EFFECTS OF ANTITUMOR AGENTS ON DNA SYNTHESIS OF MITOGEN-INDUCED HUMAN LYMPHOCYTES, IN COMPARISON WITH LEUKEMIC CELLS

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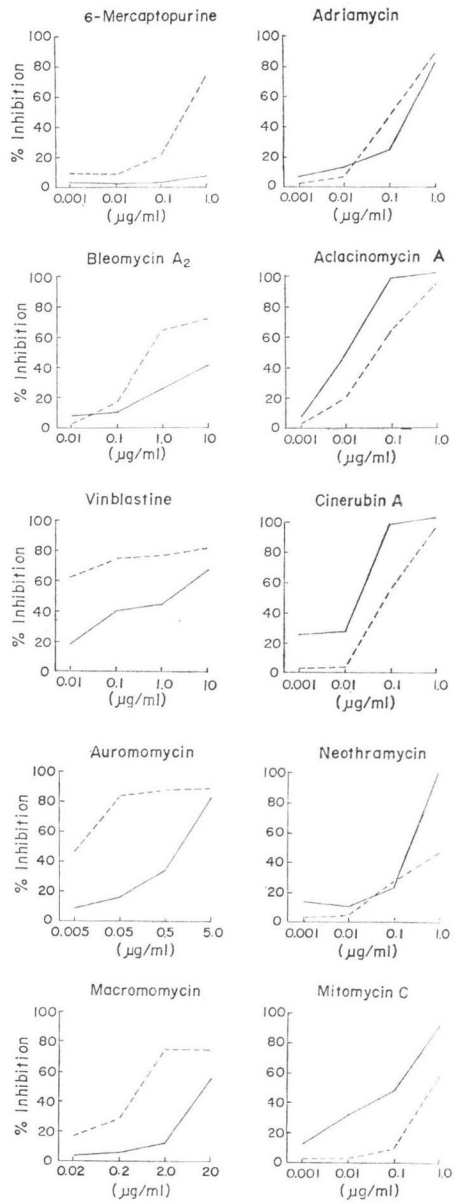
Most tumor-inhibitory drugs are known to cause bone marrow damage and immunosuppression. As one of our basic investigations on these side actions, we have examined the effects of various anticancer substances on thymidine uptake of human blood cells, particularly lymphocytes, induced by phytohemagglutinin (PHA), and compared with the effects on DNA synthesis of leukemic cells of human origin (K-562). Certain aspects of selective toxicity of the drugs have been observed with these two kinds of cells. The results are presented in this publication.

Antitumor agents used were bleomycin A_2 (Nippon Kayaku Co.), mitomycin C and adriamycin (Kyowa Hakko Kogyo Co.), aclacinomycin A and cinerubin A (Sanraku Ocean Co.), neothramycin (Meiji Seika Kaisha, Ltd.), macromomycin and auromomycin (Kanegafuchi Chemical Industry Co.), vinblastine (Shionogi & Co.), and 6-mercaptopurine (Kohjin Co.). [^{3}H] Thymidine (20 Ci/mmol) was a product of New England Nuclear, Boston, Mass. Bacto phytohemagglutinin P was purchased from Difco Lab., Detroit, Michigan, RPMI 1640 from Gibco Lab., Grand Island, New York, and Ficoll-Paque from Pharmacia Fine Chemicals, Uppsala, Sweden. Human chronic myeloid leukemia K-562 cells were generously given by Prof. KOHEI NAKANO, Jichi Medical School, Tochigi.

Human peripheral blood was obtained from healthy adults, and divided into two parts: one was used for a blastogenesis test, and plasma was separated from the other portion and used for leukemic cell growth.

The whole blood was suspended in 9-fold volume of RPMI 1640 medium, containing 10% heat-inactivated fetal calf serum, benzylpenicillin (100 units/ml), streptomycin (100 μg/ml) and PHA (20 μg/ml); and was distributed (0.2 ml each) into microwells (Nunc, Denmark) with or without antitumor drugs. The blood cells were incubated at 37°C for 48 hours in a 5% CO_2 and 95% air atmosphere, and [^{3}H] thymidine (0.2 μCi/well) was then incorporated for 24 hours. The cells were collected and washed with distilled water in a multiple automated sample harve-

Fig. 1. Effects of antitumor agents on [^{3}H] thymidine uptake by K-562 leukemic cells and PHA-activated lymphocytes: Dependency on drug concentrations. (-----) K-562 cells, (——) lymphocytes. Each point represents an average of quadruplicate cultures.



ster (Abe-Kagaku), and the radioactivity was determined in a liquid scintillation counter (Beckman). K-562 cells were grown for 18 hours in the same medium, supplemented with 5.5% human plasma, by the same procedure as described above, and [^{3}H] thymidine uptake was then carried out for 6 hours. The average of incorporation of radioactivity in quadruplicate samples was taken in each experiment.

In the absence of inhibitors, the thymidine uptake by the lymphocytes was 19,700±1,180 cpm and that by K-562 cells 14,900±1,570 cpm. As summarized in Fig. 1, the 10 kinds of antitumor agents revealed diverse degrees of DNA synthesis inhibition in PHA-blasted lymphocytes and K-562 cells. In the lymphocytes, the thymidine incorporation was not significantly affected by 6-mercaptopurine and bleomycin A_2, which prevented the thymidine uptake in the leukemic cells. Vinblastine, auromomycin and macromomycin also inhibited the thymidine incorporation in K-562 leukemic cells more markedly than in the lymphocytes. Adriamycin, aclacinomycin A, cinerubin A, neothramycin and mitomycin C blocked the thymidine uptake in the lymphocytes at a similar level as in the leukemic cells.

The activation or mitogenic stimulation of lymphocytes is known as a complex phenomenon, and PHA has been reported to induce the blastogenesis of T-cells[1,2]. The inhibition of thymidine uptake in PHA-blasted lymphocytes may be due to the prevention of DNA synthesis and/or of blastogenesis. The mechanism seems to be different with each drug. Adriamycin, aclacinomycin A, cinerubin A and neothramycin are known to block RNA and DNA syntheses by binding to DNA. Bleomycin, macromomycin and auromomycin cause DNA strand scission. Mitomycin C cross-links double strands of DNA. Vinblastine affects mitosis by interacting with tubulin and microtubules. 6-Mercaptopurine inhibits nucleotide synthesis. In the current experiments, bleomycin A_2, auromomycin and macromomycin that primarily cause DNA strand scission are preferentially active against the leukemic cell line. Whereas adriamycin, aclacinomycin A, cinerubin A, neothramycin and mitomycin C that bind to DNA show no preference. However, since only two kinds of cells have been employed in the present study, the relationship between effects on PHA-stimulated lymphocytes and molecular mechanism of action of the drugs remains to be determined.

The development of new tumor-inhibitory drugs with little immunosuppression or even with immunopotentiation is desirable, because the immunosurveillance system may kill the remaining tumor cells and prevent secondary infection in the patient undergoing cancer chemotherapy. Although the kind of tumor cells is limited, the current study suggests the selective toxicity of the antitumor agents to the lymphocytes and leukemic cells. The result with bleomycin is in accord with the previous report[3] that the antibiotic does not significantly affect immunological responses.

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