EXPERIENTIA

Vol. XXI – Fasc. 1 Pag. 1–56 15. I. 1965

New Developments in Cancer Research

Chemotherapy of Cancer: Report on the Lugano Symposium

By W. BOLLAG and A. PLETSCHER

Medizinische Forschungsabteilung, F. Hoffmann-La Roche & Co. AG, Basel (Switzerland)

A comprehensive review on the present status of the chemotherapy of cancer was given in a symposium held in Lugano (Switzerland) from April 28 to May 1, 1964. The highlights will be reported in this article.

In his opening lecture, KARNOFSKY (New York) stated that 'there seems to be little cause for optimism that a universal anticancer drug will be found or even one specific against a particular form of cancer, with the possible exception of trophoblastic tumors. This conclusion can change rapidly with new knowledge, but the great visions have yet to be seen and the great discoveries yet to be made before one can make a great prophecy of an imminent cure'. In spite of this situation, many forms of cancer are influenced and many patients with acute and chronic leukemias, malignant lymphomas, breast cancer, prostatic cancer, endometrial cancer, ovarian and testicular cancers, trophoblastic tumors and Wilm's tumor now benefit at least temporarily from drugs.

Alkylating agents. 20 years after the discovery of the anti-tumor activity of nitrogen mustard, research in this class of compounds is still progressing. BERGEL (London) gave a review on the steps leading from the first alkylating agent to the latest *developments*. Two main approaches for obtaining selective action have been chosen: (1) variation in the 'carrier' moieties for the alkylating groups and (2) search for compounds with latent effect, i.e. low activity and toxicity during transport of the drugs to the tissues where the formation of a more active metabolite takes place. Many nitrogen mustard derivatives are based on the *carrier* principle. The bis(chloroethyl)-amine group may be linked to a benzene ring with acidic, basic or amphoteric side chains, like chlorambucil (Leukeran), melphalan (Alkeran), *o*-merphalan (Merophan). Another example refers to the sugar-like polyols, such as mannitol, yielding Degranol. The principle of latency has been applied in designing compounds like Nitromin (= nitrogen mustard N-oxide) and Cyclophosphamide, which prove to be examples of rather effective antitumor agents. In addition, the mustard group as well as other active groups like ethyleneimine, methanesulfonate or epoxide have been linked to amino acids, peptides, sugars, pyrimidines, quinones, etc.

Concerning the *mechanism of action* of alkylating agents, two main theories have been discussed: BROOKES and LAWLEY (London) found that alkylation of nucleic acids occurs at the ring-nitrogen of the bases. This generally results in instability of deoxyribonucleic acid (DNA) leading to loss of 7-alkylguanine and 3-alkyladenine moieties. Difunctional alkylating agents yield di-(guanin-7-yl) derivatives, some of which result from cross-linking of guanines located in the twin strands of the DNA helix. According to HOLZER (Freiburg, Germany), alkylating agents inhibit glycolysis by a decrease of the content of nicotinamide-adenine dinucleotide (NAD, DPN) in tumor cells which is paralleled by a tumor inhibitory effect. This could be shown by investigating the effects of a given compound on resistant and non-resistant tumors, and by comparing the different activities of various cytotoxic compounds on the same tumor. The NAD content is decreased consequently by an inhibition of its biosynthesis which may be caused by an interference of alkylating agents with the activity of the enzyme ATP:NMN adenyltransferase (nicotinamide-adenine dinucleotide pyrophosphorylase). At the moment, it cannot be decided which approach to the mechanism of action of alkylating agents is more important. Perhaps the two theories – effect on DNA and effect on glycolysis – can be reconciled with each other.

From the *clinical* side, GALTON (London) gave a report on his experience with chlorambucil, melphalan, and mannitolmyleran. Chlorambucil, suitable for oral administration, is effective in a variety of conditions either in short courses or at low dosage for maintenance therapy, during years if necessary. Good results are obtained in patients with chronic lymphatic leukemia and Hodgkin's disease. Furthermore, ovarian carcinomas are favourably influenced in a rather high percentage. Melphalan seems to be of special value in myelomatosis and in solid tumors, when the drug can be used by regional administration. Mannitolmyleran was submitted to adequate trials in ovarian, mammary, and

bronchial carcinoma. It appears to be equal in efficacy to other alkylating agents. According to GERHARTZ (Berlin), tris-ethylene-iminoquinone (Trenimon) is particularly effective in the local treatment of carcinomatous effusions and in the regional infusion therapy of tumors. Cyclophosphamide is mainly indicated in Hodgkin's disease, myelomatosis, reticuloses, and in certain carcinomas, such as bronchial, mammary, and ovarian carcinoma. These two compounds differ considerably in their speed and mode of action. Trenimon reacts immediately under in vivo and in vitro conditions, whereas cyclophosphamide is transformed only after a latent period into an active cytotoxic agent.

Antimetabolites. This large class of antitumor agents has been developed on a rational basis. In the last decade, hundreds of compounds have been synthesized and tested. Only those interfering with the synthesis of nucleic acids have reached clinical importance. The three mostly used preparations are the folic acid antagonist amethopterine, the purine analog 6-mercaptopurine, and the pyrimidine analog 5-fluorouracil. HITCHINGS (Tuckahoe, USA) gave a survey on the role of *biochemical research* and 'molecular manipulation' for the development of new chemotherapeutic agents. By means of representative examples, the biochemical basis for the selective toxicity was demonstrated. In the field of 2,4-diamino-pyrimidines, for instance, which inhibit 7,8-dihydrofolate:NADP oxidoreductase (dihydrofolate dehydrogenase), appropriate chemical changes lead to highly specific toxicity for certain microorganisms. This selective discrimination is dependent on the different binding of the drugs to these oxidoreductases from various species. The antitumor activity, however, does not seem to be related to the selective binding to the above mentioned enzymes.

The *mechanism of action* of tumor inhibitory antimetabolites has been thoroughly investigated. The folic acid antagonists aminopterine or amethopterine exert a strong inhibitory effect on 7,8-dihydrofolate:NADP oxidoreductase which may be the principal mechanism inhibiting the conversion of folic acid into tetrahydrofolic acid. The fact that amethopterine-resistant tumor cells contain a higher quantity of 7,8-dihydrofolate:NADP oxidoreductase points to the same hypothesis.

Mercaptopurine seems to interfere with the purine metabolism in several ways, e.g. (a) by competition with hypoxanthine for IMP:pyrophosphate phosphoribosyltransferase (inosinic acid pyrophosphorylase), (b) by transformation of the antimetabolite to thioinosinic acid, which inhibits the formation of succinoadenylic acid from inosinic acid and its conversion to adenylic acid, (c) by inhibiting the incorporation of formate and glycine during purine biosynthesis. This multiplicity of biochemical effects renders a simple explanation of the mode of action of mercaptopurine rather difficult.

5-Fluorouracil (5-FU), described by HEIDELBERGER (Madison) as a potential pyrimidine antagonist, causes several alterations in the nucleic acid biosynthesis. The drug is incorporated by nucleotide linkage into ribonucleic acid (RNA) of ascites tumor cells, bacteria, and viruses. However, its effect on DNA synthesis seems to be of more importance. 5-FU as well as its deoxyriboside 5-fluorodeoxyuridine (5-FUDR) prevent the formation of thymine. Thus, these antimetabolites are converted into 5-fluoro-2-deoxyuridine-5'-monophosphate which causes a potent and competitive inhibition of the thymidilate synthetase. This enzyme catalyses the methylation of desoxyuridylic acid to thymidylic acid involving methylene-tetrahydrofolate as a coenzyme.

BRENNAN (Detroit) summarized the *clinical* results obtained with 5-FU. In extensive trials it has been established that the drug induces objective remissions mainly in cancers of the gastrointestinal tract, of the breast, of the female genital tract, and of the head and neck. The approximate regression rate does not seem to exceed 20%, the remissions last on the average 7 months. The toxic side effects prevent an adequate therapy in debilitated patients. Monthly courses of chemotherapy are necessary for maintaining the palliative effect. Amethopterine and 6-mercaptopurine are widely used in the treatment of acute leukemia besides steroids, leurocristine, and methylglyoxal-bis-(guanylhydrazone). There is now good agreement among most investigators on the handling of these substances in acute lymphoblastic leukemias. Chemotherapy causes a definite prolongation of life, particularly in children with acute lymphoblastic leukemia; the duration of remission is, however, limited to approximately 14 months. In spite of the fact that in many cases 'complete' hematological and clinical remissions are achieved, not all leukemic cells are eradicated. Relapses are the rule and only very few cases are known to survive 5 years. MATHÉ and AMIEL (Paris), discussing newer concepts of the treatment of acute leukemia, were rather optimistic. Accordingly, there is a certain possibility of destroying the totality of the neoplastic cells by massive chemotherapeutic doses, applying all available therapeutic means, such as steroids, antimetabolites, alkylating agents, spindle poisons, X-rays, in combination or alternating courses. A new possibility for treating patients who are resistant to chemotherapy has been developed recently. Thereby, the patient receives a whole-body irradiation with a lethal dose of X-rays. This considerably reduces the number of leukemic cells and induces a condition of immunological tolerance, whereby a homologous bone marrow graft is accepted by the host. The graft produces antibodies against leukemic cells and may thus by a 'graft against host-reaction' eradicate all neoplastic tissue. Although some encouraging preliminary success has been achieved, it is still too early to give a definite

judgment on this adoptive immunotherapy. Many complicated problems have to be solved, such as typing of the ideal bone marrow donor, prevention of the secondary syndrome with its consequences, etc.

Antibiotics. The search for antibiotics with tumor inhibitory properties is continuing all over the world. However, only very few preparations have reached clinical importance, because most of them are too toxic. Some of the products derived from streptomyces cultures have gained interest for basic research. Actinomycin D has a favorable influence on some kinds of tumors in children, such as Wilm's tumor or rhabdomyosarcoma, and exerts furthermore a sensibilizing effect on X-rays. Its mechanism of action has been thoroughly investigated, particularly the inhibition of DNA-dependent RNA synthesis, the inhibition of nucleosidetriphosphate:RNA nucleotidyltransferase (RNA polymerase), and the formation of actinomycin-nucleic acid complexes. Another antibiotic produced by a streptomyces species is Mitomycin C, the chemical structure of which has recently been elucidated. It contains an aziridine ring and seems to belong to the alkylating agents. The cross resistance of the antibiotic with this latter group supports this assumption. KENIS (Bruxelles) reported on *clinical results* with Mitomycin C in patients with solid tumors. Objective responses are obtained in a rather high percentage, although the clinical use is limited by the toxicity on the hemopoietic systems. Treatment with massive single doses at long intervals seems to be equally effective but less toxic than daily administration of low doses. As pointed out by SCHMIDT (Münster, Germany), it cannot yet be assessed whether the combination of Mitomycin with other cytotoxic agents yields better therapeutic results than treatment with the single drugs. Many other antibiotics are still under experimental and clinical investigation, e.g. carcinophilin, streptonigrin, streptovitacin, olivomycin, daunomycin, etc.

Alkaloids. Among the plant products, the alkaloids of the periwinkle *Vinca rosea* have received considerable attention. Particularly vincaleucoblastine and leurocristine, both being dimers of the indoldihydroindol type and differing only in a methyl or aldehyde group, show a marked cytotoxic effect on animal tumors. They belong to the spindle poisons, cell division being blocked in metaphase. Concerning the mechanism of action, OBRECHT (Freiburg, Germany) found an inhibition of glycolysis or a decrease of the enzyme NAD in tumors and other rapidly proliferating tissues. Both alkaloids are used particularly in the treatment of malignant lymphoma, but while vincaleucoblastine exerts its most favorable action in Hodgkin's disease, leurocristine is more effective in lymphosarcoma. The latter shows, furthermore, good results in lymphoblastic leukemia of children. Vinca alkaloids have also an influence on the growth of solid tumors. While their side effects on bone marrow are seldom severe – leucopenia is rapidly reversible and thrombocytopenia is rarely observed – the neurotoxic effects handicap the use of these very interesting alkaloids.

Hormones. Interference with the endocrine balance is of primary importance in the therapy of hormone-dependent tumors, e.g. mammary or prostatic carcinoma. HUGGINS (Chicago) summarized the history, the principles, the experimental and clinical developments of endocrine therapy which have been built up on empirical and rational bases. Basic research was facilitated by HUGGINS' discovery of an *experimental* model of a hormone-dependent animal tumor. Thus, a mammary carcinoma can be induced in rats by a single administration of 7,12-dimethylbenz(a)anthracene. These tumors respond to ovarectomy and hypophysectomy as well as to androgen administration or to a combination of estradiol and progesteron. TAGNON (Bruxelles) gave a review on the *clinical* management of endocrine therapy in disseminated mammary cancer. Ablation of endocrine organs, e.g. ovarectomy, adrenalectomy, and hypophysectomy, is the most effective treatment. Androgens, estrogens, and finally corticosteroids should be reserved for failures or recurrences in surgically treated patients. Exceptions are postmenopausal women, where a first trial with estrogens is indicated. Particular attention has been paid to the steroid Δ'-testololactone as a substitute for androgens. It induces the same percentage of remissions as testosterone with the advantage of being devoid of virilizing effects.

Miscellaneous. Several antitumor agents cannot be categorized into one of the mentioned classes of tumor inhibitory agents. *Methylglyoxal-bis-(guanylhydrazone)* has given some favorable results in the treatment of acute granulocytic leukemia. In adults, this type of leukemia is rarely influenced by other cytotoxic compounds. A review on the antitumor activity of *methylhydrazine derivatives* was presented by BOLLAG (Basel). This class of compounds, exerting a marked growth inhibitory effect on *experimental* animal tumors, leads to disturbances in the mitotic cycle by prolongation of the interphase and by chromatid breaks. Methylhydrazines depolymerize DNA in vitro only in presence of oxygen; hydrogen peroxide or OH-radicals, formed during autoxidation, seem to be responsible for this effect. MARTZ (Zürich), MATHÉ and AMIEL (Paris), KENIS (Bruxelles), and GERHARTZ (Berlin) reported that the *clinical results* with 1-methyl-2-*p*-(isopropylcarbamoylbenzyl)hydrazine are good in malignant lymphoma. No cross resistance with other cytotoxic agents has been observed. The influence on hemoblastoses and solid tumors is still under investigation. The metabolism of methylhydrazines was studied by OLIVERIO (Bethesda, USA) who also observed induction of lung adenomata in mice. A further group of new antitumor agents are the *terephthalanilides*. These

polybasic compounds, studied by HIRT (Bern) and BURCHENAL (New York), inhibit the development of experimental mouse leukemias. Their mode of action is still rather hypothetical. Terephthalanilides can be considered as analogues of short histone fragments which may interfere with these polybasic proteins. It is remarkable that derivatives with only minimal differences in chemical structure do not show cross resistance in mouse leukemia. In human leukemia, terephthalanilides are of little effect. In Burkitts lymphoma of African children, however, some impressive remissions have been observed. Toxic manifestations of the nervous system differing markedly in severity between animals and men limit the clinical application of this class of drugs.

Regional and combination therapy. In conventional cancer chemotherapy by the oral or the intravenous route, it is often not possible to reach a sufficient level of the antitumor agent at the site of the neoplastic growth without inducing systemic toxicity. In certain forms of inoperable localized cancers, the method of continuous arterial infusion yields good therapeutic results due to the high concentration and continuous action of the drug. Supralethal doses of an antimetabolite, e.g. amethopterin, can be administered by the intraarterial route, if the corresponding metabolite, e.g. citrovorum factor, is injected systemically. MILLER (New York) discussed the possibilities, indications, and complications of intraarterial infusion therapy. Out of 106 patients treated by this method – mainly with the antimetabolites amethopterin and 5-fluoro-2′-deoxyuridine – 75% experienced objective tumor regression.

Beside the regional chemotherapy, combination treatment is a further possibility for improving the results in cancer chemotherapy. This subject was dealt with by BRULÉ (Paris). Surgery in combination with chemotherapy may give better long-term results. However, it is not yet definitively assessed whether this method has a practical value in the prophylaxis of local relapses and metastases. The combination of chemotherapeutic agents with X-rays is frequently used. Many antitumor agents have been tried. Some show a purely additive effect, some seem to have a potentiating or sensibilizing action, e.g. Actinomycin D. The combination of two or more antineoplastic substances with different modes of action in simultaneous or sequential therapy may improve the direct therapeutic effect and delay drug resistance. Some preliminary encouraging results have been obtained. The statistical evaluation of combined therapy, however, is much more difficult than that of a single method of treatment.

Two further communications not directly related to present practical chemotherapy were concerned with viruses in relation to cancer and with the fixation of plasma proteins in tumors. WEIL (Lausanne) discussed some aspects of polyoma virus and its oncogenic action, whereas ISLIKER (Lausanne) outlined some possibilities of conjugating drugs to specific and nonspecific plasma proteins – antibodies, fibrinogen, albumin – in order to obtain new and better immunological approaches to cancer chemotherapy.

The general feeling at a symposium on cancer chemotherapy is always rather pessimistic. Insufficient antitumor activity and the toxicity of the present chemotherapeutic agents as well as drug resistance make definite cures impossible. Nevertheless, there are reasons for optimism, since some cases of trophoblastic tumors treated with chemotherapeutic agents, such as methotrexate, have survived more than 10 years. Furthermore, some children with leukemia – very few throughout the world – have had either long-term remissions or were even cured by chemotherapy. BURCHENAL (New York) discussed these cases as well as the possible causes for their extraordinary response. The fact that cures in these disastrous diseases seem to be within the realm of possibility gives hope and should encourage more work in cancer chemotherapy based on both approaches, the rational and the empirical one[1].

Zusammenfassung. Anlässlich eines internationalen Symposiums wurde die Chemotherapie von malignen Tumoren behandelt. Die Vorträge, welche Themen der Grundlagenforschung und der klinischen Medizin umfassten, werden im vorliegenden Artikel referiert.

[1] The Proceedings of the Symposium on the Chemotherapy of Cancer, Lugano, April 28–May 1, 1964, have been published by Elsevier Publishing Company, Amsterdam (Ed.: PL. A. PLATTNER).