loss of *Rb* function can promote spontaneous apoptosis and can enhance apoptosis induced by certain anticancer agents (*4*, *11–13*). In many of these instances, apoptosis involves *p53* (*4*, *12–15*). These observations predict that reintroduction of normal *p53* function into tumors harboring *p53* mutations will enhance apoptosis after radiation or chemotherapy, an approach that has proved successful for cisplatin in a lung carcinoma cell line (*16*).

Our results suggest a basis for the association between *p53* mutation and poor patient prognosis [for examples, see (*17–20*)] and are consistent with studies linking *p53* mutation to drug resistance in human tumors. For example, *p53* mutations dramatically reduce the probability that patients with B cell chronic lymphocytic leukemia will enter remission after chemotherapy (*19*). Mutations in *p53* are also associated with tumor relapse in acute lymphoblastic leukemia (*21*), suggesting that attenuated p53 activity confers a selective advantage on tumor cells undergoing cancer therapy. Finally, *p53* mutations are strictly associated with anaplastic Wilms tumors (*20*), an aggressive subtype that is particularly resistant to therapeutic intervention. In these tumors, *p53* loss is accompanied by attenuated apoptosis (*22*). Because *p53* mutations are among the most common alterations observed in human cancer, defects in *p53*-dependent apoptosis may be a significant impediment to successful cancer therapy.

REFERENCES AND NOTES

1. C. Dive and J. A. Hickman, *Br. J. Cancer* **64**, 192 (1991).
2. J. F. R. Kerr, C. M. Winterford, B. V. Harmon, *Cancer* **73**, 2013 (1994).
3. S. W. Lowe, H. E. Ruley, T. Jacks, D. E. Housman, *Cell* **74**, 954 (1993).
4. N. Tanaka *et al.*, *ibid.* **77**, 829 (1994).
5. S. W. Lowe, T. Jacks, D. E. Housman, H. E. Ruley, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 2026 (1994).
6. M. Hollstein, D. Sidransky, B. Vogelstein, C. C. Harris, *Science* **253**, 49 (1991).
7. We did not determine whether the two mutations in tumor 977R were in the same or in separate alleles. In human tumors harboring two *p53* mutations, the mutations are typically in separate alleles.
8. S. W. Lowe *et al.*, data not shown.
9. The *mdm-2* oncogene, which can inactivate *p53* and is amplified in many human sarcomas, was not overexpressed in any of the tumors tested.
10. H. Symonds *et al.*, *Cell* **78**, 703 (1994).
11. G. I. Evan *et al.*, *ibid.* **69**, 119 (1992).
12. X. W. Wu and A. J. Levine, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 3602 (1994).
13. T. Jacks *et al.*, *Nature* **359**, 295 (1992); E. Y-H. P. Lee *et al.*, *ibid.*, p. 288; A. R. Clarke *et al.*, *ibid.*, p. 328.
14. S. D. Morgenbesser, B. O. Williams, T. Jacks, R. A. DePinho, *Nature* **371**, 72 (1994).
15. J. Lotem and L. Sachs, *Cell Growth Differ.* **4**, 41 (1993).
16. T. Fujiwara *et al.*, *Cancer Res.* **54**, 2287 (1994).
17. S. Thorlacius, A. L. Borresen, J. E. Eyfjord, *ibid.* **53**, 1637 (1993).
18. Y. Horio *et al.*, *ibid.*, p. 1.
19. S. Elrouby *et al.*, *Blood* **82**, 3452 (1993).
20. N. Bardeesy *et al.*, *Nat. Genet.* **7**, 91 (1994).
21. M. H. Hsiao, A. L. Yu, J. Yeargin, D. Ku, M. Haas, *Blood* **83**, 2922 (1994).
22. J. Pelletier, personal communication.
23. M. E. Scheulen, V. Budach, M. Bambery, *Strahlenther. Onkol.* **165**, 529 (1989).
24. B. Bienz, R. Zakut-Houri, D. Givol, M. Oren, *EMBO J.* **3**, 2179 (1984).
25. Minimally passaged $p53^{+/+}$ and $p53^{-/-}$ mouse embryonic fibroblasts transformed by E1A and T24 H-*ras* (*3*, *5*) were detached from tissue culture plates, washed, and resuspended in phosphate-buffered saline (PBS). Cells (2×10^6) were injected into each flank of athymic nude mice 4 to 8 weeks old. Tumor volumes were estimated from caliper measurements of tumor length (L) and width (l) according to the following formula: $(L \times l^2)/2$. In general, tumors were allowed to expand to a volume of 0.15 to 0.5 cm^3 before treatment. Mice receiving ≤ 7 Gy were given a whole body dose in a Gammacell 40 irradiator containing a ^{137}Cs source (~0.8 Gy/min). For higher doses, a lead shield was used to reduce radiation toxicity to the bone marrow and thoracic cavity. Adriamycin was injected intraperitoneally at a dose of 10 mg per kilogram of body weight in PBS; thus, the amount of adriamycin approached the maximum tolerated dose for a single injection (*23*). Higher doses of adriamycin are capable of killing *p53*-deficient cells in vitro (*3*), but the concentrations required for this effect are toxic to the animal.
26. Tumor cells were dispersed with trypsin and cultured in medium containing hygromycin B (50 μg/ml) to select for tumor-derived cells [which contain hygromycin phosphotransferase (*5*)]. RNA was generally isolated from tumor cells maintained for less than 1 week in culture. For tumor 915L, RNA was isolated from frozen tumor tissue. Complementary DNA was prepared by standard methods with a primer directed to sequences within exons 9 or 11 of the murine *p53* gene (*24*). Amplification of *p53* sequences in exons 5 to 8 was accomplished by polymerase chain reaction with primers directed to exons 4 and 9. This region contains the vast majority of *p53* mutations observed in human cancer (*6*). Sequencing was performed with several primers specific to exons 5 to 8.
27. Y. Gavrieli, Y. Sherman, S. A. BenSasson, *J. Cell Biol.* **119**, 493 (1992).

28. We thank M. McCurrach for technical assistance and helpful advice. S.W.L. is an Anna Fuller postdoctoral fellow and S.B. is supported by a fellowship from the Swiss League of Cancer. Supported by grants R01CA40602 (H.E.R), 5R27CA17575 and CA14051 (D.E.H.), a Cancer Center Support (core) grant P30-CA14051 from NIH, and a grant from the General Cinemas Charitable Foundation (T.J.).

13 July 1994; accepted 9 September 1994

Transformation of Lupus-Inducing Drugs to Cytotoxic Products by Activated Neutrophils

Xiaoxia Jiang, Gus Khursigara, Robert L. Rubin*

Drug-induced lupus is a serious side effect of certain medications, but the chemical features that confer this property and the underlying pathogenesis are puzzling. Prototypes of all six therapeutic classes of lupus-inducing drugs were highly cytotoxic only in the presence of activated neutrophils. Removal of extracellular hydrogen peroxide before, but not after, exposure of the drug to activated neutrophils prevented cytotoxicity. Neutrophil-dependent cytotoxicity required the enzymatic action of myeloperoxidase, resulting in the chemical transformation of the drug to a reactive product. The capacity of drugs to serve as myeloperoxidase substrates in vitro was associated with the ability to induce lupus in vivo.

More than 40 different medications can result in unremitting muscle and joint pain and occasionally compromised heart and lung function when used for long-term therapy. Symptoms eventually resolve after the therapy is discontinued. This side effect has been termed drug-induced lupus because it is clinically indistinguishable from the idiopathic disease systemic lupus erythematosus. Both forms of lupus are accompanied by serum autoantibodies to constituents of the cell nucleus. The prototypic drugs associated with a lupus-like side effect include antiarrhythmics such as procainamide and quinidine, the antihypertensive drug hydralazine, psychotropic drugs such as chlorpromazine, antibiotics such as isoniazid, and the antithyroid agent propylthiouracil (*1*). Although some of these drugs are aromatic amines or hydrazines, there is no apparent common denominator of a pharmacologic, therapeutic, or chemical nature that links drugs with the capacity to induce lupus.

Idiosyncratic drug reactions such as drug-induced lupus may depend on metabolic transformation of the ingested agent to a reactive intermediate that displays biological activity different from the parent compound. Transformation to reactive metabolites with a common property could also explain how chemically dissimilar drugs produce the same adverse reaction. Although the liver is considered to be the main site of xenobiotic metabolism (*2*), drug-induced lupus is a systemic autoimmune disease with no clinical evidence of liver involvement (*1*). These considerations suggest three possibilities for the productive exposure of the immune system to labile metabolites: (i) Immunocytes encounter the reactive metabolite in the liver and then migrate to and expand in an immune compartment. (ii) The metabolite translocates from the liver to immune compartments in a form that preserves its activity. (iii) The reactive metabolite is produced locally within an immune compartment where it acts. Local production of the me-

W. M. Keck Autoimmune Disease Center, Department of Molecular and Experimental Medicine, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037, USA.

*To whom correspondence should be addressed.

tabolites maximizes contact between cells of the immune system and reactive intermediates, but a general mechanism for generating such products in an immune compartment is unknown.

The polymorphonuclear leukocyte (neutrophil) has access to all central and peripheral immunologic domains. These cells have the capacity to initiate extracellular oxidative reactions (*3*), and at a blood concentration of 3×10^6 per milliliter to 4×10^6 per milliliter, they represent a formidable reservoir of oxidative potential. We used a bioassay (*4*) in which the viability of EL4 cells, a mouse T cell lymphoma, was measured in the presence of the test drug and neutrophils activated with opsonized zymosan. If neutrophils metabolize the drug to a cytotoxic product or products in the extracellular medium, the indicator cells will be killed. Cytotoxicity increased when neutrophils were activated in the presence of increasing concentrations of all six therapeutic classes of lupus-inducing drugs (Fig. 1A). Drug concentrations were selected to be less than or equal to normal therapeutic blood concentrations; cytotoxicity ranged from 40 to 90%, depending on the drug. Quinidine and chlorpromazine displayed low but significant killing of target cells in the absence of neutrophils, but neutrophils enhanced toxicity of these drugs two- to fourfold. Cytotoxicity was very specific to the medically useful form of the drug; analogs that do not induce lupus (*5*, *6*) or are not therapeutics were noncytotoxic in the presence or absence of neutrophils (Fig. 1B). Promazine, like chlorpromazine, was slightly cytotoxic, but unlike the lupus-inducing parent compound, promazine toxicity was not enhanced by activated neutrophils.

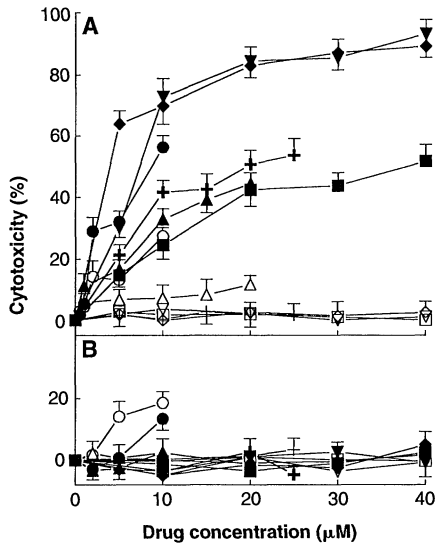

Fig. 1. Cytotoxicity of lupus-inducing drugs (**A**) and their analogs (**B**) in the presence or absence of activated neutrophils. Neutrophils from normal donors were isolated from anticoagulated blood by Ficoll-hypaque gradient centrifugation after removal of erythrocytes by dextran sedimentation and ice water lysis (*4*). Culture conditions consisted of 5×10^5 neutrophils per milliliter, 1×10^5 EL4 cells per milliliter as the targets, and increasing concentrations of test drugs in a final volume of 0.75 ml of RPMI 1640 medium containing 5% fetal bovine serum of low hemoglobin content (Hyclone Laboratories) and 0.01 M Hepes buffer. Activation was initiated by the addition of zymosan (Sigma) opsonized with normal human serum to a final concentration of 0.1 mg/ml. The pH of the medium was maintained at 8.3 for 1 hour at 37°C, and then the mixture was cultured overnight in polypropylene tubes with loosened caps in a CO_2 incubator (*23*). EL4 cell viability was measured by capacity for overnight incorporation of 5 μCi/ml of ^{3}H[thymidine]. Cells were harvested and ^{3}H levels measured by scintillation spectrometry. Percent drug cytotoxicity = 100 − 100[(cpm with drug)/(cpm control)]. Previous studies demonstrated that DNA synthetic capacity correlated with trypan blue dye exclusion as well as reduction of the vital dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (*20*). EL4 cells are not phagocytosed by neutrophils during the incubation period on the basis of studies showing the lack of cosedimentation with Ficoll-hypaque–separated neutrophils of EL4 cells prelabeled with ^{3}H[thymidine]. Filled and open symbols represent drug-mediated cytotoxicity in the presence and absence, respectively, of activated neutrophils. The drugs in panel (A) are procainamide (◆, ◇), propylthiouracil (▼, ▽), isoniazid (■, □), hydralazine (+, +), quinidine (▲, △), and chlorpromazine (●, ○). The correspondence analogs in panel (B) are *N*-acetylprocainamide (◆, ◇), propyluracil (▼, ▽), isonicotinamide (■, □), phthalazine (+, +), quinoline (▲, △), and promazine (●, ○). Variances are SD.

When the complement receptor of neutrophils is engaged by opsonized particles, respiratory burst and degranulation responses are initiated, resulting in extracellular production of various reactive oxygen species including O_2^-, H_2O_2, and OCl^- (*3*). We tested whether these agents or the biochemical reactions that produce them mediate the transformation of drugs to cytotoxic products. Elimination of O_2^- by superoxide dismutase had no effect (*7*). As shown in Table 1, removal of H_2O_2 by addition of catalase at the start of the reaction completely blocked neutrophil-dependent drug cytotoxicity. However, none of the drugs directly reacted with H_2O_2 concentrations as high as 0.1 mM, as measured by drug recovery studies or cytotoxicity (*7*). When activated neutrophils were preincubated with each drug for various lengths of time before addition of catalase and the target cells, neutrophil-mediated drug cytotoxicity became increasingly independent of H_2O_2 (Table 1). These results suggest that H_2O_2 is necessary for transformation of drugs to cytotoxic products but cytotoxicity is not due to accumulation of H_2O_2 (*8*).

Within a few minutes after activation of neutrophils, myeloperoxidase (MPO) is detectable in the extracellular medium because of its exocytosis from azurophil granules (*3*, *9*). To determine its possible role in drug transformation, we inhibited MPO activity by adding increasing amounts of NaN_3. At 50 μM NaN_3, approximately 1% of MPO activity remained, and neutrophil-mediated cytotoxicity of all the drugs was essentially neutralized. With less inhibition of MPO activity, drug-mediated cytotoxicity increased (Fig. 2). The stoichiometric relation between MPO activity and cytotoxicity suggests that neutrophil-dependent drug cytotoxicity required the enzymatic activity of MPO.

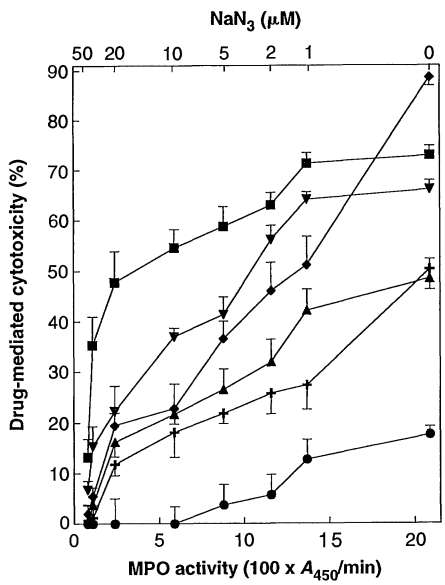

Fig. 2. Role of myeloperoxidase (MPO) in neutrophil-dependent drug cytotoxicity. The standard cytotoxicity assay was performed with activated neutrophils and the addition of increasing concentrations of NaN_3 in the presence of 30 μM procainamide (◆), 30 μM propylthiouracil (▼), 30 μM isoniazid (■), 20 μM hydralazine (+), 15 μM quinidine (▲), or 10 μM chlorpromazine (●). In the absence of neutrophils, EL4 cells were unaffected by >0.4 mM NaN_3. In a separate experiment neutrophils were activated at 4×10^7 per milliliter in Dulbecco's phosphate-buffered saline [containing glucose (1 mg/ml), bovine albumin (1 mg/ml), and 0.01 M Hepes (pH 7.2)] by addition of 1 mg of opsonized zymosan per milliliter. After 30 min at 37°C, supernatant was collected by centrifugation. MPO activity was measured after 50-fold dilution into substrate solution consisting of 300 μM H_2O_2, 250 μM *o*-dianisidine, and increasing concentrations of NaN_3. MPO activity was based on the initial velocity (V_i) of *o*-dianisidine oxidation at 37°C determined from the absorbance at 450 nm (A_{450}) recorded every 0.3 min for 2 min and an oxidized *o*-dianisidine molar extinction coefficient of 1.13×10^{-4} cm^{-1} (*24*). At [NaN_3] = 0.0 μM, MPO activity = 8.0 μIU/ml. MPO activity is plotted against percent cytotoxicity determined at the same [NaN_3]. Variances are SD.

Further support for the role of MPO en-

zymatic activity in drug transformation was obtained by enzyme kinetic studies with purified MPO. The effect on MPO activity of adding increasing amounts of test drug was determined with H_2O_2 as the primary and *o*-dianisidine as the secondary substrate. All the drugs behaved as competitive inhibitors of MPO activity, as indicated by the kinetic patterns of Lineweaver-Burk and Dixon plots; inhibition constants (K_i's) are listed in Table 2. The K_i's of hydralazine and propylthiouracil were significantly lower (that is, had stronger inhibition potency) than those of the other drugs ($P < 0.05$). At a concentration of 10 μM, all lupus-inducing drugs displayed significant MPO inhibitory activities ($P < 0.005$ compared with their respective analogs), and percent inhibition was roughly comparable with cytotoxic potency at this concentration, except for procainamide. In contrast, the analogs of these drugs that were incapable of supporting a cytotoxic reaction showed essentially no capacity to inhibit MPO activity (Table 2). The association between competitive inhibition potency and neutrophil-mediated drug cytotoxicity supports the view that these drugs undergo chemical transformation to cytotoxic products through the enzymatic action of MPO. Furthermore, the hierarchy of neutrophil-mediated cytotoxicity at 10 μM drug was similar to that of the incidence of clinical lupus during long-term therapy (*10*), suggesting that these in vitro

Table 1. Effect of catalase on neutrophil-dependent drug cytotoxicity. Neutrophils were activated in the presence of drug at the concentrations used in Fig. 2. Catalase at 50 U/ml and target cells were subsequently introduced simultaneously at various times, and the standard cytotoxicity assay was continued. Percent catalase sensitivity = 100 − 100 × (drug-mediated cytotoxicity in the presence of catalase)/(drug-mediated cytotoxicity in the absence of catalase). Variances are SD.

Drug present during neutrophil activation	Time of catalase and target cell addition (hour)	Neutrophil-dependent cytotoxicity (%)		Catalase sensitivity (%)
		Drug	Drug + catalase	
Procainamide	0.0	51 ± 3	0 ± 4	100
	0.3	53 ± 6	16 ± 2**	69
	1.0	44 ± 5	31 ± 3**	29
	2.0	30 ± 6	27 ± 1**	9
Propylthiouracil	0.0	57 ± 2	1 ± 4	98
	0.3	53 ± 6	11 ± 3*	79
	1.0	45 ± 7	28 ± 2**	37
	2.0	32 ± 3	27 ± 3**	15
Isoniazid	0.0	36 ± 3	−2 ± 0	104
	0.3	38 ± 3	8 ± 4*	79
	1.0	32 ± 8	24 ± 3**	27
	2.0	20 ± 3	18 ± 4**	8
Hydralazine	0.0	36 ± 2	−2 ± 7	104
	0.3	40 ± 4	4 ± 2	91
	1.0	31 ± 5	23 ± 3**	27
	2.0	22 ± 3	18 ± 2*	18
Quinidine	0.0	40 ± 6	−1 ± 2	102
	0.3	33 ± 4	7 ± 2*	79
	1.0	25 ± 6	22 ± 3**	12
	2.0	16 ± 2	11 ± 4*	34
Chlorpromazine	0.0	38 ± 4	2 ± 2	94
	0.3	28 ± 5	4 ± 2	84
	1.0	18 ± 6	7 ± 1*	60
	2.0	5 ± 4	2 ± 1	65

*$P < 0.05$; **$P < 0.005$, compared with time = 0.

Table 2. Inhibition of MPO activity by lupus-inducing drugs and their analogs. Dashes indicate that the compound does not induce lupus. ND, not defined.

Drug	Lupus-inducing incidence* (%)	Neutrophil-mediated cytotoxicity at 10 μM drug†	MPO activity	
			% inhibition at 10 μM drug‡	Inhibitor constant§ (K_i, μM)
Procainamide	15–20	76 ± 11	17 ± 5	31.3 ± 9.6
N-Acetylprocainamide	–	−3 ± 3	3 ± 2	ND
Hydralazine	5–10	37 ± 4	95 ± 5	0.1 ± 0.2
Phthalazine	–	−3 ± 1	2 ± 1	ND
Quinidine	<1	26 ± 4	21 ± 7	22.9 ± 3.0
Quinilone	–	1 ± 1	0 ± 3	ND
Chlorpromazine	<1	30 ± 7	29 ± 8	14.5 ± 4.3
Promazine	–	−5 ± 1	3 ± 5	ND
Isoniazid	<1	24 ± 3	40 ± 5	7.4 ± 5.1
Isonicotinamide	–	1 ± 1	1 ± 3	ND
Propylthiouracil	<1	63 ± 11	87 ± 1	1.0 ± 0.7
Propyluracil	–	−4 ± 2	0 ± 2	ND

*Approximate incidence of drug-induced lupus during 2 years of therapy (*1*). †Values at 10 μM drug were derived by interpolation of the nonlinear least squares fit of the data shown in Fig. 1 and subtraction of cytotoxicity in the absence of neutrophils. The measure of variability is the standard deviation from regression. ‡The V_i at 10 μM drug was determined at 37°C in 0.01 M borate buffer (pH 7.2) with *o*-dianisidine (100 μM) and H_2O_2 (300 μM). The reaction was initiated by addition of 10 μl of purified MPO (Calbiochem) to a final concentration of 5.7 μIU/ml. Oxidation of *o*-dianisidine was monitored at 450 nm for 2 min and converted to an initial rate (V_i) by linear regression. Percent inhibition (± SD) = 100 − 100 × (V_i in presence of drug)/(V_i in absence of drug). §K_i was determined by means of four *o*-dianisidine concentrations between 100 and 250 μM and six drug concentrations. For each drug, nonlinear least squares analysis was used to find the intersection point of the four plots of $1/V_i$ versus [drug], which = $-K_i$ (*26*). The measure of variability is the standard deviation of the estimated *x*-coordinate from the intersection point of the four regression curves.

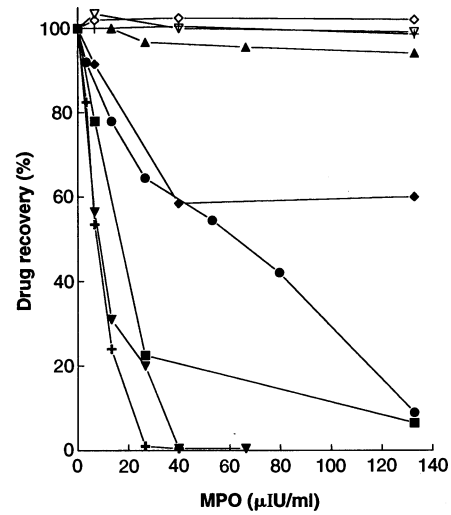

Fig. 3. Chemical transformation of drugs by MPO. Each drug was mixed with increasing concentrations of MPO in 0.01 M phosphate buffer (pH 7.3) and 0.1 mM hypoxanthine. The reaction was initiated by addition of xanthine oxidase (Sigma) at 25 mU/ml to generate O_2^-, which spontaneously dismutates into H_2O_2. After 30 min at 37°C, samples were ultrafiltered into prefrozen tubes. Samples (500 μl) were subjected to high-performance liquid chromatography on a C-18 reversed-phase column (Synchrome), and the recovery of parent compound was quantified by integration of the absorbance tracing measured at the absorption maximum and elution position of each drug (*25*). Initial concentrations were 30 μM for procainamide (◆), *N*-acetylprocainamide (◇), and quinidine (▲); 10 μM for chlorpromazine (●), isoniazid (■), hydralazine (+), and phthalazine (+); and 3 μM for propylthiouracil (▼) and propyluracil (▽).

observations have in vivo significance.

Metabolism of the drugs by MPO was demonstrated directly in a cell-free system. The enzymatic action of xanthine oxidase on hypoxanthine generated O_2^- at a rate equivalent to that generated by 3×10^6 neutrophils per milliliter activated with opsonized zymosan. Cell-free drug metabolism was measured in the absence of NaCl, thereby precluding a role for OCl^- in these processes. Addition of increasing amounts of MPO caused a dose-dependent loss of all six parent compounds (Fig. 3). Quinidine was least susceptible, with approximately 10% transformation at a MPO concentration of 0.1 mU/ml; this was followed by procainamide, chlorpromazine, isoniazid, propylthiouracil, and hydralazine. Hydralazine was essentially undetectable after the mixture was incubated with 0.025 mU of MPO per milliliter for 30 min. The analogs *N*-acetylprocainamide, propyluracil, and phthalazine (which were inert in the bioassay) were unaffected at the highest MPO concentration tested.

The inhibition kinetics suggested that the cytotoxicity of drugs exposed to activated neutrophils was due to their capacity to serve as substrates for enzymatic reactions involving MPO. Despite this substrate promiscuity of MPO, analogs of lupus-inducing drugs with blocked or missing functional groups such as $-NH_2$, $-NHNH_2$, -SH, -Cl, or $-OCH_3$ were not metabolized by MPO, and the oxidation of *o*-dianisidine by horseradish peroxidase required 5- to 100-fold higher drug concentrations to cause equivalent inhibition (*7*). Apparently, after oxidation of H_2O_2, the heme group in MPO can accept electrons from certain unnatural aromatic compounds. As a result, the drugs are chemically altered, producing numerous extra chromatographic peaks (*7*). We suggest that some of these metabolites are cytotoxic, possibly as free radicals capable of both oxidation and reduction reactions (*11*, *12*). There have been other reports on the transformation of drugs by neutrophil MPO (*12*, *13*) or other peroxidases (*14*), but no biological activity other than enhanced nonspecific binding to macromolecules was demonstrated (*15*). We have shown that MPO-mediated biotransformation is a general property of all pharmacologic classes of lupus-inducing drugs and that their metabolites display cytotoxicity at therapeutically relevant concentrations. Detection of oxidized metabolites of procainamide in serum of procainamide-treated patients (*16*) and animals (*16*, *17*) and the common finding of autoantibodies to neutrophil MPO and lactoferrin in patients with hydralazine-induced lupus (*18*) are consistent with a role for activated neutrophils in oxidative transformation of lupus-inducing drugs in vivo. Our in vitro bioassay may provide a reliable way to screen medications with high potential for idiosyncratic toxic reactions without the need for radioactively labeled drug.

The capacity to be oxidized by the extracellular MPO-H_2O_2 system of activated neutrophils appears to be a common feature of lupus-inducing drugs despite their chemical and pharmacological heterogeneity. Generation of reactive drug metabolites through neutrophil action also provides a mechanism for delivering labile compounds into immune compartments where disruption of immune tolerance presumably occurs. Cytotoxicity may induce autoimmunity by exposing autoreactive lymphocytes to abnormal forms of self-material released during premature cell death. However, it appears more likely that subtoxic concentrations of reactive drug metabolites could bring about immune disregulation. Through a general capacity to participate in oxidation-reduction reactions, labile drug metabolites may act at the lymphocyte membrane to cross-link accessible receptors involved in signal transduction (*19*). Procainamide-hydroxylamine, which is lymphotoxic at 13 μM (*20*), has been reported to be mitogenic at 0.4 to 4 μM (*21*). Identification of other reactive drug metabolites and the macromolecular targets that produce hyperimmune effects offers promise for explaining the processes that can lead to systemic autoimmune disease.

REFERENCES AND NOTES

1. S. E. Blomgren, *Semin. Hematol.* **10**, 345 (1973); A. Weinstein, *Prog. Clin. Immunol.* **4**, 1 (1980); C. E. Harmon and J. P. Portanova, *Clin. Rheum. Dis.* **8**, 121 (1982); B. C. Richardson, *Rheum. Dis. Clin. North Am.* **20**, 61 (1994).
2. K. W. Woodhouse and O. F. W. James, *Br. Med. Bull.* **46**, 22 (1990); J. P. Uetrecht, B. J. Sweetman, R. L. Woosley, J. A. Oates, *Drug Metab. Dispos.* **12**, 77 (1984); R. A. Budinsky, S. M. Roberts, E. A. Coats, L. Adams, E. V. Hess, *ibid.* **15**, 37 (1987); D. W. Hein and W. W. Weber, in *Autoimmunity and Toxicology*, M. Kammuller, M. Bloksma, W. Seimen, Eds. (Elsevier, Amsterdam, 1989), pp. 239–265.
3. J. E. Smolen and G. Weissman, in *Arthritis and Allied Conditions*, D. J. McCarthy, Ed. (Lea and Febiger, Philadelphia, 1979), pp. 282–295; B. M. Babior, *N. Engl. J. Med.* **298**, 659 (1978); J. K. Hurst and W. C. J. Barrette, *Crit. Rev. Biochem. Mol. Biol.* **24**, 271 (1989).
4. R. L. Rubin and J. T. Curnutte, *J. Clin. Invest.* **83**, 1336 (1989).
5. R. L. Woosley *et al.*, *N. Engl. J. Med.* **298**, 1157 (1978); R. Lahita, J. Kluger, D. E. Drayer, D. Koffler, M. M. Reidenberg, *ibid.* **301**, 1382 (1979).
6. D. P. Aucoin *et al.*, *J. Pharmacol. Exp. Ther.* **234**, 13 (1985).
7. R. L. Rubin *et al.*, unpublished data.
8. H_2O_2 accumulated to a peak concentration of 0.7 ± 0.05 μM within 10 min after neutrophils were activated at 10^6 per milliliter with opsonized zymosan, as determined by the scopoletin fluorescence assay [W. A. Andreae, *Nature* **175**, 859 (1955)] performed in the presence of 0.1 mM NaN_3 to prevent subsequent H_2O_2 consumption by MPO. None of the drugs caused a significant increase in H_2O_2 under these conditions. The H_2O_2 was not cytotoxic to EL4 cells until concentrations of 100 μM were reached (10% cytotoxicity).
9. R. A. Clark, *Adv. Inflammation Res.* **5**, 107 (1983).
10. Procainamide, with a drug-induced lupus incidence of up to 30%, also showed the highest neutrophil-mediated cytotoxicity of all the tested drugs [except propylthiouracil (PTU)] at a significance level of $P < 0.05$. PTU may only rarely induce lupus because its usual therapeutic dose results in a blood concentration of <10 μM for approximately 3/4 of the day [assuming an intake of 50 mg, two times a day (*22*)], although some people are treated with three times these amounts. However, greater than 50% of mongrel cats treated with comparatively high amounts of PTU develop a lupus-like disease (*6*), suggesting that PTU has a strong propensity for inducing lupus when used at high doses. Hydralazine is the next most potent lupus-inducing drug and produces the third highest neutrophil-dependent cytotoxicity.
11. J. M. van Zyl, K. Basson, A. Kriegler, B. J. Van der Walt, *Biochem. Pharmacol.* **40**, 947 (1990).
12. P. P. Kelder, N. J. De Mol, B. A. T. Hart, L. H. M. Janssen, *Chem. Biol. Interactions* **79**, 15 (1991); D. P. Barr and S. D. Aust, *Arch. Biochem. Biophys.* **303**, 377 (1993).
13. L. Waldhauser and J. P. Uetrecht, *Drug Metab. Dispos.* **19**, 354 (1991); A. H. Hofstra, S. M. Angela Li-Muller, J. P. Uetrecht, *ibid.* **20**, 205 (1992); A. H. Hofstra, L. C. Matassa, J. P. Uetrecht, *J. Pharmacol.* **18**, 1673 (1991).
14. K. Yamamoto and S. Kawanishi, *Biochem. Pharmacol.* **41**, 905 (1991); H. A. Shoeb, B. U. Bowman Jr., A. C. Ottolenghi, A. J. Merola, *Antimicrob. Agents Chemother.* **27**, 399 (1985); S. D. Nelson, D. C. Dahlin, E. J. Rauckman, G. M. Rosen, *Molec. Pharmacol.* **20**, 195 (1981).
15. D. A. Eastman and M. T. Smith, *Methods Enzymol.* **186**, 579 (1990).
16. J. F. Wheeler *et al.*, *Drug Metab. Dispos.* **19(3)**, 691 (1991); L. E. Adams *et al.*, *Lupus* **2**, 89 (1993).
17. A. J. Streeter and J. A. Timbrell, *Drug Metab. Dispos.* **11**, 184 (1983); M. Kubicka-Muranyi *et al.*, *Toxicol. Appl. Pharmacol.* **122**, 88 (1993).
18. L. Nassberger, A. G. Sjoholm, H. Jonsson, G. Sturfelt, A. Akesson, *Clin. Exp. Immunol.* **81**, 380 (1990); G. Cambridge, H. Wallace, R. M. Bernstein, B. Leaker, *Br. J. Rheumatol.* **33**, 109 (1994).
19. I. Nakashima *et al.*, *J. Immunol.* **152**, 1064 (1994).
20. R. L. Rubin, J. P. Uetrecht, J. E. Jones, *J. Pharmacol. Exp. Ther.* **242**, 833 (1987).
21. L. E. Adams *et al.*, *J. Lab. Clin. Med.* **113**, 482 (1989).
22. *USP DI* (United States Pharmacopeia Dispensing Information) (United States Pharmacopeial Convention, Rockville, MD, ed. 14, 1994), vol. I, pp. 417–422.
23. Cytotoxicity was enhanced two- to fourfold when the bioassay was performed at elevated pH. This effect was probably due to increased stability of the toxic metabolite in slightly alkaline conditions on the basis of studies with procainamide-hydroxylamine (*7*, *20*). The pH returned to neutrality by CO_2 diffusion approximately 1 hour after the tube caps were loosened.
24. C. C. Worthington, Ed., *Worthington Enzyme Manual, Enzymes and Related Biochemicals* (Worthington Biochemical, Freehold, NJ, 1988), pp. 254–255; U. Bretz and M. Baggiolini, *J. Cell Biol.* **63**, 251 (1974).
25. *N*-Acetylprocainamide or *N*-propylprocainamide was added at 5 μM to each sample as an internal standard to control for drug recovery.
26. M. Dixon and E. C. Webb, *Enzymes* (Academic Press, New York, ed. 3, 1979), pp. 345–353. Deviation from linearity of plots of $1/V$ versus [drug] was observed at low substrate and high drug concentrations. This effect may result from further reaction of the generated metabolites with MPO or its primary substrate H_2O_2 (*11*) or to the irreversible binding of the drug or metabolites to the heme group in MPO [J. M. Van Zyl, K. Basson, R. A. Uebel, B. J. Van der Walt, *Biochem. Pharmacol.* **38**, 2363 (1989)].

27. Supported by NIH grants RO1 AG09574 from the National Institute on Aging and MO1 RR00833 and a grant from the American Lupus Society. Statistical analyses by J. A. Koziol and comments of P. G. Heyworth, E. F. Johnson, and B. M. Babior are greatly appreciated. We thank D. Young of Hyclone Laboratories for providing fetal bovine serum. This is publication number 8610-MEM from The Scripps Research Institute.

25 April 1994; accepted 31 August 1994