Cancer Chemother. Pharmacol. 2, 77–79 (1979)

Cancer Chemotherapy and Pharmacology

Daunorubicin-DNA and Doxorubicin-DNA A Review of Experimental and Clinical Data

A. Trouet and D. Deprez-De Campeneere

International Institute of Cellular and Molecular Pathology and Université de Louvain, 75, avenue Hippocrate, Brussels, Belgium

Summary. *Experimental data on the pharmacokinetic, toxic and therapeutic properties of daunorubicin-DNA and doxorubicin-DNA complexes are reviewed and summarized as well as the available reports on clinical trials performed with these anthracycline-DNA complexes.*

These results are discussed in view of the further development of the drug-carrier concept of cancer chemotherapy.

Introduction

In this review we will attempt to summarize and to discuss the data available on the DNA-anthracycline (daunorubicin and doxorubicin) complexes seen from an experimental and a clinical standpoint. We will try to draw some provisional conclusions about the advantages and disadvantages of the DNA complexes, discuss the results in view of the lysosomotropic or drug-carrier concept [23] and finally enumerate some guidelines for the development of improved drug-carrier complexes.

Experimental Data

Experimental chemotherapeutic activity of daunorubicin (DNR) and doxorubicin (DOX) can very clearly be increased by administering the drugs in combination with high molecular weight DNA [8, 9, 24, 25]. The main therapeutic results, obtained in L 1210 leukemia, have shown that DOX-DNA is significantly more active than DNR-DNA.

The increase in activity observed with the DNA complexes, however, is only significant in tumor cells which are sensitive to DNR or to DOX. Binding to DNA does not make it possible for drug resistance to be overcome, and experiments on an L 1210 cell line, less sensitive to DOX, did not show a great improvement in the drug activity after binding to DNA [1].

After intravenous (IV) administration into mice, only the toxicity of DOX is reduced, in terms of LD_{50}, while the overall toxicity of DNR-DNA is equal to that of DNR [9]. DNR-DNA is more toxic in mice against the hemopoietic stem cells than DNR, while the effect of DOX-DNA is somewhat lower than that of DOX [9, 26].

These important differences between DNR-DNA and DOX-DNA are difficult to understand on the basis of the very comparable affinity of DNR and DOX for DNA, as determined in vitro [20]. They can be explained, however, on the basis of the distinct pharmacokinetic properties of the two DNA complexes [9, 26]. DNR-DNA is indeed unstable in the bloodstream after IV injection and is very rapidly dissociated into free DNR and DNA. DNR-DNA behaves much more like a slow-release prodrug of high molecular weight. The higher plasma levels obtained by combining DNR with DNA might explain the higher chemotherapeutic activity, while the availability of free DNR in the bloodstream helps to determine why the complex is as toxic as the free drug. In contrast, DOX-DNA seems to leave the bloodstream as a complex and thus better meets the requirements of a stable drug-carrier. The very much increased plasma levels achieved with DOX-DNA combined with a better stability of the complex might explain both its increased activity and its decreased toxicity. To understand a decreased toxicity in presence of increased drug levels, we must assume that normal cells, like hemopoietic stem cells, take up less DNA-complexed DOX than free drug. DNR-DNA, being a high molecular weight prodrug of DNR and being slowly released in the bloodstream, can be compared with rubidazone, which turns out to be a low molecular weight prodrug of DNR [3], with distinct toxic and therapeutic properties.

Reprint requests should be addressed to: A. Trouet

0344-5704/79/0002/0077/$ 01.00

We have to point out also that for DNR-DNA, DOX-DNA, and rubidazone as well, there is a significant decrease in accumulation of the drugs by heart tissue [3, 6, 9]. This observation gives experimental support in favor of the decreased cardiotoxicity of anthracycline-DNA complexes [11, 12], if, as we can expect, this toxicity is related to the amount of drug accumulated in the heart.

Although DOX-DNA is a stable complex in the bloodstream, we cannot determine the exact mode of its penetration into cells. The results obtained in vivo and in vitro on cultivated cells are compatible with a penetration of the complex by endocytosis with an intralysosomal hydrolysis of DNA and a release of the drug. Other mechanisms of entry, however, cannot be excluded and should carefully be investigated. DOX could first be released from its carrier in the extracellular space by deoxyribonucleases secreted by the cells or present at their surface. But, most important, DOX could dissociate from DNA by a dilution effect and this latter possibility has to be considered very carefully in view of the data [20] indicating that most of DNR and DOX is released from carrier DNA when the concentration of the DNA complex in the medium and in presence of serum approaches or falls below 0.1 μg/ml (expressed as drug concentration). This means that when the concentration of DOX-DNA in the extracellular and interstitial spaces approaches or falls below 0.1 μg/ml, DOX will be released as a free drug and enter cells as such. As a consequence, it is possible that a drug uptake through endocytosis could occur in tissues where the extracellular concentration of the complex is high and where cells are directly or very closely in contact with the bloodstream (bone marrow, spleen, liver). An uptake of drugs could occur simultaneously by diffusion in the tissues where the extracellular concentration of the complexes is very low. This latter situation is likely to occur in tissues where the passage of the complex from the bloodstream to the interstitial space is very slow because of capillary barriers relatively impermeable to high molecular weight DNA.

Similar considerations are valid for interpreting the experiments studying the interaction (drug uptake; cytotoxicity) of DNA-bound drugs and cells in vitro [2, 4, 19, 21]. At a drug DNA concentration exceeding 0.1 μg of drug/ml, the effects analyzed will concern most probably the interaction of cells with DNA-bound drugs, while at lower concentration, effects of free drug released from DNA will be studied.

Clinical Data

In the last few years, a variety of clinical results obtained with anthracycline-DNA complexes have been reported [5, 7, 10, 13–18, 22], but only recently have results been published about randomized trials [5, 10].

In agreement with the results obtained on L 1210 leukemia, it appears that given in combination with other drugs in acute lymphoblastic leukemia, DOX-DNA is significantly more active than DNR-DNA in giving long-term remissions [10]. No significant differences could be found between DNR and DNR-DNA during the treatment of acute nonlymphoblastic leukemia when given in combination with cytosine arabinoside [10]. On the other hand, DOX-DNA seems to be as active as DOX in the treatment of bronchogenic carcinoma [5].

Fragmentary data indicate moreover that very good results can be obtained with DOX-DNA in the treatment of acute nonlymphoblastic leukemia and of a variety of childhood solid tumors [15], of metastatic breast carcinoma [16], and of non-Hodgkin lymphomas [18].

From the experimental results and the available clinical data, it is obvious that what is urgently needed is a randomized study of the activity of DOX-DNA in various hematologic neoplasia, with special emphasis on acute nonlymphoblastic leukemia. A variety of solid tumors, such as breast carcinoma, should be studied similarly.

The toxicity of DOX-DNA and DNR-DNA seems to be very similar to that of the free drug as far as the bone marrow, the gastrointestinal tract, and the mucosae are concerned. It is becoming more and more evident, however, on the basis of the 588 patients treated with DNA complexes, that the cardiotoxicity of DNR and of DOX can be reduced when administered in combination with DNA. Of these patients, 54 received a cumulative dosis of DOX exceeding the critical dosis of 550 mg/m^2, and no cardiac failures related to the administration of DOX were observed. In 16 patients who received more than 500 mg of DOX-DNA/m^2, Lie et al. [15] could not find any heart lesions at autopsy.

No evidence has been reported concerning the occurrence of allergic reactions and the presence of anti-DNA antibodies.

Conclusions

The experimental results show clearly that the toxicity and the chemotherapeutic activity of DNR and DOX can be modified by associating these drugs with high molecular weight DNA. The most stable complex is obtained in vivo with DOX, and this DOX-DNA combination gives the most striking experimental results, yielding both lower toxicity and higher therapeutic efficiency. The clinical data clearly suggest that the activity of DNR and DOX is not decreased by association with DNA, and that their cardiotoxicity could be reduced. But systematic trials with DOX-DNA are needed in

order to determine whether this complex really has a therapeutic advantage over DOX.

It is also evident that a lot of research and development is needed in order to obtain a drug-carrier complex which would be truly 'tumor cell specific'. We can summarize the lines of further research in this difficult, but promising, field as follows:

1. Development of drug-DNA complexes with a higher in vivo stability. Preliminary results obtained in our laboratory with detorubicin-DNA suggest that this complex is more stable than DOX-DNA.

2. Development of linkage methods enabling drugs to be bound covalently to a variety of carriers. Such a bind should be stable in vivo and enable the drug to be released in an active form by physical (pH) or enzymatic processes at the target site, i.e., after endocytosis.

3. Suitable carriers which interact selectively with tumor cells should be looked for or synthesized [23].

Acknowledgements: This work has been supported by the Fonds Cancérologique de la Caisse Générale d'Epargne et de Retraite, Brussels, Belgium, and by Rhone-Poulenc, Paris, France.

References

1. Atassi, G., Tagnon, H. J., Trouet, A.: Comparison of adriamycin with the DNA-adriamycin complex in chemotherapy of L 1210 leukemia. Eur. J. Cancer **10,** 399 (1974)
2. Barlogie, B., Drewinko, B., Benjamin, R. S., Freireich, E. J.: Kinetic response of human lymphoid cells to adriamycin-DNA complex in vitro. Cancer Res. **36,** 2450 (1976)
3. Baurain, R., Deprez-De Campeneere, D., Trouet, A.: Distribution and metabolism of rubidazone and daunorubicin in mice: A comparative study. Cancer Chemother. Pharmacol. **2,** 37–41 (1979)
4. Beran, M., Andersson, B., Eksborg, S., Ehrsson, H.: Comparative studies on in vitro killing of human normal and leukemic clonogenic cells (CFU_c) by daunorubicin, daunorubicinol and daunorubicin-DNA complex. Cancer Chemother. Pharmacol. **2,** 19–24 (1979)
5. Bosly, A., Prignot, J., Ledent, C., Sokal, G., Trouet, A.: Adriamycin and adriamycin-DNA in inoperable bronchogenic carcinoma. A randomized study with cyclophosphamide vinblastine. Eur. J. Cancer **14,** 639 (1978)
6. Brown, I., Ward, H. W. C.: The comparative toxicity and therapeutic efficacy of adriamycin and the adriamycin-DNA complex in the chemotherapy of C_3H mice with transplanted mammary adenocarcinoma. Cancer Lett. **2,** 227 (1977)
7. Cornu, G., Michaux, J. L., Sokal, G., Trouet, A.: Daunorubicin-DNA: Further clinical trials in acute non-lymphoblastic leukemia. Eur. J. Cancer **10,** 695 (1974)
8. Deckers, C., Mace, F., Deckers-Passau, L., Trouet, A.: Antitumor effect of adriamycin and adriamycin-DNA complex in rat immunocytoma and leukemia. In: Adriamycin review. Staquet, M. et al. (eds.), p. 79. Ghent, Belgium: European Press Medikon 1975
9. Deprez-De Campeneere, D., Baurain, R., Huybrechts, M., Trouet, A.: Comparative study in mice of the toxicity, pharmacology and therapeutic activity of daunorubicin-DNA and doxorubicin-DNA complexes. Cancer Chemother. Pharmacol. **2,** 25–30 (1979)
10. Ferrant, A., Hulhoven, R., Bosly, A., Cornu, G., Michaux, J. L., Sokal, G.: Clinical trials with daunorubicin-DNA and doxorubicin-DNA in acute lymphoblastic leukemia of childhood, acute nonlymphoblastic leukemia and bronchogenic carcinoma. Cancer Chemother. Pharmacol. **2,** 67–71 (1979)
11. Langslet, A., Øye, I., Lie, S. O.: Decreased cardiac toxicity of adriamycin and daunorubicin when bound to DNA. Acta Pharmacol. Toxicol. (Kbh.) **35,** 379 (1974)
12. Langslet, A., Øye, I., Lie, S. O.: Reduction of the immediate cardiac toxicity of adriamycin and daunorubicin when bound to DNA. In: Adriamycin review. Staquet, M. et al. (eds.), p. 143. Ghent, Belgium: European Press Medikon 1975
13. Lie, S. O., Lie, K. K., Langslet, A.: Experiences with DNA-adriamycin complex in malignant disorders of childhood. In: Adriamycin review. Staquet, M. et al. (eds.), p. 226. Ghent, Belgium: European Press Medikon 1975
14. Lie, S. O., Lie, K. K., Glomstein, A.: Adriamycin-DNA complex: A new principle in the therapy of childhood malignancies (Meet. Abstr.). Pediatr. Res. **11,** 1019 (1977)
15. Lie, S. O., Lie, K. K., Glomstein, A.: Clinical and pharmacologic studies with adriamycin-DNA complex in children with malignant disease. Cancer Chemother. Pharmacol. **2,** 61–66 (1979)
16. Longueville, J., Maisin, H.: Combined therapy with adriamycin-DNA, vincristine and medroxy-progesterone acetate in metastatic breast cancer. In: Adriamycin review. Staquet, M. et al. (eds.), p. 260. Ghent, Belgium: European Press Medikon 1975
17. Michaux, J. L., Cornu, G., Trouet, A., Sokal, G.: Clinical trials with the daunorubicin-DNA complex in human leukaemia. A preliminary report. In: Therapy of acute leukemias, p. 815. Proc. Int. Meet., Rome, December 6–8, 1973
18. Michaux, J. L., Cornu, G., Sokal, G., Trouet, A.: Preliminary clinical trials with the adriamycin-DNA complex in human leukemias and non-Hodgkin lymphomas. In: Adriamycin review. Staquet, M. et al. (eds.), p. 216. Ghent, Belgium: European Press Medikon 1975
19. Peterson, C., Noel, G., Zenebergh, A., Trouet, A.: Uptake of daunorubicin-DNA complex in cultured fibroblasts. Cancer Chemother. Pharmacol. **2,** 3–6 (1979)
20. Schneider, Y.-J., Baurain, R., Zenebergh, A., Trouet, A.: DNA binding of daunorubicin and doxorubicin in the conditions used for studying the interaction of anthracycline-DNA complexes with cells in vitro. Cancer Chemother. Pharmacol. **2,** 7–10 (1979)
21. Seeber, S., Brucksch, K. P., Seeber, B., Schmidt, C. G.: Cytostatic efficacy of DNA complexes of adriamycin, daunomycin and actinomycin D. I. Comparative studies in Novikoff hepatoma, human mammary carcinoma cells and human leukemic leukocytes. Krebsforsch. Klin. Onkol. **89,** 75 (1977)
22. Sokal, G., Trouet, A., Michaux, J. L., Cornu, G.: DNA-daunorubicin complex: Preliminary trials in human leukaemia. Eur. J. Cancer **9,** 391 (1973)
23. Trouet, A.: Increased selectivity of drugs by linking to carriers. Eur. J. Cancer **14,** 105 (1978)
24. Trouet, A., Deprez-De Campeneere, D., de Duve, C.: Chemotherapy through lysosomes with a DNA-daunorubicin complex. Nature [New Biol.] **239,** 110 (1972)
25. Trouet, A., Deprez-De Campeneere, D., De Smedt-Malengreaux, M., Atassi, G.: Experimental leukemia chemotherapy with a 'lysosomotropic' adriamycin-DNA complex. Eur. J. Cancer **10,** 405 (1974)
26. Trouet, A., Deprez-De Campeneere, D., Baurain, R., Huybrechts, M., Zenebergh, A.: Desoxyribonucleic acid as carrier for antitumoral drugs. In: Carriers in biology and medicine. Gregoriadis, G. (ed.). (in press, 1978)