Peptides, Amides, and Esters of Chloroethylamino Derivatives of Amino Acids and of Phenylalkanecarboxylic Acids

A New Class of Antitumor Compounds

L. F. Larionov

(Institute of Experimental and Clinical Oncology, Academy of Medical Sciences of the U.S.S.R.)

SUMMARY

The first stage of these studies consisted in the development of antitumor drugs of the type of alkylating metabolites 1952–55 (5, 6, 13). These are metabolites or other natural or biologically active compounds which are combined with a cytotoxic group (e.g., the chloroethylamino group). Sarcolysin and dopan are representative of drugs of this type. In the former, the essential amino acid, phenylalanine, acts as a metabolite carrier; and in the latter, uracil (or 4-methyl-uracil). Sarcolysin and dopan have different actions on the spectrum of animal and human tumors. Thus, dopan is effective in Hodgkin’s disease and chronic myelogenous and lymphatic leukemia (8), and sarcolysin is ineffective in Hodgkin’s disease but gives positive results in seminoma, reticulosarcoma, multiple myeloma, Ewing’s bone tumor, primary cancer of the liver and ovaries (1, 15). These differences suggest a definite role of the “carrier” of the cytotoxic group in the antitumor activity.

These as well as other data accumulated in modern cancer chemotherapy indicate great variability in the response of different tumor forms to anticancer drugs, even of tumors of the same organ or of similar histological structures.

These differences in susceptibility are presumably due to certain differences in chemical composition, in the enzyme activity and metabolism of different tumors. It is possible that the variability in the sensitivity of various tumors to chemotherapeutic drugs is due in particular to fine differences in the structure of DNA proteins, since the latter represent the major substrates affected by cytotoxic compounds.

The above data and conclusions indicate the necessity for the creation of a great number of drugs for the treatment of different groups or even varieties of the same form of malignant tumors. Individual compounds may exhibit not only considerable differences but also insignificant ones in accordance with the fine structural peculiarities of the nucleoproteins of various tumors.

In 1956, in order to develop compounds of this type, it was decided, in collaboration with Prof. I. L. Knunianz, to make use of more complex carriers of the cytotoxic group. At first a number of peptides of N-formyl-sarcolysin and of esters of certain amino acids, such as valine, phenylalanine, and methionine, were synthesized in the chemical laboratory headed by Prof. I. L. Knunianz of the Institute of Element-Organic Compounds of the U.S.S.R. Academy of Sciences (4). Later, in the Chemical Department (Prof. A. J. Berlin in charge) of the Institute of Experimental and Clinical Oncology, peptides of N-acetyl-sarcolysin have been synthesized.1

A study of these compounds carried out in our laboratory at first by Z. P. Sophina and the author2 showed the following results (see also 7, 10, 11, 14, 16):

1 Synthesis of these compounds and of those mentioned below has been carried out by I. L. Knunianz, O. V. Kildisheva, and N. E. Golubeva.

2 In further study of the compounds the collaboration of G. N. Platonova, I. G. Spasskaya, Fang Chi-chao, E. I. Khomenkovsky, S. A. Degteva, and N. A. Lesnaya is to be mentioned.
Peptides of N-acyl derivatives of sarcolysin showed low toxicity. The LD_{100} of these compounds for rats upon intraperitoneal administration was 150–1000 mg/kg of body weight and more (versus 25 mg/kg for sarcolysin). The LD_{100} for mice was higher than that for rats (1000 mg/kg and more). The compounds were less toxic in case of oral administration.

Though showing low toxicity, the peptides of N-acyl-sarcolysin in daily doses of 7–200 mg/kg greatly inhibited the growth of a number of transplantable tumors in mice and rats and even brought about complete regression of some of them (Sarcomas 45 and 298, the Walker carcinosarcoma).

The antitumor action was attended by a relatively slight inhibitory effect upon bone marrow hemopoiesis, which is indicative of the higher selectivity of antitumor action of the new compounds compared with that of sarcolysin. Of still greater significance is the fact that the peptides of sarcolysin and of various amino acids exhibited distinct differences in the tumor spectrum. This is illustrated by Chart 1 with regard to the action spectrum of three peptides of N-acetyl-sarcolysin.

Along the vertical line, inhibition indices (arranged on a logarithmic scale) are given with the respective percentages of inhibition of tumor growth. Along the horizontal line, various tumor strains of rat and mouse tumors are presented. The indices and per cent inhibition of tumor growth of the same compounds have been connected, for demonstration purposes, by a continuous line which has no mathematical meaning.

Chart 1 shows that the valine peptide of N-acetyl-sarcolysin in maximal tolerated doses brought about complete regression of tumors.
about a complete cure of Sarcoma 45 (inhibition index, 250—e.g., a complete regression). This peptide greatly inhibited the growth of Sarcomas M-I and 298 (indices 50 and 70, per cent of inhibition 98 and 98.6, respectively) and only slightly inhibited the growth of Sarcoma 37 (index 2, per cent of inhibition, 50). The phenylalanine peptide was much less effective in Sarcoma 45 (index, 20); it showed the same effect as valine peptide in sarcoma M-I, while it was almost ineffective in Sarcoma 298. The same chart shows that the action of the methionine peptide of \( N \)-acetyl-sarcolysin on the same tumors was different from that of the valine and of the phenylalanine peptides.

It will appear from the diagram that the differences in the strength of action of different peptides upon the same tumor, as well as the differences in the action spectra of a peptide on various tumors, depends on the kind of amino acid which is combined with sarcolysin by means of a peptide bond.

In 1957 Bergel and Stock synthesized several peptides of sarcolysin (2). Their method of synthesis (through azlactone) was different from that used in the synthesis of the above mentioned peptides (the Sheehan and Hess method). The authors found that \( N \)-benzoyl-sarcolysil-glycine in a dose of 5 mg/kg in rats showed no action on the Walker carcinosarcoma (this was probably due to the lower doses used) (17).

In 1958 sarcolysin peptides with a free amino group of sarcolysin (all are hydrochlorides) were synthesized by Knunianz, Kildisheva, and Golubeva. Our investigations (12) show that their toxic and therapeutic doses are close to those of sarcolysin. Their antitumor effect was found to be very high.

Chart 2 shows the spectra of action of three sarcolysin peptides of this type compared with that of sarcolysin in adequate doses. It will appear from the chart that (a) the spectrum of action of the sarcolysin peptides differs from that of sarcolysin;
(b) the action of the peptide formed by sarcolysin with a free amino group differs from the action of peptides of N-acetyl-sarcolysin (compare Charts 1 and 2). Thus, of the three N-acetyl-sarcolysin peptides (Chart 1) the valine peptide showed the strongest action on Sarcoma 45 (index, 250).

Next followed the methionine peptide (index, 150). The phenylalanine peptide influenced the tumor in the smallest degree (index, 20). Out of sarcolysin peptides with a free amino group (Chart 2) the phenylalanine peptide was the one to produce in adequate (maximum tolerated) doses the strongest action on Sarcoma 45 (index, 200); next followed the methionine peptide (index, 17); the valine peptide was the least active of the three.

Hence, acylation of the amino group of sarcolysin in dipeptides greatly influenced the toxicity as well as specificity of action of peptides on various tumors.

To ascertain the role of the primary carrier of the chloroethylamino group in the antitumor effect, peptide-like compounds were synthesized in which phenylacetic acid was taken as the primary carrier instead of phenylalanine, and the same amino acids served as the terminal compound (3). It will appear from Chart 3 that the effect of the peptides of p-di(2-chloroethyl)aminophenylacetic acid and of valine was different from that of the N-acetyl-sarcolysin peptide with the same amino acid (11).

The peptides of p-di(2-chloroethyl)aminophenylacetic acid and of various amino acids likewise differed in their action upon various tumors, but less than did the respective sarcolysin peptides.

The peptide-like compounds of p-di(2-chloroethyl)phenylbutyric acid and of valine exhibited a higher activity than the respective peptide of phenylacetic acid.
Then tripeptides containing the chloroethylamino group were synthesized and studied (Chart 5). It will appear from the diagram that the strength and spectrum of action of various tripeptides depend on the kind of amino acid used as a terminal compound, as well as on the sequence of the same amino acids in the peptides.

My assumption was (9, 12) that a still greater diversity in the action spectrum of the drugs could be achieved by using some other biologically active substances as terminal groups, such as vitamins or their components, instead of amino acids. On the basis of this assumption the amide of nicotinic acid and of sarcolysin and the amide of sarcolysin and aminothiasol were synthesized.

Thus, N-acetyl-sarcolysyl, the amide of 2-aminothiazol, inhibited the solid form of Ehrlich carcinoma more than did N-acetyl-sarcolysil-valine and a N-nicotinoyl-sarcolysinisopropyl ester. However, it does not inhibit Sarcoma 180 and even stimulates the growth of this tumor.

The amides of chloroethylaminophenylacetic acid and of a number of drugs or their components, such as anesthesin (the ethyl ester of paraaminobenzoic acid), of 6-methoxy-8-aminoquinoline, and of the chloramphenicol residue, were also synthesized. The first two of these three amides exhibited a high activity with a different spectrum, while the third proved inactive.

Finally, the ester of cholesterol and chloroethyl-amino acid was synthesized and investigated. This compound in daily doses of 50–70 mg/kg, injected subcutaneously in oil solution, inhibited the growth of certain tumors, its action spectrum being different from that of peptides and amides (Chart 7).

Thus, a number of anticancer compounds of a new type have been produced which can be called complex alkylating metabolites. The constituents of the complex carrier are connected by a peptide, amide, or ester bond. Graphically these compounds may be presented as follows (Chart 8).

Clinical tests of our drugs are under way at the Institute of Experimental and Clinical Oncology under the guidance of Prof. N. N. Blokhin. So far it has been found that, for example, N-acetyl-sarcolysil-valine ethyl ester, when administered orally in a daily dose of 2 gm., is effective in seminoma, reticulosarcoma, multiple myeloma, and Ewing's bone tumor. No depressive effect on he-
mopoiesis has been noted in patients who have undergone treatment.

The results of our investigations show that the strength and spectrum of antitumor action of new drugs depend on the structure and the properties of the given compound, particularly on the nature of the primary carrier of the cytotoxic group and on the nature of the terminal compound (11, 12). The effect of the compound also depends on the structure of the cytotoxic group. Since the primary carrier and the terminal compound, as well as the cytotoxic group, can be varied indefinitely, wide prospects are opened for the development of almost unlimited numbers of anticancer drugs. These drugs of a new type have a low toxicity with fairly high selectivity for antitumor effect and with different actions on the spectras of various tumors.

REFERENCES


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