Oxisuran: A Differential Inhibitor of Cell-Mediated Hypersensitivity (36264)

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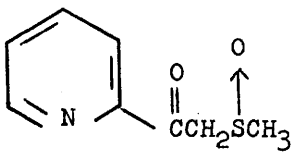
Compounds presently characterized as "immunosuppressive" inhibit both humoral antibody formation and cell-mediated, delayed, hypersensitivity. Recent reviews (1, 2) have detailed the origins and modes of action of available immunosuppressive agents. That these substances, recognized largely from cancer screening, should fail to discriminate between the two limbs of the immune response is understandable from their profound cytotoxicity as antimetabolic, antiproliferative agents.

On the assumption that there was no first principle prohibiting the separation of humoral antibody formation and cell-mediated immunity, we undertook to seek compounds which might effect selective suppression. This initial report describes the suppression of allograft immunity, without concomitant suppression of humoral antibody formation, by oxisuran, the first compound we recognized as a differential inhibitor of cell-mediated immunities.

Materials and Methods. Oxisuran, 2-[(methylsulfinyl)acetyl] pyridine,



was prepared by adding one equivalent of ethyl picolinate to two equivalents of the anion (3) of dimethyl sulfoxide. The mixture was stirred at room temperature for 1 hr and then was poured into water and extracted with chloroform. Removal of the chloroform and recrystallization from ethyl acetate afforded a crystalline solid, melting point 71–72°. The elemental analysis showed (%): C, 52.51; H, 4.93; N, 7.69; S, 17.68. Calculated values for $C_8H_9NO_2S$ are: C, 52.44; H, 4.95; N, 7.64; S, 17.50. The spectral characteristics are: infrared ($CHCl_3$) 1692 cm^{-1} (C = O) and 1046 cm^{-1} ($s \rightarrow o$); nuclear magnetic resonance (ppm): ($CDCl_3$) 2.7 (s,3,C–CH_3), 4.5 (q,2;J = 13 Hz, $\overset{O}{\overset{\|}{C}}—CH_2—\overset{O}{\overset{\uparrow}{S}}$) 7.2–8.2($m$,3 C(2,3,4)-aromatic H and 9.0 (d,1;J = 5 Hz, C(5)-aromatic H). The 3- and 4-positional isomers of oxisuran were also synthesized by the above procedure. However, they did not show the differential biological activity of the 2-isomer.

Allograft survival was determined in female HaICR (H-2^d) mice (Charles River) given tail skin allografts (4) from C57Bl/6 (H-2^b) female mice (A. R. Schmidt), and in CBA (H-2^k) female mice as recipients of tail skin allografts from A/J (H-2^a) donor female mice (Jackson Labs.). We are indebted to Dr. D. L. Ballantyne, Jr., New York University Medical Center, for providing the data on the effect of oxisuran on survival of full-thickness skin allografts transplanted from (L × BN) Fl to L female rats (5). Influence on antibody formation was studied in HaICR female mice immunized with 10^8 sheep erythrocytes (sRBC), iv, by assay of spleen cell suspensions for numbers of hemolytic antibody-producing cells (6, 7). Response to immunization was determined at several closely spaced intervals, in separate groups of mice, during graft-bearing and rejection and at the same times in identically treated nongrafted controls. In additional experiments, drug influence on the peak primary response, on priming for a subsequent secondary response, and on the secondary response in normally primed mice

TABLE I. Influence of Oxisuran and 6-MP on Allograft Survival in H-2 Histoincompatible Mice.

Drug treatment started 7 days before grafting and continued daily to rejection; 5 mice/group.

Expt.	Treatment	Allograft survival (days ± SE)
C57Bl/6→HaICR	None	7.4 ± 0.24
	6-MP	13.8 ± 0.86[a]
	Oxisuran	17.2 ± 0.92[a]
A/J→CBA	None	9.9 ± 0.41
	6-MP	16.0 ± 0.45[a]
	Oxisuran[b]	19.6 ± 0.68[a]

[a] $p \leq .001$.

[b] Treatment through day 19.

was measured.

Oxisuran was administered ip at a daily dose of 50 mg/kg in all the experiments in mice and at 100 mg/kg in Dr. Ballantyne's experiment in rats; 6-mercaptopurine (6-MP) was used as a positive control at a daily dose of 15 mg/kg, sc. The protocols for individual experiments are given in the tables.

The LD_{50} of oxisuran for the mice was 2510 mg/kg, confidence limits 2873.9–2192.1; and for the rat, 1800 mg/kg, confidence limits 1983.6–1633.3. The compound produced no changes in relative or absolute peripheral blood lymphocyte levels, or in any of the formed elements of the blood or in bone marrow or lymphoid tissues, even at the end of a 13-week subacute toxicological study. Teratogenic studies in mice and rats produced no evidence of abnormalities in the offspring. With particular reference to the lymphocyte, studies on cell transfer of delayed hypersensitivity, to be reported separately, have revealed that oxisuran-suppressed sensitized donors have available fewer, by order of 10^4, lymphoid cells capable of transferring specific delayed hypersensitivity but not fewer total lymphocytes.

Results. Data on allograft survival in mice are given in Table I. Survival was significantly extended, over that for the untreated controls, by daily drug treatment starting on day −7 and for both H-2 histoincompatibility barriers. From the data provided by Dr. Ballantyne (Table II), oxisuran produced a chronic course of rejection for most treated rats, with almost half the group rejecting at over 60 days in a model in which control survival ranged from 8 to 12 days. Because of the broad distribution of values in the treated group, calculation of mean survival time was considered inappropriate and the individual graft survival times are given in the table.

The data in Table III establish that, in separate groups of HaICR mice immunized on days 0, 2, 7, or 9, antibody formation in graft-bearing and in intact control mice was not suppressed by treatment with oxisuran but was suppressed, in all instances, by 6-MP. In these experiments, drug treatment was started on day −7 (grafting from C57Bl/6 on day 0) and continued daily; nongrafted controls were immunized and assayed at the corresponding drug-treatment times. These data also demonstrate that, in the absence of drug treatment, the early antibody response of these graft-bearing mice to sRBC was not different from that of normal nongrafted controls. In the experiments reported in Table III, spleens were assayed 42 hr after immunization, for early detection at a number of intervals during the limited period of graft survival and rejection. The data in Table IV show the influences of oxisuran and 6-MP on the peak primary and the secondary responses to sRBC in normal HaICR mice. For the primary response, drug treatment was started the day of immunization (day 0) and continued daily through day 3

TABLE II. Influence of Oxisuran on Survival of Skin Allografts Transplanted from (LXBN)F1→L Rats.

Drug treatment started 7 days before grafting and continued daily to rejection.

Treatment	No. of rats	Allograft survival [days; (range) and mean, or individual days]
None	52[a]	(8–12) 9.8
Oxisuran	20	13, 14,[b] 18, 23, 24, 25, 26, 26, 28,[b] 35, 41,[b] 44,[b] 65, 65, 65, 65, 67, 67, 67, 67

[a] Two deaths.

[b] Died, graft intact.

TABLE III. Influence of Oxisuran and 6-MP on Numbers of Hemolytic Plaque-Forming Cells per One-Fifth Aliquot of Total Spleen-Cell Suspension in Normal and Graft-Bearing Mice.

Separate groups of 5 mice each immunized with sheep RBC on days 0, 2, 7, or 9 and assayed 42 hr later.

Group and treatment	No. of hemolysin-producing cells (mean ± SE); day immunized: 0	2	7	9
Normal HaICR				
None (background)	1 ± 0.5	2 ± 1.0	1 ± 0.4	1 ± 1.0
sRBC, only	74 ± 2.6	64 ± 3.1	74 ± 2.6	70 ± 2.1
+ oxisuran	82 ± 2.1	73 ± 3.5	79 ± 3.6	80 ± 3.4
+ 6-MP	40 ± 1.7[a]	32 ± 2.4[a]	34 ± 1.2[a]	24 ± 0.8[a]
Grafted HaICR (from C57Bl/6)[b]				
None (background)	1 ± 1.0	2 ± 0.8	2 ± 0.8	2 ± 1.0
sRBC, only	79 ± 2.4	83 ± 2.6	76 ± 2.8	62 ± 2.4
+ oxisuran	79 ± 1.9	80 ± 4.1	82 ± 1.9	93 ± 4.6[a]
+ 6-MP	36 ± 2.6[a]	26 ± 2.1[a]	31 ± 1.0[a]	12 ± 2.3[a]

[a] $p \leq .001$.

[b] Grafted on day 0.

with assay on day 4. For priming and for the secondary response, previously immunized mice were reimmunized with 10^8 sRBC on day 14, having been treated with drug either only on days 0–4 and 7–11 (influence on priming) or only on days 14–16 (influence on secondary response in normally primed mice); assay was on day 17. The peak primary response, priming and to a lesser extent the secondary response, were suppressed significantly by treatment with 6-MP but not by oxisuran.

Discussion. The present data demonstrate that allograft survival may be significantly prolonged by chemical intervention without incurring suppression of antibody formation. The HaICR mice described in Tables I and III were studied at the same time, sampling from a common pool of identically treated animals. Oxisuran was no less effective in extending allograft survival than was 6-MP but, unlike the latter, did not under the same conditions inhibit antibody production. The data in Table IV further extend and illustrate this distinction for the same doses of the two compounds as were used in the transplantation experiments reported in Table I. The data in Table II clearly demonstrate that skin allograft survival is prolonged by oxisuran in the rat, as well as in the mouse.

In this initial report, allograft survival has been presented as a model of cell-mediated immunity, and this has also been studied for skin in the rabbit, fetal heart in the mouse, and mammary gland in the rat. That the influence of oxisuran is, indeed, on cell-mediated immunity is supported by additional studies, to be reported, on cutaneous delayed reactivity in guinea pigs and rats previously sensitized to ovalbumin or tuberculin and on contact hypersensitivity to DNCB in guinea pigs. Mechanism of action is not yet known, but oxisuran is not cytotoxic or antiproliferative *in vitro* or *in vivo*, nor does it exhibit antiinflammatory activity. It is of interest that the 3- and 4-positional isomers do not share the distinctive biological activity in laboratory rodents of the 2-isomer.

Summary. Oxisuran, 2-[(methylsulfinyl)acetyl]pyridine, has been shown to suppress allograft rejection in the rodent without causing concomitant suppression of the hemolytic antibody response to sheep erythrocytes. This simple synthetic compound thus differs from presently defined "immunosup-

TABLE IV. Influence of Oxisuran and 6-MP on Numbers of Hemolytic Plaque-Forming Cells per Spleen for Primary, Priming, and Secondary Responses to Sheep RBC in Normal Mice. Groups of 5 mice each, immunized on days 0 and 14.

Treatment	No. of hemolysin-producing cells (mean ± SE); day of assay		
	4	17	
	Primary	Priming	Secondary
sRBC, only	211,400 ± 6880	423,000 ± 18,340	
+ oxisuran	193,500 ±10,740	454,000 ± 17,710	417,000 ± 20,710
+ 6-MP	96,600 ± 2290[a]	117,500 ± 12,800[a]	232,500 ± 14,210[a]

[a] $p \leq .001$.

pressants," such as 6-mercaptopurine with which it has been directly compared, which from their modes of action fail to discriminate between humoral and cell-mediated immune responses.

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