Deutsche Zeitschrift für
Onkologie 5
Journal of Oncology

22. Jahrgang
Oktober 1990
Dtsch. Zschr. Onkol.
ISSN 0931-0037

vfm
Verlag für Medizin
Dr. Ewald Fischer
6900 Heidelberg 1

Centre de Recherche Biologique (CERBIOL), Ivry-Sur-Seine

Cancer Therapy: A New Approach

Krebstherapie: Eine neue Annäherung

M. Beljanski

Zusammenfassung

Die meisten Krebsmedikamente haben sekundär toxische Effekte auf normale Zellen und verursachen Leukopenie und Thrombozytopenie in Säugetieren. Wir haben ein kurzkettiges RNS-Fragment präpariert und charakterisiert, das als „Primer" für die in vitro Replikation von Knochenmarks- und Milz-DNS wirkt, jedoch nicht auf Krebszellen. Andererseits haben wir Substanzen natürlichen Ursprungs gefunden und isoliert, die unter in vitro Bedingungen selektiv Krebszellen erkennen und deren proliferative Kapazität zerstören, während normale Zellen nicht beeinflußt werden. Die Selektion solcher Substanzen war möglich aufgrund der Beobachtung, daß DNS von Krebszellen im Vergleich zu Normalzellen destabilisierte Moleküle sind (mehr Bereiche mit ungepaarten DNS-Ketten). Selektiv krebshemmende Mittel, die wir isolierten, heilen eine relativ große Zahl von Mäusen mit YC8 Lymphomzellen. Dieser Effekt wird wesentlich verstärkt durch klassische Krebsmittel, welche DNS-Ketten öffnen, was dazu führt, daß unsere selektiven Komponenten leichter gebunden werden.

Schlüsselwörter

RNS-Primer für DNS Synthese, Leukozyten und Thrombozyten Genesis, Leukopoese, selektiv krebshemmende Mittel, Leukopenie, Thrompzytopenie.

Summary

Most classic anticancer drugs exhibit a secondary toxic effect on normal cells and induce appearance of leukopenia and thrombocytopenia in mammals. We have prepared and characterized short-chain RNA fragments that act as primers for in vitro replication of DNA from bone marrow and spleen without affecting that of cancer cells. In animals and humans under chemotherapy they induce leukocyte and platelet genesis without impeding the effect of cancer drugs. On the other hand, we have selected and isolated substances from natural origin that distinguish cancer cells whose proliferative capacity is destroyed in *in vitro* conditions, while normal cells are ignored. The selection of

such substances was possible due to the observation that cancer DNA is a destabilized molecule (more areas of impaired DNA chains) in comparison with normal cell DNA. Selective anticancer drugs we isolated cure a relatively important number of mice bearing YC8 lymphoma cells. Their effect is highly potentiated by classic anticancer drugs which induce cancer DNA chain opening thus facilitating the binding of our selective compounds.

Keywords

RNA primers for DNA synthesis, leukocyte and platelet genesis, leukopoiesis, selective anticancer drugs, leukopenia, thrombocytopenia.

Introduction

Therapeutic drugs and ionizing radiation used for conventional cancer therapy are non selective, that is, they destroy or incapacitate any rapidly dividing cell, whether malignant or normal. This induces adverse side effects. Prominent among these is destruction of hemopoietic cells, which induces leucopenia and thrombocytopenia. A consequence of this is that conventional therapies need to be temporarily suspended to allow for blood cell regeneration, during which time cancer cells are once more free to multiply [1-3].

1. Discovery of specific agents for protection and enhancement of hemo-poiesis

15 years ago, our fundamental research on cell biochemistry and particularly enzymology has progressed far enough for us to choose as our first objective a quest for nontoxic substances which would protect normal hemopoietic cells and induce and accelerate their genesis in patients undergoing cancer therapy.

DNA replication, the primary step of cell division: is carried out by DNA dependent DNA polymerase, which has shown over fifteen years ago to require a short RNA priming sequence [4-6]. Our research on short-chain RNAs enabled us at the time to produce a whole range of biologically active RNA fragments, a number of which could be used as primers by DNA dependent DNA polymerase. These shortchain primers RNAs exhibited specificity, both large-scale (they primed either mammalian, bacterial or viral DNA replication) and close (some primed replication of a specific DNA) [4]. Thus we discovered RNA fragments which selectively primed the replication of DNA derived from normal hemopoietic cells of the bone marrow and spleen. In several papers [7-9], we described a procedure for large scale preparation of RNA primers active on DNA replication in bone marrow and spleen cells. Pancreatic ribonuclease was used to hydrolyse purified *Escherichia coli* ribosomal RNA, which was thus cleaved into short purine-rich fragments. Cells similary produce short RNA primers by enzymic degradation of longer RNAs.

Our RNA fragments were characterized by chemical and physical methods and their *in vitro* and *in vivo* activities were reported by us (5)(8). Extensive experimental work carried out in our laboratory demonstrated their biological efficiency as primers.

Results of in vitro and in vivo assays

1. *In vitro*, these RNA fragments are used by DNA-dependent DNA polymerase I as primers to initiate the replication of DNAs derived from rabbit bone marrow and spleen cells. In contrast, they do not prime replication of DNAs derived from a number of other normal tissues and from malignant cells (fig. 1).

2. Intravenous administration of these RNA fragments restores normal circulating leukocyte counts after they have been depleted by high doses of cyclophosphamide (CP) in healthy rabbits. On account of this activity, these RNA fragments were termed „Beljanski Leukocyte Restorers" (BLRs) (fig. 2).

Peak BLR-driven leukocytosis occurs after 48 hours and, in spite of daily CP administration, leukocyte counts remain within normal physiological limits for 3 to 5 days. Granulocyte/lymphocyte balance, upset by CP, is also restored by BLRs.

3. Platelet counts, when decreased by Daunorubicin (fig. 3) or by CCNU, may also be restaured to normal using appropriate BLR concentrations.

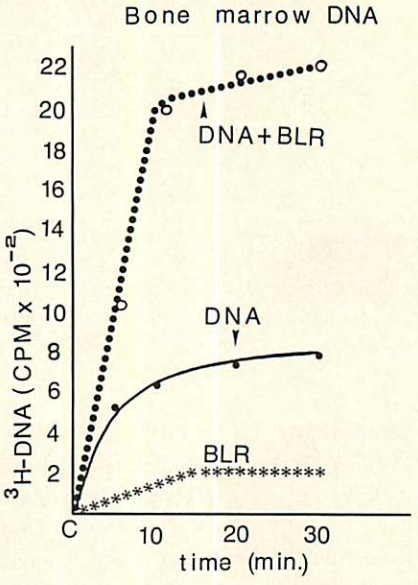

Fig. 1: Bone marrow DNA *in vitro* replication in the absence and presence of BLRs.
BLRs act *in vitro* as primers for bone marrow DNA replication. Incubation mixture contained per 0.15 ml: Tris-HCl buffer ph 7.65, 25µMoles; $MgCl_2$: 2 µMoles; four d-XTP : each 5 nanomoles (+ (^{3}H)-TTP, 50,000 CPM); DNA : 0.2 µg; BLRs : 4µg; DNA dependent DNA polymerase I, 80 µg. Incubation: 10, 20 and 30 min. at 36°C. TCA (trichloroacetic acid) – precipitable material was filtered on GF/C glass filter, washed, dried and radioactivity measured with a Packard liquid spectrometer (Prias). Analysis were carried out in triplicate for each incubation time.

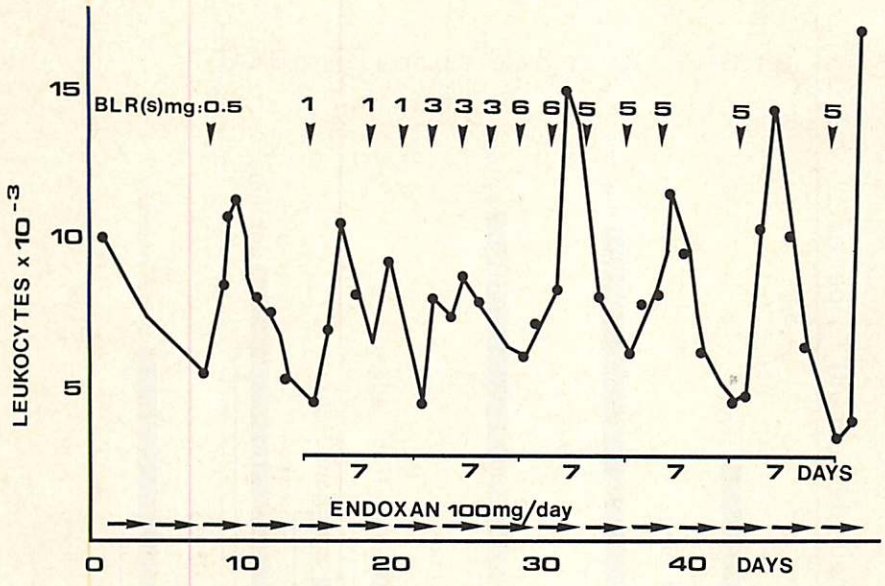

Fig. 2: Leukocyte formation in cyclophosphamide treated rabbits. Effect of BLRs.
Leukocyte count in the Endoxan treated rabbits receiving varying i.v. doses of BLRs every second day. After leukocyte count had been strongly decreased, a 3.5 kg Endoxan treated rabbit (100 mg/day) received varying doses of BLRs ranging from 1 to 6 mg every second day as shown by the arrows. Circulating leukocytes were counted daily with a Coulter Counter. The results given in this Fig. are an average obtained with 10 rabbits. The mean increase in leukocyte count was 172 % ± 17 % as standard error. The confidence interval calculated using paired sample student's test : $p < 0.001$.

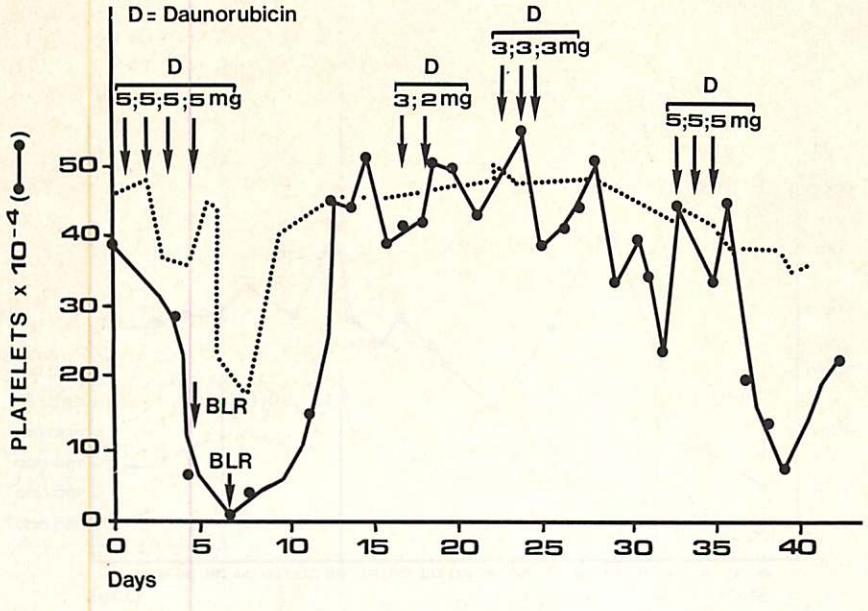

Fig. 3: Platelet and leukocyte formation in the presence of Daunorubicin. Effect of BLRs.
Effect of BLRs on platelet count in the rabbit pretreated with high dosages of Daunorubicin. A 4 kg rabbit received i.v. 5 mg Daunorubicin daily for 4 consecutive days. At time shown by arrows, the rabbit received two BLR dosages (5 mg i.v. and 20 mg per os). The leukocytes count which was low in the drug-pretreated animal, was brought back to 10,000 within 48 h. After treating with BLRs, several doses of Daunorubicin were injected i.v. (see arrows). The same experiment was repeated on another rabbit. Platelet count were performed for both animals. Three control rabbits treated only with Daunorubicin died at the 6th to 9th day following the first daunorubicin injection.

4. No toxicity is ever observed. Numerously repeated BLR doses exhibit no cumulative effect and do not induce any tolerance phenomenon.

5. BLRs concentrate in the bone marrow and spleen. *In vivo*, they are not transcribed into DNA and, after priming DNA replication, they are removed from template and degraded (fig. 4).

In vivo assays were typically performed as follows:

New Zealand white rabbits were given daily, high dose CP injections (23-35 mg/kg). This alkylating agent currently used (but in proportionately much lower doses) for human cancer therapy exerts, like almost all antimitotic drugs, an adverse side effect on white blood cell (WBC) production (see fig. 2).

These high CP doses induced a progressive decrease in rabbit circulating leukocyte counts, which, starting from a mean value of 10 000/mm^3, were decreased to 3500-5000/mm^3 on day 8, i.e. to about half their normal value. If rabbits were not given BLRs, they eventually died on day 13 (2 out of 10 rabbits).

If, when leukocyte count was at its lowest, rabbits were injected i.v. with 1 mg/kg of BLRs in sterile physiological saline, leukocyte count increased and reached a normal value after 48 hours. WBC count remained for a few days at its highest normal value, then decreased on account of daily CP administration. Mean leukocyte count increase was 154 % ± 11 % ($P < 0.01$ by standard Student's test). BLR – induced leukocyte count modification lasted about 4 to 6 days. Another BLR injection was then required to ensure rabbit survival. BLR therapy could be repeated every seventh day for 2 or 3 month without loss of activity and without adverse side effects.

BLR-induced leukocyte count elevation never exceeded 300 % of the decreased count in CP-treated rab-

bits and 100% of normal count in untreated controls.

Very high BLR doses induced the same response as 0.3 mg/kg. This might be explained in two ways. It might be objected that BLRs simply release stored leukocyte. If this were true, it should be expected that, in rabbits treated long-term with very high CP doses, several consecutive BLR injections would empty leukocyte reserves and lead to the animal's death; but this is not the case. The alternative and true explanation is that BLRs, being analogous, if not wholly identical, to endogenous primers, respond to the body's powerful regulatory mechanisms. Leukocyte count elevation in the presence of BLRs is always seen to remain within physiological limits.

Further analysis of BLR activity at the cytological level indicated that they enhance leukocyte and platelet genesis. They are however inactive on erythropoiesis.

Corresponding results were obtained when BLRs were later used in man (perlingual route). Low doses are sufficient to protect patients undergoing radiotherapy or intensive chemotherapy: adverse haemotologic side effects are avoided and patients can lead a normal life, without even having to interrupt their work.

Antimitotic therapies do not need to be periodically suspended and thus become more efficient. Because they only act on normal blood stem cells, BLRs have also been used in leukemia patients (fig. 5).

Yet BLRs must be given sufficiently early during antimitotic therapy. When their protection is lacking, many cells are destroyed, and ribonucleases are synthesized to degrade cell debris. During longterm therapy, these enzymes become extremely abundant, so that when BLRs are given too late, they are degraded at once.

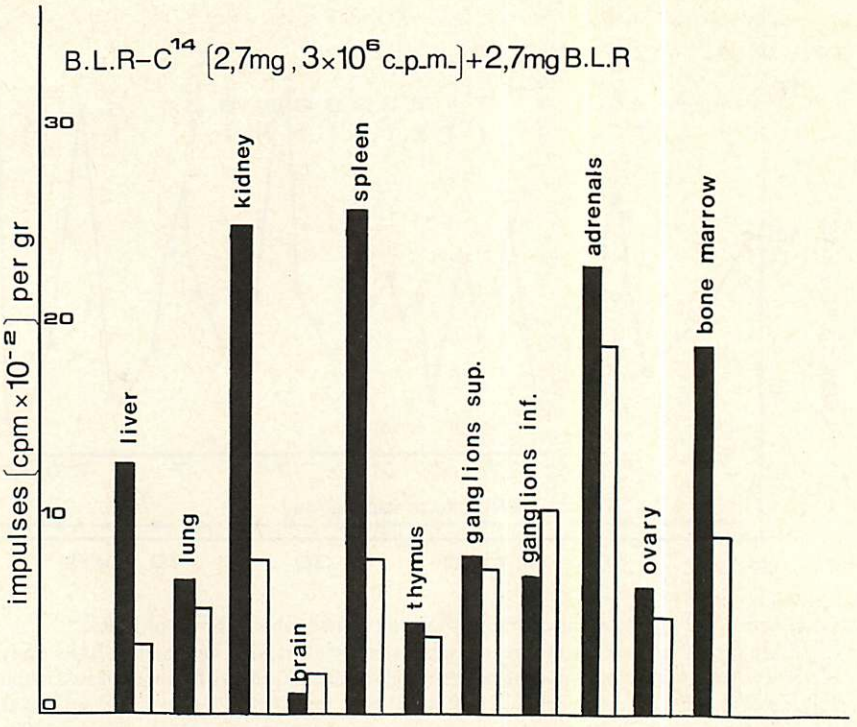

Fig. 4: *In vivo* localization of radioactive BLRs in the rabbit.
5 mg of (^{3}H)-BLRs(3x10^6 CPM)(labelled with (^{3}H)-guanine and (^{3}H)-uracil) are injected into a 3 kg normal rabbit. 24 h later, the animal is anesthesized and sacrificed. Samples of 0.2 to 0.5 g of organs are analysed for hot trichloroacetic acid soluble radioactivity (5% final conc. of TCA, 100°C, 30 min). The results (mean values for three rabbits) are expressed as CPM/g of wet material. Urine was collected from the bladder for radioactivity measurement.

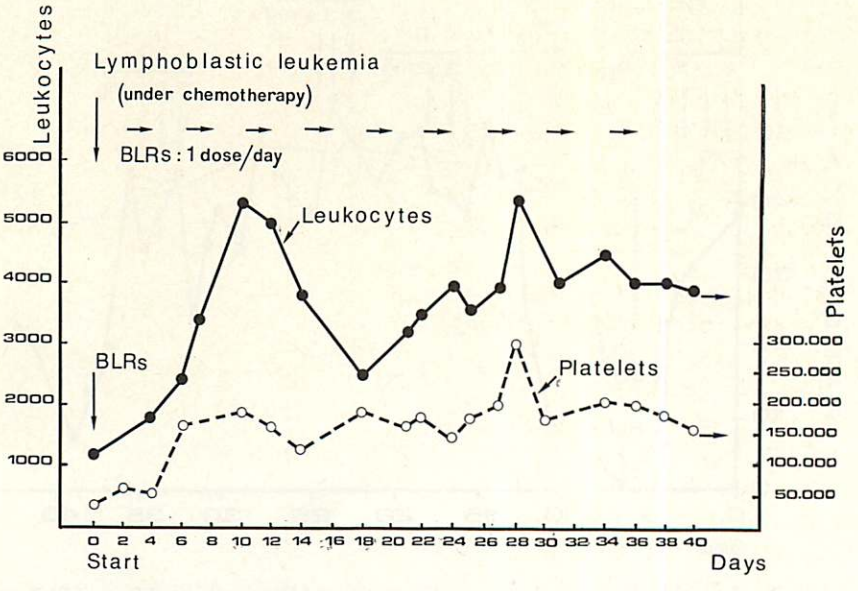

Fig. 5: Effect of BLRs in human lymphoblastic leukemia treated by chemotherapy.
Twenty years old female patient was treated by conventional chemotherapy. Once long term aplasia occured, BLRs have been administered by perlingual route as indicated in the figure (1 dose = 15 mg of BLRs).

It was recently proved that BLRs favor the production of natural killer (NK) lymphocytes which attack malignant cells. BLRs have also been

successfully used in patients suffering from congenital or pharmalogically induced medullary aplasia.

2. Discovery of selective and nontoxic anticancer drugs

A cell's behavior reflects that of its DNA: the rapid and uncontrolled mulitplication of cancer cells, and the synthesis of molecules which their normal counterparts would not produce, exactly match the biological behavior of their DNA, which undergoes enhanced replication and unprogrammed gene activation.

These characteristic properties of cancer DNA are classically accounted for by the occurrence of mutations in its nucleotide sequences, i.e. by primary structure alternations. Yet most of these mutations, which were extensively studied by the scientific community for the past thirty years, seem rather to result from the malignant process than to be its fundamental and universal cause.

On the other hand, cancer may be linked to modifications of DNA secondary structure, that is, to conformational alterations of the molecule; in this view, there is no need for mutations.

We came to propose such a model after reviewing the evidence collected over many years of extensive research on the compared replication and structure of cancer and normal DNAs [10].

In a normal DNA molecule, the strands separate, locally and briefly, for replication and for gene expression. Hydrogen bonds between the strands are temporarily broken. The more such "open" areas, the more the molecule absorbs 260 nm UV radiation. We demonstrated that UV absorption by cancer DNA far exceeds measures. This means that a cancer DNA molecule contains many more "open" areas than normal DNA ever does, even while it replicates or while its genes are being transcribed. The cancer DNA helix may be pictured as having wide areas which remain permanently untightened, with their hydrogen bonds broken; in a word, it is *destabilized* molecule. It should be noted that "open", i.e. distended areas of the cancer DNA, helix are flanked on either side-short overtight regions in which gene function and replication are hampered; this adds to the general imbalance of the cancer cells (fig. 6a + b).

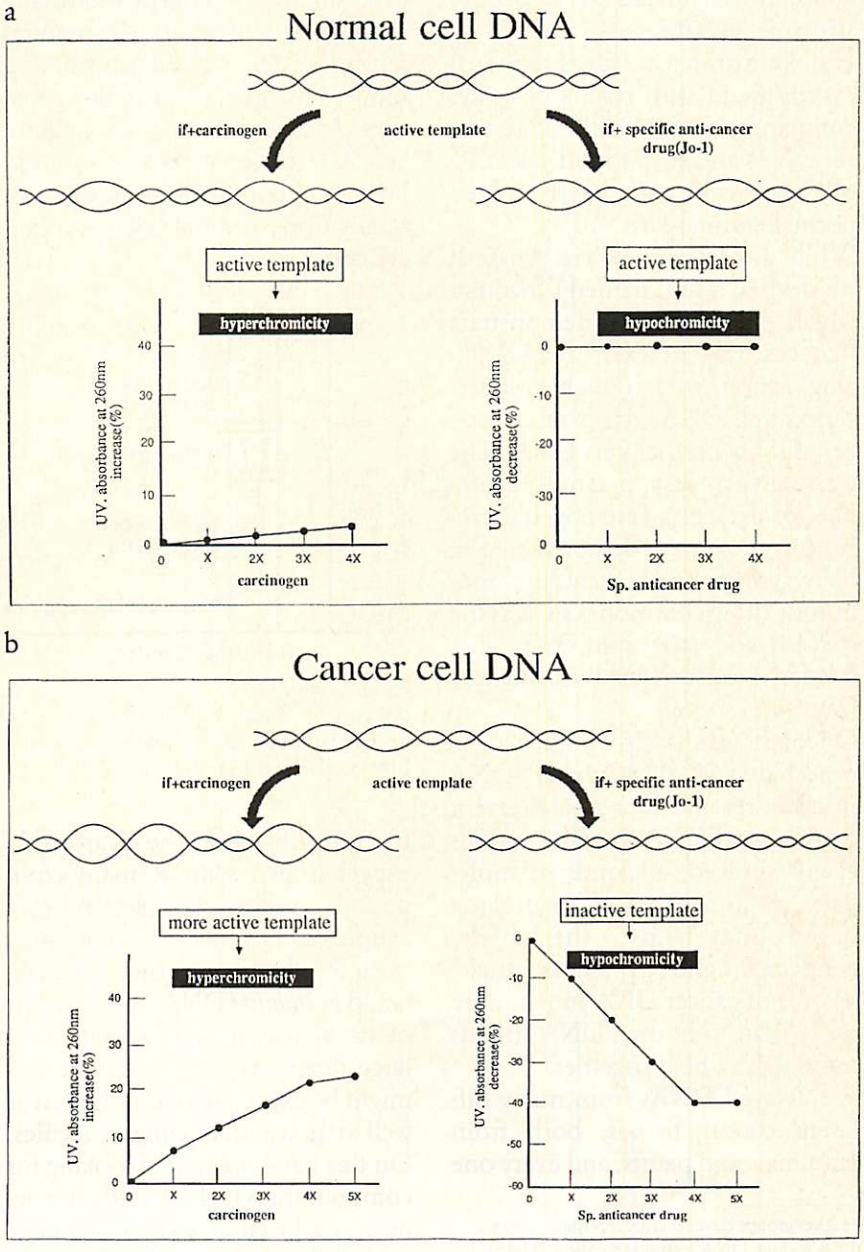

Fig. 6a+6b: *In vitro* behavior of DNA from normal and cancer tissues.
DNAs from human normal and cancer tissues were isolated by phenol and chloroform method as previously described (10). DNA was dissolved in 0.01 M Tris-HCl buffer solution (ph 7,5). The substances to be tested were dissolved in 10 ul of the same buffer and were then added to the blank and the DNA solution. The mixture was gently agitated at 20°C temperature and its UV spectrum determined. The concentrations inducing the maximal increase or/ and decrease in UV absorbance at 260 nm were determined (%).

In the "open" areas, new, normally unused initiation sites become exposed and available to enzymes involved in replication and in gene transcription; these initiation sites are then specific to cancer DNA. In addition, genes located in single stranded areas (i.e. where hydrogen bonds are broken) are more susceptible to mutations, which they appear as facilitated by secondary structure alterations.*

Hyperchromicity measurements exactly fit in with results of assays comparing *in vitro* synthesis of cancer DNAs and *in vivo* multiplication of cancer cells with those of their normal counterparts [10].

While this research was in progress, we devised a test, named Oncotest, which enabled us to demonstrate that carcinogens exert a destabilizing action, which highly affects cancer cell DNA, but is rarely perceptible in normal cell DNA. The Oncotest is a easy, practical technique for detecting carcinogenic properties; these were demonstrated in many compounds, including antimitotic drugs, hormones such as the steroids and calcitonin, as well as formerly unsuspected substances [10].

Oncotest results also point to the expected fact that cancer DNA reacts faster and to a greater extent than normal DNA to many various agents. Indeed, all kinds of molecules, prominent among them carcinogens, may bind to the loosely-connected, and in places single, strands of cancer DNA much more easily than to normal DNA strands more tightly held together.

We assayed DNAs from many different cancer tissues, both from mammals and plants, and every one of them proved to be destabilized. Carcinogens were shown to increase this prior destabilization in a progressive manner. Exposure of a DNA to several different carcinogens induces both additive and cumulative effects (fig. 7). This also applies to the slight destabilizing action exerted on normal DNAs, so that long-term and/or intensive exposure to carcinogens will finally overwhelm a normal cell's natural repair and defense mechanisms and induce its malignant transformation. *In vivo*, for instance, carcinogen A + carcinogen B + a steroid hormone will, in the long run, finally turn a normal cell into a cancer cell.

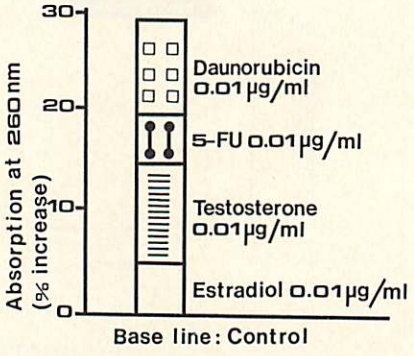

Fig. 7: Cancer DNA strand separation in the presence of various compounds. Human neurocarcinoma DNA strand separation [10](see also legend to fig. 6).

It could however be reasonably expected that, while so many compounds exerted a destabilizing action on DNA, other substances existed which, on the contrary, could *re-stabilize* DNA. And, in view of its increased susceptibility to a large range of agents, cancer DNA might be expected to respond just as well to these restabilizing molecules. On this basis, we started looking for compounds which would "recognize", i.e. be attracted to, the "open" areas of the destabilized cancer DNA, where the strands carrying the genes are largely unpaired, and would be able to "close" them by binding to the strands and bringing them closer together, back to their normal position. This "retightening" of the helix would restabilize the cancer DNA molecule, thus preventing unprogrammed replication and gene activation.

Our restabilizing molecules would also be required to bind preferentially to specific cancer DNA initiation sites, the majority of which, as we demonstrated, characteristically are rich in guanine (G) and adenine (A) nucleotides.

These two conditions would ensure that the restabilizing "bolt-molecules" would specifically act on cancer DNA and have no effect on normal DNA.

Using our Oncotest, we were able to discover such molcules. To this day, we found about half a dozen specific anticancer compounds which proved highly efficient *in vivo*. All are plant-derived and most are alkaloids, such as alstonine, serpentine, or sempervirine, obtained from various *Apocynacea*.

In vitro assays demonstrate that these specific anticancer drugs efficiently decrease cancer DNA hyperchromicity and inhibit its replication (as measured by synthesis of ^{3}H-labeled DNA) [19].

They are inactive on normal DNA replication. They were shown to have a high affinity for G- and A-rich nucleotides [19]. By binding to A- and G-rich specific cancer initiation sites, they induce either death of the malignant cell, which can no more multiply and express its genes, or recovery of cells in which the malignant process has not progressed so far that they are unable to recuperate.

In cell cultures conducted under identical conditions, cancer cell multiplication is inhibited by the anticancer drug, while normal cell multiplication is unaffected [20].

These results were obtained with mammalian cell lines and with human cells derived from cancer patients; a human chemotherapy-resistant leukemia clone was shown to respond to the anticancer drug. There was no sign of any toxic effect

* Other groups have recently reported that various drugs induce DNA strand separation [11-15]; for instance, CP induces *in vivo* strand separation, which lasts for 6 to 9 hours before the DNA molecule recovers its normal structure [14]. In a multidrug resistant Chinese hamster ovary cell line, overexpression and amplification of five genes has been observed [17]. Some drugs have been seen to facilitate tumor cell development *in vivo* [10, 16].

on bone marrow cells used as controls.

In vivo assays were carried out in mice bearing either YC lymphoma or *Ehrlich* carcinoma cells. In a high, or very high, percentage of mice, malignancies completely disappeared and no adverse side effects were detected [19].

Human patients were treated with these specific anticancer drugs, with similarly good results; no toxicity was observed.

Synergy with classic anticancer drugs

Our specific anticancer drugs may be used alone or together with conventional anticancer therapies. We demonstrated that low doses of antimitotic drugs destabilize cancer DNA [10, 11], and so do therapeutic radiation doses. By binding to A and G rich specific cancer initiation sites, they induce either death of the malignant cell, which can no more multiply and express its genes, or recovery of cells in which the malignant process has not progressed so far that they are unable to recuparate. This exposes initiation sites to which our anticancer drugs may bind. Thus paradoxically, the efficiency of our nontoxic drugs may be increased by synergistic association with conventional chemotherapeutic agents or radiation used in such limited doses that adverse side effects can be avoided. Enhanced efficiency of this association was first demonstrated in mice, then confirmed in human cancer patients.

Fig. 8 shows results of combined conventional + specific therapy in mice. Only long-term survival (100 days and over) was taken into account. Follow-up of the animals for 3 or 4 month after termination of treatment showed a high percentage

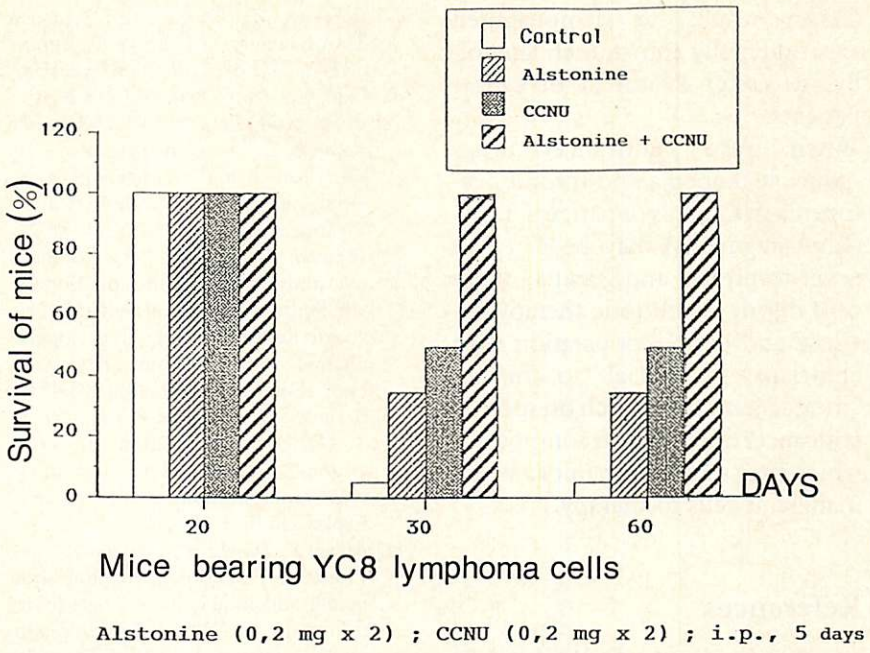

Fig. 8: Synergistic effect of alstonine with classic anticancer drug (CCNU).
Balb C mice bearing YC8 lymphoma cells were treated as described elsewhere [18].

of complete recovery with recuperation or normal health. Association of alstonine or sempervirine with either CCNU, CP, 5-FU or Daunorubicin gave excellent results and no side effects were observed.

Very encouraging results were similarly obtained in human patients treated with a combination of specific anticancer drugs and conventional chemo- or radiotherapy; no adverse side effects were noted.

Conclusion

In the last few years, the need for new, different approaches has made itself felt in cancer therapy, for the simple reason that, for a very long time already, no real improvement in the cure of this desease, particularly in adults, has been obtained with conventional therapies. So the scientific community recently began to look for specific, nontoxic anticancer compounds.

Yet already fifteen years ago, we had proposed a wholly novel approach to cancer therapy, using highly specific anticancer drugs. The activity of our nontoxic compounds of natural origin is targeted to the very root of the malignant condition, which is the destabilization of the cancer DNA molecules. Our "bolt-molecules" are restabilizing agents.

For this reason they differ from conventional therapeutic agents on several interdependent points:

1) They restabilize the destabilized cancer DNA.

2) They are efficient because they are specifically targeted to malignant cells.

3) They are not toxic because they have no effect on normal cells.

Lack of specificity for malignant cells, coupled to high toxicity, are the main drawbacks of conventional anticancer therapies, which, as we were able to demonstrate, further destabilize cancer DNA. Yet the fact that they increase cancer DNA destabilization may become an asset inasmuch as this enhances binding of our anticancer drugs to the malignant molecule, and the combined use of our specific anticancer compounds with limited doses of conventional therapies gives very satis-

It was recently reported that CP induces *in vivo* DNA strand separation, which lasts for 6 to 9 hours before the DNA molecule recovers its normal structure [14].

factory results, as demonstrated experimentally at first, then later on by successful treatment of cancer patients.

When classic anticancer drugs induce leukopenia and thrombocytopenia in animals or patients, BLRs (RNA-fragments) may be given in order to protect and generate these cells during antimitotic therapy.

Close and active cooperation with clinicians is crucial to further advancement of research on specific anticancer drugs and on compounds which may sensitize chemoresistant malignant cells to therapy.

References

[1] *I. J. Filder, Gernsten, D. M., Hart, I. R.*: The biology of cancer invasion and metastasis. In: *Klein, Weinhouse*: Advances on cancer research. Academic Press, New York 1978, pp. 149-250.

[2] *Giraldi, T., Sava, G.*: Selective antimetastatic drugs (review). Anticancer Res. 1 (1981) 163-174.

[3] *Braun, D., Harris, J. E.*: Modulation of the immune response by chemotherapy. Pharmac. Ther., 14 (1981) 89-122.

[4] *Sugino, A., Hirose, S., Okazani, R.*: RNA-linked nascent DNA fragments in Escherichia coli. Proc. Nat. Acad. Sci. USA 69 (1972) 1863-1867.

[5] *Beljanski, M., Beljanski, M. S., Plawecki, M., Bourgarel, P.*: ARN-fragments amorceurs nécessaires à la réplication "in vitro" des ADN. C.R.Acad.Sci. Paris 280 (1975) 363-366 (série D).

[6] *Chargaff, E.*: Initiation of enzymatic synthesis of deoxyribonucleic acid by ribonucleic acid primers. Prog. Nucl. Acid. Res. Mol. Biol. 16 (1976) 1-24.

[7] *Beljanski, M., Plawecki, M.*: Particular RNA-fragments as promoters of Leukocyte and Platelet formation in rabbits. Expl. Cell. Biol. 47 (1979) 218-225.

[8] *Beljanski, M., Plawecki, M., Bourgarel, P., Beljanski, M. S.*: Leukocytes Recovery with short-chain RNA fragments in cyclophosphamide-treated rabbits. Cancer Treatment Reports 67 (1983) 611-619.

[9] *Plawecki, M., Beljanski, M.*: Comparative study of Escherichia coly Endotoxin, hydrocortisone and Beljanski Leukocyte Restorers activity in cyclophosphamide-treated rabbits. Proc. Soc. Expl. Biol. Med. 168 (1981) 408-413.

[10] *Beljanski, M., Bourgarel, P., Beljanski, M. S.*: Correlation between in vitro DNA synthesis, DNA strand separtion and in vivo multiplication of cancer cells. Expl. Cell. Biol. 49 (1981) 220-231.

[11] *Mong, S., Daskal, Y., Prestayko, A. W., Crooke, S. T.*: DNA supercoiling shortening and induction of single-strand regions by cis-Diaminedichloroplatinium (II). Cancer. Res. 41 (1981) 4020-4026.

[12] *Neubort, S., Liebeskind, D., Mendez, F., Hsu, K. C., Bases, R.*: Morphological transformation, DNA strand separation and antinucleoside immunoreactivity following exposure of cells to intercalating drugs. Molex. Pharmacol. 21 (1982) 739-743.

[13] *Center, M. S.*: Induction of single-strand regions in nuclear DNA by adriamycin. Biochem. Biophys. Res. Commun. 89 (1979) 1231-1238.

[14] *Pillans, P. I., Ponzi, S. F., Parker, M. I.*: Cyclophosphamide induced DNA strand breaks in mouse embryocephalic tissue in vivo. Carcinogenesis. 10 (1989) 83-85.

[15] *Rebouleau, C. P., Shapiro, H. S.*: Chemical inducers of differentiation cause conformational changes in the cromatin and deoxyribonucleic acid murine erythroleukemia cells. Biochemistry. 22 (1983) 4512-4517.

[16] *De Ruiter, J., Cramer, S. J., Smink, T., Van Putten, L. M.*: The facilitation of tumor growth in the lung by cyclophosphamide in artificial and spontaneous metastasis models. Eur. J. Cancer. 15 (1979) 1139-1149.

[17] *Van der Bliek, A., Van der Velde-Koerts, T., Ling, V.*, et al.: Overexpression and amplification of five genes in a multidrug resistant chinese hamster ovary cell line. Mol. Cell. Biol. 6 (1986) 1671-1678.

[18] *Beljanski, M., Beljanski, M. S.*: Three Alkaloids as selective Destroyers of cancer cells in mice. Synergiy with classic anticancer drugs. Oncology. 43 (1986) 198-203.

[19] *Beljanski, M., Beljanski, M. S.*: Selective inhibition of in vitro sythesis of cancer DNA by alkaloids of B carboline class. Expl. Cell. Biol. 50 (1982) 79-87.

[20] *Beljanski, M., Beljanski, M. S.*: Three alkaloids as selective destroyers of the proliferative capacity of cancer cells. IRCS, Med. Sci. 12 (1984) 587-588.

Postscript:

New data supporting our new approach in cancer therapy are described in the following book:

Anticancer Drugs. Eds. *Tapiero, H., Robert, J., Lampidis, Th. J.* eds.: Colloque Inserm, 191 (1989) 1-351. Les Editions INSERM. John Libbey Eurotext, London-Paris.

Correspondence to:

Prof. Dr. *M. Beljanski*, Centre de Recherche Biologique (CERBIOL), F-94200 Ivry-Sur-Seine.