NOTES

Immunosuppressive Effect of 6-Mercaptopurine and Mitomycin C

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6-Mercaptopurine and mitomycin C were used in combination drug therapy to produce a prolongation of skin allograft survival.

Immunosuppressive drugs have long been employed to increase the longevity of allografts in animals and humans. Unfortunately, many of these drugs possess toxic side effects which limit their effectiveness. Combinations of these drugs have been examined for possible synergistic effects (1, 2, 10). Resulting studies show that two important requirements of immunosuppressive drugs, relatively low toxicity and effective immunodepression, may be fulfilled through the synergistic effect of various drug combinations.

Mitomycin C, an antibiotic isolated from *Streptomyces caespitosus*, is an effective though highly toxic, antineoplastic agent, and a known inhibitor of deoxyribonucleic acid (DNA) synthesis (3, 6, 8). It produces covalent linking of the complimentary DNA strands, thereby causing an inhibition of DNA synthesis (3). 6-Mercaptopurine (6-MP) is a purine antagonist commonly used for the treatment of leukemia and for producing immunosuppression (4). In the present investigation, 6-MP and mitomycin C were used synergistically in an effort to prevent skin allograft rejection in mice. The results demonstrate a synergistic effect of definite value in prolonging the survival of such allografts.

Full-thickness skin allografts of 1 cm² were exchanged between 3-month-old mice of C57BL-6J and C3H-HeJ strains (Jackson Laboratory). Four series, each consisting of 19 to 21 female animals weighing 15 to 30 g each, were used. These included a control series, a series receiving an intraperitoneal (i.p.) dose of 20 mg of 6-MP per kg per day (mean lethal dose; LD₅₀), a third series receiving an i.p. dose of 1

mg of mitomycin C per kg per day (LD₅₀), and a fourth series combining 6-MP and mitomycin C at a dosage of 20 mg/kg and 1 mg/kg, respectively. Fresh preparations of 6-MP in physiological saline were made daily (9). Mitomycin C was similarly prepared in 0.85% NaCl buffered to pH 6.8 with 0.01 M sodium phosphate (5). In all cases, treatment commenced on the day of grafting and continued with daily administration until rejection. Rejection was assessed macroscopically with grafts being inspected daily. Contraction and ischemic necrosis of the skin allograft were used as the determining signs of graft rejection.

Figure 1 depicts the mean survival time (MST) of skin allografts in the different series of mice. MST of the grafts in control animals was 7.1 ± 0.23 (standard deviation) days, whereas the MST of grafts in animals receiving 6-MP was 11.3 ± 1.26 days ($P < 0.001$). In the third series, mice receiving mitomycin C showed a graft survival time of 8.2 ± 0.24 days. Although this represented a slight increase over the survival time of control animals, the difference was not statistically significant. The fourth series, which received both drugs, had a MST of 14.1 ± 0.24 days ($P < 0.001$).

Different phases of the immune response, from the initial processing of antigen by macrophages to subsequent lymphoid cell proliferation and antibody production, are liable to interference by various chemical agents. Treatment regimens which interfere with the development of the immune response without lethal toxicity may be of value in subsequent clinical application. One means of avoiding toxic effects in immunosuppressive regimens is the use of combinations of drugs which give synergistic effects.

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FIG. 1. Synergistic immunosuppressive effect of 6-mercaptopurine and mitomycin C. Figures in each series indicate the mean survival time. Each bar series is labeled according to the treatment received.

In the present report, a synergistic effect of combined drug therapy is demonstrated at the LD_{50} . When compared with control animals, 6-MP increased the longevity of skin allografts by 59%, whereas mitomycin C caused an increase of only 15% in graft survival time. However, combined use of these drugs increased skin allograft survival time by 99% ($P < 0.001$). The drug combination studies did not possess increased toxicity in these short-term experiments. Percent mortality from the drug combination was approximately the same as when

6-MP of mitomycin C was used alone. Other studies, with 6-MP or a substituted derivative in combination with other immunosuppressive drugs, have also reported a synergistic effect (1, 10). Thus, combinations of various immunosuppressive drugs may often achieve more profound immunosuppression while causing no discernible increase in toxicity. Results of this study suggest that immunotherapy with combinations of drugs might effect a more marked disruption of the immune response without any increase in toxicity over that observed from treatment with one of the immunosuppressive drugs used alone.

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