MECHANISMS OF RESISTANCE TO METHOTREXATE

Pages with reference to book, From 348 To 350

M. Perwaiz Iqbal (Department of Biochemistry, The Aga Khan University Medical College, Karachi.)

Methotrexate (MTX) is a folate antagonist which kills the proliferating cell by binding tightly to the enzyme dihydrofolate reductase (DHFR). Due to this binding the pathway of de novo DNA synthesis is blocked[1-3]. The drug has produced spectacular results controlling choriocarcinoma, Burkitt's lymphoma, acute leukemia and-psoriasis. But continued administration to patients often results in emergence of drug-resistance. Because of this problem investigators have, over the past two decades, studied a variety of possible mechanisms for the development of resistance. As a result of these intensive studies, five mechanisms have been put forward.

The first mechanism involves a structural change in the enzyme, DHFR, such that the normal high affinity for MTX is lost. When this happens the enzyme would not be inhibited by the drug. Evidence for this mechanism has been reported by a number of laboratories[4-9] . Albrecht and his associates by a multi-step selection procedure have isolated a methotrexate-resistant Chinese hamster cell line which contained structurally altered DHFR[9]. The mutant enzyme had a single pH optimum for the reduction of dthydrofolate as compared to double pH optima observed in the wild-type enzyme. The mutant enzyme was also found to have a dramatically altered affinity for MTX. It was shown that to achieve a 50% inhibition of the enzyme from the mutant cells, a 10-fold excess of MTX was required when compared to the amount of drug required to achieve a 50% inhibition of enzyme activity in parental cells.

In the second mechanism there is a structural change in a transport protein for MTX which is located in the surface membrane of the cell. In this case, the uptake of the drug by the cell may be remarkably reduced and the enzyme inside the cell again would not be inhibited. With regards to this mechanism of resistance, evidence has been provided by Sirotank and his associates who succeeded in isolating mutants of D. pneumoniae exhibiting levels of resistance to MTX of up to 100-fold as compared to the wild type strain[10].

The uptake of the drug by these mutants was remarkably slow and far less than the wild type indicating that the resistance was due to changes in the transport system of the drug. Similar evidence has been presented by Hill and his associates[11].

The third possible mechanism is an increase in the levels of DHFR in the cells. The sensitivity of the enzyme towards the drug remains the same. However there would be an excess of the enzyme with respect to the concentration of the drug in the cells, and some of the enzyme would be available to reduce dihydrofolate into tetrahydrofolate and hence, the pathway of de novo DNA synthesis would continue. Evidence for this mechanism of resistance has been reported by a number of laboratories[12-24] Schimke, Bertino and their associates have shown that although the increased level of the enzyme, DHFR, can be due to decreased catabolism of the enzyme due to its stabilization as a result of binding to the inhibitor, yet enzyme induction is the major cause of this increase in the enzyme levels[12] This enzyme induction takes place as a result of gene amplification, the process whereby a small part of the genome, representing one or more genes, is duplicated locally within a chromosome[12,13] Such an amplification of genes is stable if it is localized in a specific region of a chromosome or unstable if localized in the nucleus as extrachromosomal DNA[13,25].

The fourth possible mechanism of drug resistance is decreased polyglutamation of MTX inside the cell. Since polyglutamation facilitates longer retention of the drug by the cells, a decrease in that would result in rapid efflux of the drug and the enzyme, DHFR, would not be completely inhibited by the drug. Cowan and Jolivet have shown that the resistance to MTX exhibited by a human breast cancer

cell line was due to decreased formation of MTX polyglutamates in these cells[26].
The fifth possible mechanism of resistance to methotrexate is due to the induction of another molecular form of diliydrofolate reductase exhibiting low affinity for methotrexate. There is some evidence that methotrexate resistant murine leukemia cells have increased levels of a form of DHFR having low affinity for MTX[27,28].
Overproduction of this low affinity form of enzyme has also been shown in cultured mouse fibroblasts and Chinese hamster cells[29,30].
These studies for the elucidation of the mechanism of resistance to MIX are important because they give us a basis for a better understanding of general mechanisms of drug resistance. They also provide us with a rationale for the principles of cancer chemotherapy. For example, combination chemotherapy in most neoplastic diseases is usually considered to be superior to single drug treatments because in combination chemotherapy most of the neoplastic cells are likely to be killed and the chances for the development of resistance to the drugs are considerably reduced. It also tells us that a treatment should be carried out only as long as is necessary and the thug should be immediately changed if resistance develops. Over exposure of the cells with the drug is going to impart a stable form of resistance to the drug and, hence, must be avoided.

REFERENCES

1. Osborn, M.J., Freeman, M. and Huennekens, F.M. Inhibition of dihydrofolic reductase by aminopterin and amethopterin. Proc. Soc. Exp. Blot. Med., 1958;97: 429.
2. Whaler, R J. Anticancer agents and nucleic acid metabolism of isolated human leukocytes, in the leukemias, etiology, pathophysiology and treatment. Henry Ford Hospital International Symposium. Edited by Rebuck, J.W., Bethel, F.H. & Monto, R.W. New York, Academic Press, 1957; p. 567.
3. Dunlap, R.B., Harding, N.G. and Huennekens, F.M. Thymidylate synthetase and its relationship to dihydrofolate reductase. Ann. N.Y. Acad. Sci., 1971;186: 153.
4. Hanggi, U.S. and Littlefield, .T.W. Isolation and characterization of the multiple forms of dihydrofolate reductase from methotrex ate-resistant hamster cells. S. Biol. Chem., 1974; 249: 1390.
5. Flintoff, W.F. and Essani, K. Methotrexateresistant Chinese hamster ovary cells contain a dihydrofolate reductase with an altered affinity for methotrexate. Biochemistry, 1980;19:4321.
6. Sirotnak, F.M., Donati, G.J. and Hutchison, D.J. Dihydrofolate reductase in genotypically distin guishable amethopterin resistant Diplococcus pneumoniae. S. Biol. Chem., 1964; 239: 2677.
7. Albrecht, A.M., Palmer, J.L. and Hutchison, D.J. Differentiating properties of dthydrofolste reductases of amethopterin-resistant streptococcus faecalis/Ak and the sensitive parent strain. J. Biol.Chem., 1966;241: 1043.
8. Fan C.C., Vitols, K.S. and Huennekens, F.M. Inhibition of dihydrofolate reductase by metho trexate; a new look at an old problem. Adv.Enzyme Regul., 1980; 18: 41.
9. Albrecht, A.M., Biedler, J.L. and Hutchison, D.J.Two different species of dihydrofolate reductas in mammalian cells differently resistant to amethropterin and methasquin. Cancer Res., 1972; 32: 1539.
10. Blakley, ltL. The Biochemistry of folic acid and related pteridines. London, North Holland IishingCo, l969;p. 501.
11. Hill, B.T., Bailey, B.D., White, S.C. and Goldman I.D. Characteristics of transport of 4-amino antifolates and folate compounds by two lines of L5 178Y lymphoblasts, one with impaired transport of methotrexate. Cancer Res., 1979; 39: 2440.
12. Schimke, RT., Kaufman, R.S., Alt, F.W. and Kellems, R.F. Gene amplification and drug resistance in cultured murine cells. Science1978;202: 1051.
13. Schimke, R.T. Gene amplification and thug resis tance. Sci.Am., 1980;243:60.
14. Barsoum, S., Levinger, L. and Varshavsky, A. On the chromatin structure of the amplified,

transcriptionally active gene for dihydrofolate reductase in mouse cells. J. Biol. Chem., 1982; 257: 5274.
15. Bostock, C.J. and Clark, E.M. Gene amplification in metotrexate-resistant mouse cells. V. Intact amplified units can be transferred to and amplified in metotrexate sensitive mouse L cells. Chromo soma, 1983;88: 31.
16. Brown, P.C., Tlsty, T.D. and Schimke, R.T. Enhancement of methotrexate resistance and dihydrofolate reductase gene amplification by treatment mouse 3T6 cells with hydroxyurea. Mol. Cell. Biol., 1983; 3: 1097.
17. Cowan, K.H., Goldsmith, M.E.. Levine, R.M., Aitken, S.C., Douglass, E., Clendeninn, N., Nienhuis, A.W. and Lippman, M.E. Dihydrofolate reductase gene amplification and possible rearrangement in estrogen-responsive methotrexateresistant human breast cancer cells. J. Biol. Chem., 1982;257: 15079.
18. Crouse, G.F., Simonsen, C.C., McEwan, R.M. and Schimke, R.T. Structure of amplified normal and variant dthydrofolate reductase genes in mouse sarcoma Sl80 cells. J. Biol. Chem., 1982; 257: 7887.
19. Domin, B.A., Grill, S.P. and Cheng, Y. Establishment of dihydrofolate reductase-increased human cell lines and relationship between dihydrofolate reductase levels and gene copy. Cancer Res., 1983;43: 2155.
20. Flintoff, W.F., Weber, M.K., Nagainis, C.R., Essani, A.K., Robertson, D. and Salser, W. Overproduction of dthydrofolate reductase and gene amplification in methotrexate-resistant Chinese hamster ovary cells. Mo1. Cell. Biol., 1982; 2: 275.
21. Flintoff, W.F., Livingston, E., Duff, C. and Worton, R.G. Moderate level gene amplification in methotrexate-resistant Chinese hamster ovary cells is accompanied by chromosomal translocations at or near the site of the amplified DHFR gene. Mol. Cell. Biol., 1984;4: 69.
22. Lewis, J.A., Biedler, J.L. and Melera, P.W. Gene amplification accompanies low level increases in the activity of dihydrofolate reductase in antifolate-resistant Chinese hamster lung cells containing abnormally banding chromosomes. J. Cell. Biol., 1982;94: 418.
23. Mullner, F., Hofbauer, R. and Wintersberger, E. Increased levels of dihydrofolate reductase mRNA can be measured in normal, growth-stimulated mouse fibroblasts. Biochem. Biophys. Acta, 1983; 740: 436.
24. Trent, J.M., Buick, R.N., Olson, S., Horns, R.C. Jr. and Schimke, R.T. Cytologic evidence for gene amplification in methotrex ate-resistant cells obtained from a patient with ovarian adenocarcinoma. J. Clin. Oncol., 1984; 2: 8.
25. Kaufman, R.J., Brown, P.C. and Schimke, R.T. Amplified dihydrofolate reductase genes in unstably methotrex ate-resistant cells are associated with double minute chromosomes. Proc. Natl. Acad. Sci. USA., l979;76: 5669.
26. Cowan, K.Fl., and Jolivet, J. A noval mechanism of resistance to methotrexate in human breast cancer cells: lack of methotrexate polyglutamate formation. Clin. Res., 1983; 31: 508a.
27. Dedhar, S. and Goldie, J.H. Overproduction of two antigenically distinct forms of dihydrofolate reductase in a highly methotrexate-resistant mouse leukemia cell line. Cancer Res., 1983; 43: 4863.
28. Duffy, T.H., Beckman, S.B. and Huennekens, F.M. Multiple forms of L1210 dihydrofolate reductase differing in affinity for methotrexate. Biochem. Biophys. Res. Commun., 1984; 119: 352.
29. Haber, D.A., Beverley, S.M., Kiely, M.L. and Schimke, R.T. Properties of an altered dihydrofolate reductase encoded by amplified genes in cultured mouse fibroblasts. J. Biol. Chem., 1981;256: 9501.
30. Melera, P.W., Lewis, J.A., Biedler, J.L. and Hession, C. Antifolateresistant Chinese hamster cells; evidence for dlliydrofolate reductase gene amplification among independently derived sublines overproducing different dihydrofolate reductases. J. Biol. Chem., 1980; 255: 7024.