PRIMARY SELECTION OF ANTI-TUMOR ANTIBIOTICS

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Of special importance in the search for anti-tumor antibiotics is the primary selection of their producers. The success of eliciting the active producers of anti-tumor substances depends on the employment of a reliable, technically simple method which offers quick results. At the present time we do not have such a generally accepted method, and various laboratories employ a great variety of tests.

The most reliable results are achieved by testing the anti-tumor effect of investigated substances in animals with experimental tumors. Results thus obtained attest quite conclusively to the therapeutic effect of the investigated preparations. This method is widely used in the study of chemotherapeutic anti-tumor preparations, and, on the basis of data obtained the expediency of transfering them to the clinic for treating patients with malignant tumors is determined.

However, such methods cannot be employed extensively in the study of a very large number of preparations, for instance, in the investigation of anti-tumor antibiotics; here, an immense quantity of culture fluids has to be studied and an excessively large number of animals would be required. Furthermore, in the selection of a procedure, if one should limit oneself to testing the effect of the investigated material on some single tumor, one risks the loss of active preparations ineffective for this particular tumor, but possessing anti-tumor action on other tumors. Besides, the response is slow, often requiring two to three weeks.

It is necessary to note that this method, the most reliable of those available at present, is not without a very substantial defect: not infrequently preparations which

are very active on animals with experimental tumors prove to have little or no anti-tumor effect on human tumors. This difference results from the fact that experimental tumors of animals and humans, while frequently of an analogous morphological structure, differ markedly in their biochemical characteristics and their metabolism. And since anti-tumor preparations function by impairing metabolic links specific for each preparation, it is quite understandable that, while impairing the cellular metabolism of an animal tumor, they may have no harmful effect on the different metabolism of a human tumor.

Therefore, numerous attempts are being made to find simpler methods which would permit the investigation of a large number of culture fluids and make possible a quick decision of whether further study of various culture media is necessary.

The majority of these methods is based on the contact of the culture fluids with tumor cells. They differ from each other in the method of evaluating the anti-tumor action.

In some methods the morphological changes of tumor cells, submitted to the action of the tested material, serve as a criterion of its anti-tumor activity. A representative of these methods is one suggested by Schrak -- live staining with eosin of a suspension of tumor cells which has been in contact with the tested material. The harmful effect of the material on tumor cells is judged by the percentage of stained cells as compared to the control. In the Soviet Union L. M. Shabad and other researchers developed a method of selecting culture fluids by means of microscopic examination of tumor cells following their contact with the tested material in non-stained form. Certain definite changes are observed in this case in tumor cells which enable one to judge the degree of activity of the tested culture fluids. The cells of the ascitic form of Ehrlich carcinoma are usually employed in this method.

The defect of these methods, as of contact methods in general, is in the use of cells of one tumor only. Thus, as stated above, the unavoidable possibility exists of losing a number of preparations which do not affect the cells of a given tumor but are active on other tumors.

More reliable results are obtained in the evaluation of the anti-tumor activity of the tested culture fluids according to the loss of inoculative property by tumor cells after contact with them. Such a method was developed in our laboratory by V. A. Talyzina which enables us to effect the selection by using a number of experimental

tumors, solid as well as ascitic. A comparison of results using this method with the results of testing the same preparations in work on animals with experimental tumors, demonstrated the possibility of employing it for primary selection of the producers of anti-tumor antibiotics. Its defects, however, are first, the need for a large number of animals though in much smaller numbers than in tests on animals with experimental tumors, and, second, the possibility of obtaining results in only 10 days following the inoculation of animals with the tumor cells which had been subjected to the action of the tested preparations.

The use of cultures of tumor tissues as means for testing the anti-tumor activity of culture fluids is essentially another contact method. This method has been lately finding increasing application in some laboratories. Its advantage is the possibility of employing cultures of human tumors. The HeLa strain of cells is most frequently used for this purpose. However, there is at present only a limited quantity of cultivated in-vitro strains of human tumors. Therefore, this method involves the possibility of loss of anti-tumor antibiotics which do not inhibit the growth of cultures of tumor tissues subjected to testing, but are active on other human tumors.

Lately there have been developed in Japan contact-methods using agar media in which the inhibition of the property of tumor cells to reduce certain chemical compounds which change their color in the process, serves as an indicator of the activity of the tested culture fluids. One of these is the cup method, developed by Umezawa, which uses small cylinders in which the tumor cell dehydrase reduces the 2-dichlorphenol-indophenol. There is also the analogous Miuamura method in which methylene blue is used as an indicator. In both methods the cells of the Ioshida ascitic sarcoma or the cells of Ehrlich ascitic form of tumor are used.

The disadvantage of these methods is in their unfitness for detecting substances which are incapable of diffusion in agar, with the formation of a larger or smaller zone of nonreduced indicator around the small cylinders containing the tested material.

The last defect was eliminated in the modification suggested by Aran and Suzuki. This modification is much simpler than the cup method since it does not require a special agar nutritive medium; instead nutritional agar is used. The experiment is conducted in test tubes in which agar tinted with methylene blue is mixed with a suspension of Ehrlich ascitic cancer cells and the tested culture fluid.

The absence of discoloration of the test tube content serves here as an indicator of the activity of the tested material. This modification makes possible the demonstration of the activity of anti-tumor substances incapable of diffusion in agar.

As demonstrated in our laboratory by V..A. Talyzina, the Aran and Suzuki method may have eliminated the basic defect -- the employment of ascitic tumor cells alone. With adequate preparation there is the possibility of obtaining a suspension of cells of solid tumors fully suitable for the arrangement of this test. This makes possible the selection of culture fluid with practically any tumor.

A comparison of results obtained by this method with the results of testing activity on experimental animal tumors, showed that it is possible to select culture fluid possessing anti-tumor activity in vivo. Discrepancy in the results is observed with a material of low activity. Therefore, this method can be used for the primary selection of active culture fluids with mandatory arrangement of the test using a suspension of several tumors. In our laboratory the selection was usually made from the Ehrlich ascitic tumor cells, ascitic sarcoma 180, LIO-1 lymphosarcoma, M-1 and 45 rat sarcomata, and Geren Garcinoma.

The Japanese authors of this method think that, upon contact of active substances with live tumor cells, the latter perish causing cessation of activity of their dehydrase. This explanation of the mechanism of this test seemed to us incorrect, since it is hardly possible that they could retain their viability after a 24-hour study of tumor cells within the agar nutritive medium at 37°. It is more likely that we deal here with the inhibition of dehydrase activity independently of cell viability. To clarify this problem, V. A. Talyzina conducted tests which showed that to obtain reduction of methylene blue the addition of suspension of tumor cells was not necessary, but that it sufficed to add cellular salt extracts which contained no cells. In this instance, just as in the use of a tumor cell suspension, the reduction of methylene blue did not take place in the presence of active anti-tumor substances.

The results obtained demonstrated that the test could be successfully made with tumor extracts in which the dehydrase activity persists. Thus we were able to develop a more convenient method of primary selection without the use of tumor cell suspension, by using prepared tumor extracts. The extracts can be made from any tumors which make possible a primary selection with the employment

of several tumors.

Another advantage of employing extracts of tumors, instead of suspensions of tumor cells, is the possibility of prolonged preservation of extracts in a frozen state (several months). Thus the tests can be made at any time without depending on the availability of suitable tumors for a test at a given moment.

Some authors also studied the possibility of using various microorganisms for a primary selection of producers of anti-tumor antibiotics. However, no positive results were obtained because they did not establish a correlation between the inhibition of tumor growth under the effect of anti-tumor substances and their antimicrobic action.

Of considerably greater interest are the investigations of G. F. Gause who studied the effect of anti-tumor substances on microorganisms. Certain mutants which he had obtained approximated tumor cells in the character of their respiration. It is interesting to note that anti-tumor substances exerted marked bacteriostatic effects on the mutants of some microorganisms without affecting the initial cultures. Further observations are needed for establishing the possibility of using mutants of microorganisms for a primary selection of anti-tumor antibiotics. It is difficult to assume that a correlation can exist between the effect of some anti-tumor substance on one mutant and its effect of this same substance on all tumors. If it is possible to establish such correlation concerning at least one human tumor, a basis will have been made for the hope that a whole series of mutants can be selected and used for developing anti-tumor substances which would be effective against certain human tumors.

As can be seen from the above brief review, of those methods of primary selection of anti-tumor antibiotics in most frequent current use, there is still no method available which would enable one to select with certainty preparations effective on human tumors. This situation results from the fact that the selection is done mainly by means of experimental animal tumors which differ markedly in their biological characteristics from human tumors.

The development of more reliable methods of primary selection of anti-tumor antibiotics must, obviously, be conducted in several directions. First various possibilities of using human tumors in the form of tissue cultures or by other means, need to be studied. Furthermore, one must ascertain which experimental animal tumors as well as human tumors are affected by the anti-tumor substances. The establishment of such correlations would open wide

possibilities of selection of preparations possessing anti-tumor activity for definite human tumors. Finally, as has been stated, it is necessary to investigate whether analogous correlations exist in the case of selection by microbial mutants.

Since any anti-tumor substance affects some definite metabolic link, there is no reason to believe we can count on finding a universal preparation which would act on all tumors. Each preparation may have only a certain circle of action which includes one or several tumors. Therefore, it is more expedient to search not for anti-tumor preparations in general, but for those which act on various human tumors. Such a trend of investigations will advance the possibility of finding preparations which can be utilized in the clinic for the treatment of some malignant tumor patients.

Extensive and intensive work is needed in this direction.

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