and the fourth elution with 2 liter of $CHCl_3$ gave respectively 0.47 g, 1.20 g, and 1.07 g of pale yellow semi-solids (II). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1615, 1550 and 1500. NMR in $CDCl_3$ (τ): 5.15 (–CO–CH=C<). Picrate, mp 175—177° (decomp.) (from iso-PrOH). *Anal.* Calcd. for $C_{19}H_{22}O_8N_4$: C, 52.53; H, 5.10; N, 12.90. Found: C, 52.26; H, 5.05; N, 12.89. The total yield of II was 2.74 g (30%).

The fifth elution with 1.4 liter of $CHCl_3$–EtOH (20:1) and the sixth elution with EtOH (500 ml) gave respectively 0.99 g and 0.48 g of a brown semi-solid (III). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1640 (sh), 1625, 1560, and 1525. NMR in $CDCl_3$ (τ): 3.85 (–CO–CH=C<, 1H, singlet). Picrate, yellow needles from EtOH–acetone, mp 225—228° (decomp.). *Anal.* Calcd. for $C_{19}H_{20}O_8N_4$: C, 52.78; H, 4.66; N, 12.96. Found: C, 52.65; H, 4.79; N, 12.65. The total yield of III was 1.57 g (16%).

4a-Methyldodecahydro-6*H*-benzo[*c*]quinolizin-6-one (IV)——To the Grignard reagent prepared from Mg (219 mg, 9.0 matoms), CH_3I (1.28 g, 9.0 mmoles) and anhydrous ether (20 ml) was added Cu_2Br_2 (140 mg, 0.97 mmole) in one portion. A solution of the compound (II) (370 mg, 1.8 mmoles) in anhydrous ether (20 ml) was added dropwise to the above mixture at R.T. and then refluxed with stirring for 1.5 hr. The reaction mixture was decomposed with aq. saturated NH_4Cl solution under ice-cooling. The ethereal layer was separated and the aq. layer was shaken with ether (30 ml × 4). The combined ether extracts were washed with brine and dried over anhydrous K_2CO_3. Evaporation of ether gave a brown oil (300 mg), which was chromatographed on alumina (15 g). The first elution with 300 ml of benzene gave a pale yellow oil (IV) (80 mg, 21%). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2800, 2755 and 1710. NMR in $CDCl_3$ (τ): 9.05 (3H, singlet, >C–CH_3). Picrate, yellow needles from acetone, mp 219—220° (decomp.). *Anal.* Calcd. for $C_{20}H_{26}O_8N_4$: C, 53.33; H, 5.82; N, 12.44. Found: C, 53.19; H, 5.70; N, 12.51.

The second elution with 500 ml of $CHCl_3$ gave the starting material (200 mg, 54%). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1615 and 1550.

5-Acetyl-1,2,3,4,6a,7,8,9,10,10a-decahydro-6*H*-benzo[*c*]-quinolizin-6-one (V)——A solution of the compound (II) (560 mg) in Ac_2O (10 ml) in the presence of BF_3·ether (2.5 ml) was refluxed for 1.5 hr. After cooling, the reaction mixture was poured into ice-water, basified with 20% NaOH and shaken with $CHCl_3$ (50 ml × 5). The combined extracts were washed with H_2O and dried over anhydrous K_2CO_3. Evaporation of $CHCl_3$ afforded a dark brown solid (570 mg), which was chromatographed on alumina (30 g). The first elution with 300 ml of benzene gave the starting material (70 mg, 13%). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1615 and 1550.

The second elution with 200 ml of benzene–$CHCl_3$ (1:1), and the third elution with 350 ml of $CHCl_3$ gave respectively 45 mg and 315 mg of a pale pink colored solid (V), which was recrystallized from *n*-hexane–benzene (4:1) to give pink colorled needles, mp 119—120° (decomp.). *Anal.* Calcd. for $C_{15}H_{21}O_2N$: C, 72.84; H, 8.56; N, 5.66. Found: C, 72.74; H, 8.42; N, 5.66. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1635, 1615, 1550. NMR in $CDCl_3$ (τ): 7.55 (3H, singlet, >C–CO–CH_3). The total yield of V was 360 mg (54%).

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Antitumor Activity of Psychotropic Drugs and Their Synergic Action with Cyclophosphamide

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It has been reported that chlorpromazine has weak antitumor activity on Walker 256[2] and Sarcoma 37,[3,4] and also some other phenothiazine derivatives have the same effect on

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Sarcoma 180[5] and Walker 256.[2,4] Moreover, chlorpromazine[6] and imipramine[7] have been found to enhance the activity of cyclophosphamide.

The purpose of the present studies is to investigate the possible synergistic antitumor activity of chlorpromazine and other psychotropic drugs with cyclophosphamide.

Experimental

Materials and Methods

Female mice of ddN strain weighing 20 ± 2 g and Sarcoma 180 ascites tumor were used. The procedure is as follows: Each mouse, twelve animals in each group, was transplanted intraperitoneally 3×10^7 tumor cells, and was injected the drug to be tested intraperitoneally, one-third dose of each LD_{50}, once daily for 5 days, starting 6 hours after implantation. For the combination therapy, 20 mg/kg/day of cyclophosphamide, corresponding to ED_{20}, was injected intraperitoneally once daily for 5 days, starting 24 hours after implantation, while the drug to be tested was injected in the same procedure as above mentioned.

Tumor growth inhibition was evaluated with the total packed cell volume (TPCV) ratio, which was measured at the 8th day after implantation.[8] Ascites volume was measured in graduated cylinder, and ascitocrit was determined in capillary tube in a microhematocrit centrifuge. TPCV was calculated simply as the product of above two values. When ascites was nondetectable, it was defined as "regression," and the regression ratio was expressed by the number of regressed animals per total survivors at that day.

The following thirteen psychotropic drugs, mainly phenothiazine derivatives, were used: chlorpromazine, promethazine, and prochlorperazine (Shionogi Co., Ltd.), chlorprothixene and methotrimeprazine (Yoshitomi Pharmaceutical Ind., Ltd.), imipramine, desmethylimipramine, and opipramol (Fujisawa Pharmaceutical Co., Ltd.), amitriptyline (Nippon Shinyaku Co., Ltd.), promazine (Banyu Pharmaceutical Co., Ltd.), meprobamate (Daiichi Seiyaku Co., Ltd.), and pentobarbital (Tanabe Seiyaku Co., Ltd.).

Results and Discussion

As shown in Table I, chlorpromazine was found to be weakly active on Sarcoma 180 ascites tumor in inhibiting its growth. Chlorprothixene and methotrimeprazine were more active than chlorpromazine, and the tumor regression was also observed in over a half of the animals. Desmethylimipramine and prochlorperazine inhibited the tumor growth. Promazine, opipramol, amitriptyline, imipramine, and prothipendyl were weakly active almost equal to chlorpromazine. Promethazine, meprobamate and pentobarbital were inactive.

As a result, some of the phenothiazine and iminodibenzyl derivatives were found to have stronger activity than chlorpromazine.

Mechanism of action of these drugs on the tumor was not clear, and their potencies did not correlate to their pharmacological activities such as tranquilizing and antidepressing activities.

Tumor growth was inhibited slightly (about 20%) with low dose of cyclophosphamide (20 mg/kg/day $\times$ 5 days). When one-third of LD_{50} of psychotropic drug was combined with cyclophosphamide, sometimes, not only tumor growth inhibition was increased, but also tumor regression was increased. Such effect was produced by the following four drugs: desmethylimipramine, prochlorperazine, chlorpromazine, and imipramine. Also, almost of other drugs enhanced tumor growth inhibition but regression was not so affected. Tumor growth inhibiting activity of cyclophosphamide was not depressed with any of the psychotropic drugs tested.

The enhancement of antitumor activity, including growth inhibition and regression, of cyclophosphamide in combination of psychotropic drugs was supposed not to be the simple

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Table I. Antitumor Activity of the Psychotropic Drugs and Their Combination Effect of Cyclophosphamide plus One-third of LD_{50} of the Drugs

Drug	Dose (mg/kg/day)	Inhibition in TPCV[a] Drug alone	Inhibition in TPCV[a] Combination	Regression ratio[b] Drug alone	Regression ratio[b] Combination
Chloroprothixene	25.0	++	+++	8/12	9/12
Desmethylimipramine	45.0	++	+++	3/ 9 [3]	7/8[c] [4]
Methotrimeprazine	25.0	++	+++	7/11 [1]	6/10 [2]
Prochlorperazine	50.0	++	+++	2/12	10/11[d]
Promazine	40.0	++	++	3/12	6/12
Opipramol	50.0	+	++	2/12	5/12
Amitriptyline	30.0	+	+	2/11	2/12
Prothipendyl	50.0	+	+	0/11	2/11
Chlorpromazine	25.0	+	++	1/10	7/12[c]
Imipramine	40.0	+	++	1/11	6/11[c]
Pentobarbital	40.0	−	−	0/10	0/12
Promethazine	25.0	−	+	1/12	2/12
Meprobamate	100	−	−	1/11	0/11
Cyclophosphamide	20.0	−		0/12	

a) −: 34% or less in inhibition ($P>0.05$), +: 35—59% ($P<0.05$), ++: 60—89% ($P<0.01$), +++: 90% or more ($P<0.001$)
b) Evaluated at the 8th day after implantation.
c) Effect of combination treatment was greater than that of drug alone ($P<0.05$).
d) Effect of combination treatment was greater than that of drug alone ($P<0.01$).
[ ] Number of animals died from drug toxicity.

addition of antitumor activity, but rather to be due to other effects of drugs such as modification of cell membrane permeability, as reported previously in the case of imipramine.[9]

In conclusion, some of the derivatives of phenothiazine and iminodibenzyl were found to be especially active in tumor growth inhibition and regression in combination with cyclophosphamide.

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