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IMMUNOSUPPRESSION BY COMPOUNDS WHICH COMPLEX WITH DEOXYRIBONUCLEIC ACID

L. D. ZELEZNICK,* J. A. CRIM and G. D. GRAY†

Department of Hypersensitivity Diseases Research, The Upjohn Company, Kalamazoo, Mich. and Alcon Laboratories, Fort Worth, Tex., U.S.A.

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Abstract—Compounds selected for their ability to bind to DNA, as measured by the displacement of methyl green from its DNA complex, were tested for immunosuppressive activity in the graft vs. host reaction (GVHR). Seven compounds of the nineteen compounds tested showed significant immunosuppressive activity. Oral administration of the compounds was less effective than intraperitoneal injection in suppressing the GVHR. None of the compounds was immunosuppressive when humoral antibody response to sheep red blood cells was measured. There was good correlation between the ability to suppress the GVHR *in vivo* and the ability to bind to DNA as measured by methyl green displacement *in vitro*. However, this does not prove that the immunosuppressive mode of action of the compounds depended on binding to nucleic acid *in vivo*.

COMPOUNDS which bind to DNA have been shown to have pronounced biological effects in both isolated enzyme systems as well as the whole animal. For example, the antibiotics actinomycin D, nogalamycin and daunomycin, and synthetic drugs such as ethidium bromide, quinacrine, chloroquine and acriflavine inhibit the enzymes DNA polymerase, RNA polymerase and DNase.[1–4] The compounds are also active *in vivo* as trypanocides, antimalarials or antitumor agents. In a series of papers, Farr *et al.*[5–7] have studied the immunosuppressive effects of one such drug, acriflavine, on humoral antibody response in rabbits to bovine serum albumin and bovine gamma globulin. This is the only report of the immunosuppressive properties of any of the synthetic compounds which bind to DNA.

In the present communication we have studied several synthetic compounds, selected by their ability to bind to DNA, for effects on the graft vs. host reaction (GVHR) and humoral antibody response to sheep red blood cells in mice. The GVHR appears to be a model system for cellular immune mechanisms, e.g. the type involved in host vs. graft rejections, rather than for humoral immune mechanisms.[8, 9] Although many characteristic changes have been described after the onset of a GVHR such as anemia, dermatologic changes, retardation of growth and splenomegaly,[8, 9] only the latter parameter was measured in the current experiments.

MATERIALS AND METHODS

All compounds tested for the ability to bind to DNA were obtained from commercial sources, except for compounds 8, 10, 11, 13, 14, 16 and 17, which were generously made available by Dr. J. H. Burckhalter, University of Michigan. Salmon sperm

* Present address: Alcon Laboratories, Fort Worth, Texas.
† To whom reprints should be addressed.

DNA was a product of the Worthington Biochemical Corp. Methyl green-DNA substrate (MG-DNA) was prepared as described by Kurnick.[10] The displacement of methyl green from its complex with DNA was measured as previously described.[4, 11]

The GVHR was performed as described by Gray *et al.*[12] using male C57Bl donors and female C_3BF_1 hosts which were obtained from Cumberland View Farms, Tenn. Splenomegaly was measured in groups of animals on day 8 after spleen cell injection. Compounds were administered as a single daily dose i.p. for 5 consecutive days, beginning 1 day after donor spleen cell administration. When a compound did not inhibit the GVHR-induced splenomegaly (parent spleen cells → F_1 hosts), the inhibition was described as 0 per cent. When a compound reduced the GVHR-induced splenomegaly to normal spleen/body weight ratios of syngeneic controls (F_1 spleen cells → F_1 hosts), the inhibition was designated 100 per cent.[12] Spleen (mg) to body weight (g) ratios varied from 3·5 to 5·1 in control mice, and from 8·6 to 13·0 in GVHR mice.

Humoral hemagglutinating antibody formation to sheep red blood cells (S-RBC) was measured by standard techniques on serum obtained 8 days after i.p. injection. Groups of five Upjohn ICR male mice (20 g) were given the drug in a single dose i.p. on days 1 through 5 after the i.p. administration of 0·2 ml of a 30% (v/v) suspension of S-RBC in saline. The hemagglutinating antibody titer is expressed as the $\log_2$ of the reciprocal of the highest dilution demonstrating a positive agglutination.

RESULTS

The compounds selected for their ability to bind to DNA are given in Table 1. They are arranged in order of decreasing affinity for DNA. The most potent compound found was ethidium bromide, which is known to bind to DNA.[3, 13] The displacement of methyl green from its complex with DNA by these compounds seems to depend on the ability of the competing compound to intercalate into the DNA helix.[4]

The per cent inhibition of splenomegaly by the compounds administered by i.p. injection is shown in Table 2. Arabinosyl cytosine (cytarabine), which does not bind to DNA, was included as a positive immunosuppressant control.[12, 14–16]

The effect of oral administration of the most active compounds on the GVHR is presented in Table 3. All the compounds tested orally in the GVHR were much less active at dosage levels which had resulted in fair to good immunosuppression upon intraperitoneal administration.

Compounds that suppressed the GVHR, as well as the other compounds (Compounds 1–8, 11, 12, 15–19) which bind to DNA, were then tested for their immunosuppressive effects on humoral antibody formation. None of the compounds had any significant effect on humoral antibody response in mice to sheep red blood cells. Typical results are shown in Table 4.

The immunosuppressive effects of acriflavine on the antibody response of rabbits to a soluble protein antigen[5–7] were not observed in the current experiments at 10 mg/kg/day for 5 days when the hemagglutinin responses of mice, hamsters, rats and gerbils were measured. The discrepancy may be due to a species difference or a difference in the antibody response to a soluble vs. a particulate antigen.

DISCUSSION

Compounds which bind to DNA might be expected to have profound biological

activities. Among the compounds selected by their ability to bind to DNA were several known antimalarial agents (Compounds 5, 13, 15), antiparasitic agents (Compounds 1, 3, 6), and compounds which show profound cytotoxicity (ID_{50} = 10 μg/ml) in cell culture against KB epidermal cancer cells (Compounds 3, 4, 12), using a

TABLE 1. DISPLACEMENT OF METHYL GREEN FROM DNA BY VARIOUS COMPOUNDS*

	Compound name	Mol. wt.	DNA added μmoles/mg	% Displacement of methyl Green
1.	Ethidium bromide	394	1·27	92
			0·6	86
2.	6-Chloro-9-amino-2-methoxy acridine	258	0·2	33
			0·1	15
3.	Prothidium bromide	597	0·19	20
			0·09	7
4.	Acriflavine	234	2·15	92
			1·07	74
5.	Quinacrine	436	1·15	75
			0·57	60
6.	Proflavine	325	1·5	59
			0·7	32
7.	6-Methoxy-1,9-dimethyl-9H-pyrido[3,4-b] indole · HCl	262	1·9	58
			0·95	42
8.	2-[[5-(7-Chloro-4-quinolylamino)-2-hydroxy-benzyl]ethylamino]ethanol	372	1·35	50
			0·65	41
9.	8-[2-(Diethylamino)ethyl]-3-[(diethylamino) methyl)-2-methyl-4-quinolinol	416	1·2	38
			0·6	29
10.	7-Chloro-4-[3-(diethylaminomethyl-*p*-anisidino]-quinoline	370	1·35	34
			0·65	23
11.	α(Aminomethyl)-*p*-(7-chloro-4-quinolylamino) benzyl alcohol · HCl	396	1·25	32
			0·65	23
12.	6-Chloro-9-(2-hydroxyethylamino)-2-methoxy acridine	302	1·65	31
			0·8	30
13.	8-[(4-Amino-4-methylpentyl)amino]-6-methoxy quinoline · HI	529	0·96	31
			0·48	28
14.	7-Chloro-4-*p*-hydroxyanilino-1-methylquino-linium iodide	413	1·2	32
			0·6	35
15.	Chloroquine	516	0·97	22
			0·48	14
16.	1-(7-Chloro-4-quinolylamino)-3-diethylamino-2-propanol	504	1·0	21
			0·5	11
17.	7-Chloro-4-[3-[(2-chloroethyl)aminomethyl]-*p*-anisidino]quinoline · HCl	449	1·1	18
			0·55	12
18.	3-(Dimethylamino)-2′propionaphtone	264	1·9	24
			0·95	15
19.	1-Methyl-9H-pyrido[3,4b]indole (Harmane)	182	2·7	28
			1·4	13

* The rankings of compounds is based on the displacement of methyl green at two concentration levels of compound which were tested. This should not be considered to be an absolute ranking since the actual displacement curves may not be linear.[4]

standard cytotoxicity assay.[17] Thus, it was not too surprising that some of the compounds demonstrated immunosuppressive activity, especially since acriflavine had been so reported.[5–7] However, the degree of correlation between the immunosuppressive activity and the ability to bind to DNA *in vitro* was unexpected. As can be seen in Table 2, the compounds which displaced methyl green to the greatest degree from its complex with DNA were also the most active suppressants in the GVHR. Only two exceptions were found; namely, Compound 2, which complexed

TABLE 2. GVHR IN MICE INJECTED I.P. WITH COMPOUNDS WHICH BIND TO DNA

Compound*	Dose (mg/kg)†	% Inhibition
Normal GVHR		"0"
Syngeneic controls		"100"
Cytarabine (standard)	40	63
1	10	54
2	12	0
3	8	44
4	20	83
5	100	40
6	20	40
7	10	31
8	200	5
9	25	0
10	120	0
11	40	0
12	60	0
13	12	0
14	120	0
15	20	31
16	40	0
17	20	0
18	20	0
19	12	0

* Listed in order of decreasing binding affinity for DNA. See Table 1 for compound name.

† Except for cytarabine, these doses represent one-fifth of the acute LD_{50}.

TABLE 3. EFFECT OF COMPOUNDS ADMINISTERED ORALLY ON GVHR

Compound*	Dose (mg/kg)		% Inhibition
	Oral	Intraperitoneal	
1		10	54
	10		0
	50		20
3		8	44
	10		6
	50		28
4		20	83
	20		10
	100		43
5		100	40
	100		21
	200		41
	200		71
6		20	40
	20		0
	100		45
15		20	31
	20		2
	100		18
Cytarabine		20	35
(standard)		40	81
	100		7
	300		37
	400		62
	500		70

* See Table 1 for compound name.

TABLE 4. EFFECT OF DOSAGE SCHEDULE ON HUMORAL ANTIBODY RESPONSE TO SHEEP RBC*

Compound†	Dosage (mg/kg/day)	Days of injection	Hemagglutination $\log_2$ titers
Saline			7
1	10	0, 1, 2, 3	7
	10	−2, −1, 0, 1, 2	6 2
3	8	0, 1, 2, 3	7
	4	−2, −1, 0, 1, 2	6 2
4	10	0, 1, 2, 3	8
	10	−2, −1, 0, 1, 2	7
5	100	0, 1, 2, 3	8
	100	−2, −1, 0, 1, 2	6·5
6	20	0, 1, 2, 3	7·75
	20	−2, −1, 0, 1, 2	7
15	20	0, 1, 2, 3	7·6
	20	−2, −1, 0, 1, 2	7·8

* Sheep RBC administered on day 0 and mice bled on day 7.
† See Table 1 for compound name.

strongly but had no activity, and Compound 15, which complexed less strongly but demonstrated immunosuppressive activity.

The inability of these compounds to inhibit humoral antibody response to S-RBC was both puzzling and encouraging. The results are encouraging in the search for drugs which inhibit cell-mediated immune responses, such as transplantation rejection, but do not affect humoral antibody responses, which are primarily involved in defense against bacteria and viruses. The results may reflect specific differences in the cell type involved in these two immune responses and, therefore, differences in their sensitivity to inhibition by the compounds which bind to DNA. The work is suggestive but does not in any way prove that the ability to bind to DNA is related to the mechanism of action of these compounds in suppressing the GVHR.

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REFERENCES

1. I. H. GOLDBERG, *Am. J. Med.* **39**, 722 (1965).
2. S. N. COHEN and K. L. YIELDING, *Proc. natn. Acad. Sci. U.S.A.* **54**, 521 (1965).
3. D. C. WARD, E. REICH and I. H. GOLDBERG, *Science, N.Y.* **149**, 1259 (1965).
4. L. D. ZELEZNICK and C. M. SWEENEY, *Archs Biochem. Biophys.* **120**, 292 (1967).
5. J. S. SAMUELSON, P. B. STEWART, S. C. KRAFT and R. S. FARR, *J. Immun.* **94**, 314 (1965).
6. R. S. FARR, J. S. SAMUELSON and P. B. STEWART, *J. Immun.* **94**, 682 (1965).
7. J. S. SAMUELSON, S. C. KRAFT and R. S. FARR, *J. Immun.* **95**, 1013 (1966).
8. M. SIMONSEN, *Prog. Allergy* **6**, 349 (1962).
9. R. A. MCBRIDE, *Cancer Res.* **26**, 1135 (1966).
10. N. B. KURNICK, *Meth. biochem. Analysis* **9**, 15 (1962).
11. N. B. KURNICK and I. E. RADCLIFFE, *J. Lab. clin. Med.* **60**, 669 (1962).
12. G. D. GRAY, J. A. CRIM and M. M. MICKELSON, *Transplantation* **6**, 818 (1968).
13. M. J. WARING, *Biochim. biophys. Acta* **87**, 358 (1964).
14. D. S. FISCHER, E. P. CASSIDY and A. D. WELCH, *Biochem. Pharmac.* **15**, 1013 (1966).
15. J. S. EVANS, E. A. MUSSER, L. BOSTWICK and G. D. MENGEL, *Cancer Res.* **24**, 1285 (1964).
16. G. D. GRAY, M. M. MICHELSON and J. A. CRIM, *Transplantation* **6**, 805 (1968).
17. C. G. SMITH. W. L. LUMMIS and J. E. GRADY, *Cancer Res.* **19**, 843 (1959).