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p-Alkoxyphenols, a New Class of Inhibitors of Mammalian R2 Ribonucleotide Reductase: Possible Candidates for Antimelanotic Drugs

STEPHAN PÖTSCH, HANNELORE DRECHSLER, BRUNHILDE LIERMANN, ASTRID GRÄSLUND, and GÜNTER LASSMANN

Max Delbrück Center of Molecular Medicine, D-13122 Berlin-Buch, Germany (S.P., H.D., B.L., G.L.), Max Volmer Institute of Biophysical and Physical Chemistry, Technical University of Berlin, D-10623 Berlin, Germany (G.L.), and Department of Biophysics, Stockholm University, S-10691 Stockholm, Sweden (A.G.)

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SUMMARY

The inhibition by different *p*-alkoxyphenol derivatives of the growth-regulating enzyme ribonucleotide reductase (RR) in purified *Escherichia coli* and mouse R2 protein preparations was studied by EPR spectroscopy. The inhibitor-induced inactivation of the catalytic subunit protein R2 was measured at 77°K by observing the decrease of the typical EPR signal from the functionally essential protein-linked tyrosyl free radical. *p*-Methoxy-, *p*-ethoxy-, *p*-propoxy-, and *p*-allyloxyphenol were about 2 orders of magnitude more effective in inhibiting mouse R2, compared with *E. coli* R2. Among the *p*-alkoxyphenols studied, *p*-propoxyphenol was the most effective inhibitor of mouse R2 (IC_{50}, 0.7 μM) and *p*-methoxyphenol was the least effective (IC_{50}, 11 μM); *p*-ethoxy- and *p*-allyloxyphenol were intermediate. The observed half-maximal inhibition values characterize *p*-alkoxyphenols as a new class of strong inhibitors of the R2 protein of mammalian RR. *p*-Propoxy-, *p*-ethoxy-, and *p*-allyloxyphenol could be considered as new candidates for anticancer drugs. A special cellular inhibition assay of RR in proliferating tumor cells, in which the tyrosyl radical of R2 at natural concentration was monitored by EPR, showed that the four *para*-substituted alkoxyphenols also inhibited the enzyme with high efficiency in tumor cells (IC_{50}, between 0.5 μM and 5 μM). Our results with inactivation of protein R2 of RR imply that the cytostatic effect of *p*-alkoxyphenols on melanoma cells, which has been hitherto explained by inhibition of tyrosinase [*Melanoma Res.* **2**:295–304 (1992)], may be caused at least partly by inhibition of RR. Protein R2 of RR may be considered as an additional target that could be used for future cancer chemotherapy.

The enzyme RR catalyzes the reduction of ribonucleotides to their corresponding deoxyribonucleotides. RR has a key role in DNA synthesis and is important for the regulation of cell proliferation (1–5). RR from *Escherichia coli* and mammalian organisms consists of two nonidentical subunits. The large subunit R1 contains the redox-active cysteines and binding sites for substrates and allosteric regulators. The active form of the catalytic smaller subunit R2 harbors a stable phenoxy-type free radical on a tyrosyl residue. This radical is essential for the enzyme activity (6). The enzyme activity of RR is directly proportional to the amount of protein-linked tyrosyl free radicals. These radicals exhibit a characteristic EPR signal, which can be used for spectroscopic quantitation of enzymatic activity of RR (7). In close proximity to the tyrosyl radical is a μ-oxo-bridged binuclear iron(III) complex (8, 9), which is also essential for enzyme function but which is EPR silent. The iron/radical site is formed *in vitro* in a reaction involving apoprotein R2, iron(II), and O_2 (8, 9). The three-dimensional crystal structure of the *E. coli* protein R2 subunit shows that the iron/tyrosyl site is deeply buried inside the tightly folded structure, about 10 Å from the nearest protein surface (10).

RR activity is maximal in the early S phase of proliferating cells, when deoxyribonucleotides are needed for DNA synthesis (11). Therefore, RR represents a prime target for the development of S phase-specific cytostatic agents that can be used to inhibit the growth of tumors (12, 13) and bacteria (14).

4-HA has been used clinically for treatment of malignant melanoma (15, 16). A current hypothesis assumes that tyrosinase is the target for the inhibitory action of 4-HA. Tyrosinase catalyzes the biosynthesis of melanin (17). RR has also been discussed as an alternative target for 2-, 3-, and 4-HA, instead of or in addition to tyrosinase (18). We have shown that mammalian RR in Ehrlich ascites tumor cells is a target for 4-HA (19). Clinical melanoma therapy with 4-HA has been discontinued, because treatment with higher doses caused toxic side effects (20, 21). The potential medical importance of 4-HA for cancer therapy has, however, received new attention, be-

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ABBREVIATIONS: RR, ribonucleotide reductase; HU, hydroxyurea; 4-HA, 4-hydroxyanisole (*p*-methoxyphenol); HIV-1, human immunodeficiency virus type 1; dNTP, deoxyribonucleoside triphosphate.

cause *p*-substituted derivatives with longer alkyl chains showed improved cytotoxicity against melanoma cells *in vitro* (22, 23) and *in vivo* (24), compared with the methyl derivative.

The aim of the present study was to quantitate the inactivation of the catalytic R2 subunit of RR by 4-HA derivatives in a cell-free enzyme system, to further elucidate the target function of RR. The effects of different *p*-alkoxyphenols (methyl, ethyl, propyl, and allyl derivatives), which have recently been reported as being highly cytostatic against melanoma cells (23, 24), were examined. Species-specific inhibition was studied by comparison of the effects on R2 protein from mouse and *E. coli*. The studies were also extended to Ehrlich ascites tumor cells. The present paper demonstrates that *p*-alkoxyphenols are potent inhibitors of mammalian R2.

Materials and Methods

Chemicals. HU and *p*-methoxyphenol were purchased from Sigma; *p*-ethoxyphenol and *p*-propoxyphenol were purchased from Aldrich; *p*-allyloxyphenol [4-(2-propenyloxy)phenol] was kindly provided by Dr. W. Radeck, WITEGA-Laboratorium Berlin-Adlershof GmbH.

Proteins and assay conditions. The R2 protein of *E. coli* was prepared from an overproducing *E. coli* strain (*C 600 pBS1*) (25) kindly provided by Prof. B. M. Sjöberg (Stockholm University). The mammalian R2 protein was prepared from a recombinant *E. coli* strain containing a mouse structural gene [*BL 21 (DE3) pET M2*] (26), kindly provided by Prof. L. Thelander (University of Umeå). The cell preparation and purification of *E. coli* and mouse proteins were performed according to published procedures (25, 26). The recombinant mouse R2 protein was reactivated by addition of a Fe^{2+} solution and oxygen as described (9). The protein concentration was 10 μM for both R2 proteins, in 50 mM Tris·HCl, 100 mM KCl, pH 7.6.

The purity of the prepared proteins, mouse R2 and *E. coli* R2, was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (single band), the EPR spectrum of the tyrosyl radical, and the 410-nm peak in the UV/visible spectrum.

Protein samples (200 μl) were incubated in Eppendorf tubes at room temperature for 20 min, in the presence of the inhibitor (concentrations from 0.01 μM to 100 mM), under aerobic conditions. After incubation, the solution was pipetted into quartz tubes and frozen slowly to 77°K for EPR investigation.

A previously developed special cellular inhibition assay (13) was used to quantitate by EPR the tyrosyl radicals of RR in Ehrlich ascites mouse mammary carcinoma cells (early S phase). A dilute suspension of cells in a physiological sodium chloride solution at room temperature was exposed to the inhibitor and centrifuged for 5 min at 1000 rpm, and the pellet was frozen to 77°K in the EPR tube. The incubation time at room temperature, including centrifugation and sample transfer, was 20 min; the same time was used in the cell-free system.

EPR spectroscopy. EPR spectra of the tyrosyl radicals were recorded from samples (R2 proteins or cell pellets) in liquid nitrogen at 77°K with a Varian E3 X-band spectrometer, using a finger cryostat. The modulation amplitudes were 10 G (mouse R2) and 6 G (*E. coli* R2), and microwave power settings of 100 mW (mouse R2) and 10 mW (*E. coli* R2) were used. Quartz sample tubes with an internal diameter of 3 mm, corresponding to a sample volume of 200 μl, were used.

The relative amplitude of the characteristic EPR doublet spectrum of tyrosyl radicals in the active R2 protein from mouse or *E. coli* (Fig. 1, *inset*) (normalized to 100% for the control without inhibitor) was used to measure the inactivation of R2 at various concentrations of the different inhibitors. Half-maximal inhibition values were used to compare the efficiency of the inhibitors for purified RR and cellular RR.

Results

Inactivation of the catalytic R2 subunit of RR by *p*-alkoxyphenols. The EPR signal of the essential enzymatic tyrosyl radical in the catalytic R2 subunit of RR has been used to study the inactivation of R2 by *p*-alkoxyphenols with varying alkyl chain lengths. The following preparations were used: 1) isolated and purified R2 protein from mice, 2) isolated and purified R2 protein from *E. coli*, and 3) Ehrlich ascites tumor cells from mice. *p*-Methoxyphenol, *p*-ethoxyphenol, *p*-propoxyphenol, *p*-allyloxyphenol, and HU in different concentrations were incubated with the R2 protein of mouse and *E. coli* and with tumor cells. The intensity of the residual EPR signal of the tyrosyl radical after incubation, relative to that of the untreated sample, is shown for the three different preparations in Figs. 1 and 2.

Isolated mammalian R2 protein. The mouse R2 protein is inactivated by all *p*-alkoxyphenols with high efficiency, exhibiting half-maximal inhibition values between 0.7 and 11 μM (Fig. 1A; Table 1). The inhibition increases significantly with the length of the alkyl chain. The most effective quenching of tyrosyl radicals occurs with *p*-propoxyphenol, followed by *p*-ethoxyphenol, *p*-allyloxyphenol, and *p*-methoxyphenol. *p*-Propoxyphenol quenches the tyrosyl radical in the mouse R2 protein 215 times more effectively than does HU (Fig. 1A; Table 1).

Isolated *E. coli* R2 protein. The *E. coli* R2 protein is also inactivated by *p*-alkoxyphenols (IC_{50}, 280–1100 μM), but 2 orders of magnitude less effectively than the mammalian R2 protein (Fig. 1B; Table 1). The derivatives with longer alkyl chains are also more effective in *E. coli* R2, compared with the methyl derivative, but to a lesser extent (factor of 4) than with mouse R2 (factor of 16). HU inactivates *E. coli* R2 to a similar extent as does *p*-allyloxyphenol.

RR in tumor cells. The inactivation of tyrosyl radicals in the isolated catalytic R2 subunit of mouse RR by *p*-alkoxyphenol derivatives (cell-free system) (Fig. 1A) has been compared with the inhibition of holo-RR in the cytoplasm of whole tumor cells of mice. Tyrosyl radicals of RR in Ehrlich ascites mouse mammary carcinoma cells are quenched by all *p*-alkoxyphenols with slightly higher efficiency (IC_{50}, 0.5–5 μM) (Fig. 2) than are those in the cell-free mouse R2 protein (Fig. 1A; Table 1). In Ehrlich ascites tumor cells the derivatives with longer alkyl chains are more effective in inactivating the purified mouse R2 protein than is the methyl derivative. The most effective quenching of tyrosyl radicals of RR in tumor cells is observed with *p*-propoxyphenol (IC_{50}, 0.5 μM) (as in mouse R2 protein), followed by *p*-allyloxyphenol, *p*-ethoxyphenol, and *p*-methoxyphenol.

Discussion

RR as a target for cytostatically active *p*-alkoxyphenols. The increasing incidence of skin cancer produced by enhanced UV exposure and the small number of cytostatic agents that are effective against melanoma emphasize the need for improved drugs in the treatment of malignant melanoma. The drug 4-HA has been shown *in vitro* (27) and *in vivo* (15) to have cytostatic effects on melanoma cells, presumably by targeting tyrosinase, an enzyme involved in melanogenesis (17).

In clinical applications a partial regression of malignant melanomas on the skin of extremities of patients treated by local intra-arterial infusion with 4-HA was reported (16). Nevertheless, clinical chemotherapy has ceased, due to toxic side effects associated with slightly increased doses (20, 21). On the other hand, *para*-substituted oxyphenols with longer alkyl chains have recently been shown to be highly cytostati-

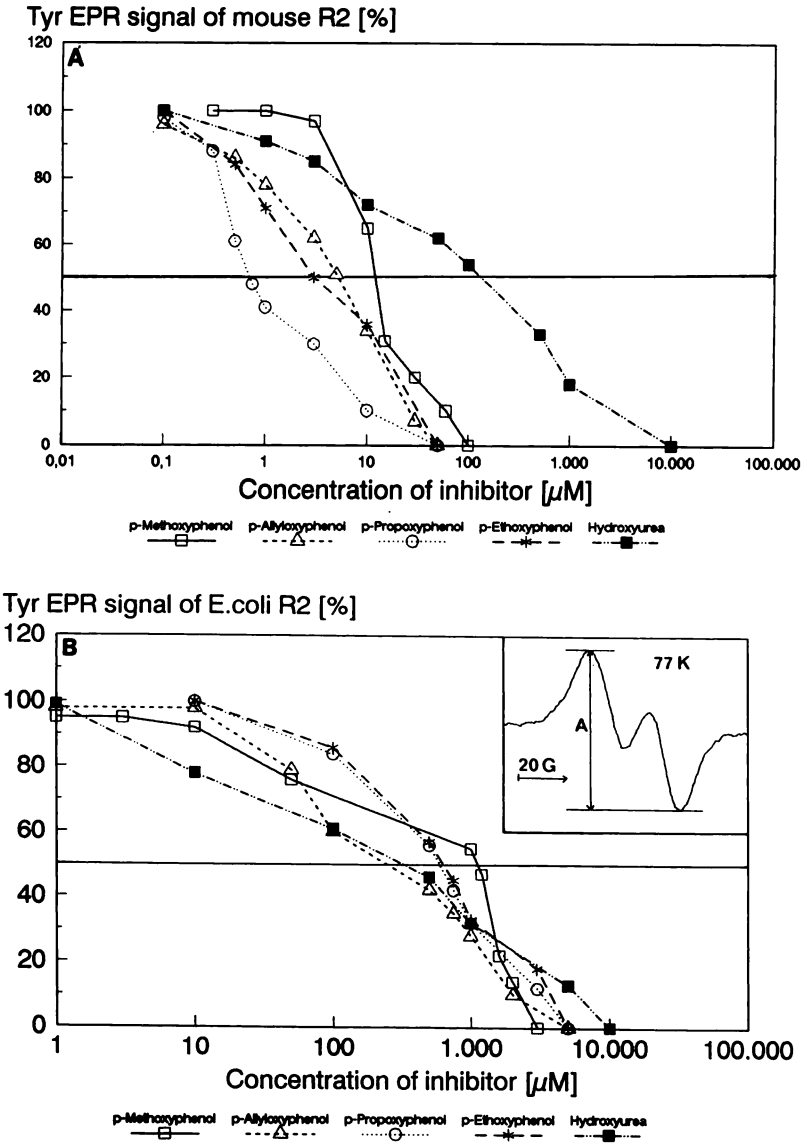

Fig. 1. Inactivation of the mouse R2 (A) and *E. coli* R2 (B) catalytic subunits of RR by *p*-alkoxyphenols and HU. The relative amplitude of the EPR doublet from the tyrosyl radical at 77°K is calculated as a function of the concentrations of inhibitors. *Points* are averages of three independent measurements, and the standard error is 5–10%. IC_{50} values are presented in Table 1. *Inset*, EPR doublet from the tyrosyl radical of *E. coli* R2 of RR at 77°K. Modulation amplitude, 10 G; microwave power, 10 mW.

cally active in *in vivo* (24) and *in vitro* experiments (22, 23); they are more efficient than the methyl derivative. In a previous study we showed that 4-HA quenches the tyrosyl radicals of RR in Ehrlich ascites tumor cells as well as in melanoma tissue (19); therefore, RR has been considered as a possible target for the cytotoxicity of 4-HA. To test this hypothesis, the present study was designed to investigate the effects of *p*-alkoxyphenols with varying alkyl chains on the tyrosyl radicals of pure catalytic R2 subunits of RR from mouse and *E. coli*, as well as Ehrlich ascites cells. We show that all four *p*-alkoxyphenols studied are potent inhibitors of RR, particularly of the mammalian enzyme.

p-Propoxyphenol is the most effective inhibitor of mouse R2 among the 4-HA derivatives studied (Fig. 1; Table 1). This compound inhibits mammalian R2 protein 215-fold more effectively than does the clinically used antimelanotic drug HU (28) and 16-fold more effectively than does *p*-methoxyphenol, previously used in melanoma chemotherapy (local perfusion) (17, 20, 21). Our results on cell-free mouse R2 protein are in remarkable agreement with *in vivo* inhibition experiments in mice bearing transplantable B16 melanoma and Harding Passey melanoma; those studies showed 72% regression of the tumors after treatment with *p*-propoxyphenol, whereas treatment with dacarbazine, a clinically applied antimelanotic drug, showed only 28% regression of the tumors (24).

Our cellular inhibition assays with Ehrlich ascites mouse tumor cells show that *p*-propoxy-, *p*-allyloxy-, *p*-ethoxy-, and *p*-methoxyphenol quench tyrosyl radicals of RR in tumor cells with an overall slightly higher efficiency than that for the purified mouse R2 protein. This indicates that the required passage of the inhibitor through the diffusion barrier of the plasma membrane does not hinder the access of the inhibitor to RR in the cytoplasm.

The quenching of catalytically essential tyrosyl radicals in purified mammalian R2 protein, as well as in tumor cells, by *p*-alkoxyphenols suggest that 1) radical reactions are dominant for inactivation of R2 and 2) the cytostatic action of the *p*-alkoxyphenols is due to the targeting of R2 of RR. Detailed studies of *p*-alkoxyphenols as inhibitors of tyrosinase in cell-free systems and in melanoma cells have previously shown that tyrosinase is a target for cytotoxicity (17, 22–24). The data presented here show clearly that RR is another target for the

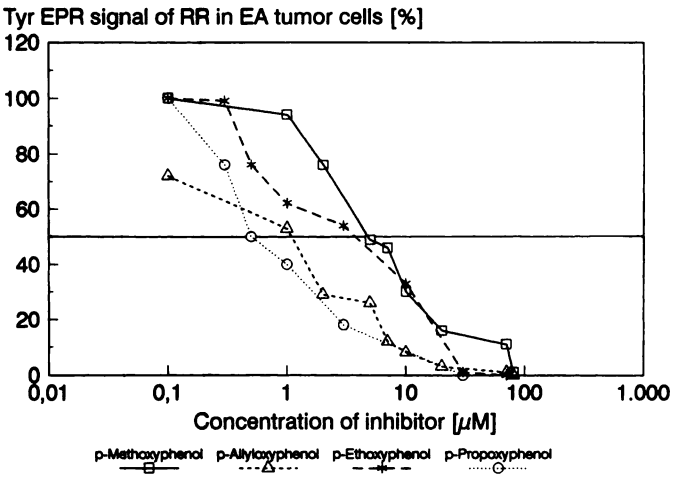

Fig. 2. Quenching of the tyrosyl radical signal from RR in proliferating Ehrlich ascites mouse mammary carcinoma tumor cells by *p*-methoxy-, *p*-ethoxy-, *p*-propoxy-, and *p*-allyloxyphenol. *Points* are averages of five independent measurements, and the relative error is about 15–20%. IC_{50} values are presented in Table 1.

TABLE 1

Half-maximal inhibition values of HU and *p*-alkoxyphenols for the isolated R2 catalytic subunits of RR from mouse and *E. coli* and for RR in Ehrlich ascites tumor cells

Inhibitor	IC_{50}		
	Mouse R2	RR in Ehrlich ascites tumor cells	*E. coli* R2
		μM	
p-Methoxyphenol	11 ± 1	5 ± 0.8	1100 ± 50
p-Ethoxyphenol	3 ± 0.2	4 ± 1	650 ± 50
p-Allyloxyphenol	5 ± 0.2	1 ± 0.2	280 ± 50
p-Propoxyphenol	0.7 ± 0.05	0.5 ± 0.1	580 ± 60
HU	150 ± 15		280 ± 20

p-alkoxyphenols, possibly in addition to their previously known action on tyrosinase. This is the first time that the efficient inhibitory activity of alkyl-substituted 4-HA derivatives on RR has been reported.

The high efficiency of *p*-alkoxyphenols in inhibiting RR may improve a new strategy for anti-acquired immunodeficiency syndrome therapy against HIV-1 proposed very recently by Gallo and associates (29). In their study it was shown that HU depletes the cellular pool of dNTPs in HIV-1-infected stimulated lymphocytes. Under such conditions of suboptimal concentrations of dNTPs, the reverse transcriptase activity was found to result in incomplete HIV-1 DNA synthesis (discrete fragments rather than high molecular weight DNA). This effect was hypothesized to increase the therapeutic effect of nucleoside analogs such as 3′-azido-3′-deoxythymidine, which are provided in a deoxy state and do not need RR for their metabolism. Because the depletion of the dNTP pool by HU is caused by inhibition of RR, more effective inhibitors of RR, such as the *p*-alkoxyphenols (up to 215 times more effective than HU) described in the present paper, may prove to be even more effective therapeutic agents against HIV-1, because considerably lower doses of inhibitors would lead to the same extent of dNTP depletion as with HU.

Molecular mechanisms of inactivation of R2. The question arises regarding the molecular mechanisms of the inhibition of RR by *p*-alkyl-substituted oxyphenols. The quenching of tyrosyl radicals requires the transfer of an electron from the reducing inhibitor to the Tyr-122 residue in R2, which is deeply buried in the protein tertiary structure (10). For the known RR inhibitor HU it has been shown that the reaction with tyrosyl radicals is generally slow but is 3 times faster in mouse R2 than in *E. coli* R2 (30). This indicates better steric accessibility to Tyr-122 for the inhibitor in mouse R2 than in *E. coli* R2. A similar tendency is even more obvious for the IC_{50} values of *p*-alkoxyphenols in mouse and *E. coli* R2 (a difference of a factor of 100). The much more effective inhibition of mouse R2 by *p*-alkoxyphenols, compared with HU, could be explained by a favored access to Tyr-122 in R2 by *p*-alkoxyphenols.

The increasing efficiency of inactivation of R2 with increasing size of the hydrophobic alkyl substituents of the inhibitors may be related to the distinct hydrophobic environment of the tyrosyl radical (10), which may facilitate access of the agent to the radical. A similar pattern of inhibition was previously observed for another class of RR inhibitors, i.e., alkyl-substituted hydroxamic acids (31).

In conclusion, the results demonstrate that *p*-alkoxyphenols represent a very potent group of inhibitors of mammalian RR. These agents may prove useful as low-dose chemotherapeutic drugs against tumors, including malignant melanoma.

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References

1. Thelander, L., and P. Reichard. Reduction of ribonucleotides. *Annu. Rev. Biochem.* **48**:133–158 (1979).
2. Eriksson, S., and B. M. Sjöberg. Ribonucleotide reductase, in *Allosteric Enzymes* (G. Hervé, ed.). CRC Press, Boca Raton, FL, 189–215 (1989).
3. Stubbe, J. Ribonucleotide reductases. *Adv. Enzymol.* **63**:349–419 (1989).
4. Thelander, L., and A. Gräslund. Ribonucleotide reductase in mammalian systems, in *Metal Ions in Biological Systems* (H. Sigel and A. Sigel, eds.). Marcel Decker, Inc., New York, in press.
5. Reichard, P. Regulation of deoxyribonucleotide synthesis. *Biochemistry* **26**:3245–3248 (1987).
6. Gräslund, A., M. Sahlin, and B. M. Sjöberg. The tyrosyl free radical in ribonucleotide reductase. *Environ. Health Perspect.* **64**:139–149 (1985).
7. Ehrenberg, A., and P. Reichard. Electron spin resonance of the iron-containing protein B2 from ribonucleotide reductase. *J. Biol. Chem.* **247**:3485–3488 (1972).
8. Peterson, L., A. Gräslund, A. Ehrenberg, B. M. Sjöberg, and P. Reichard. The iron center from ribonucleotide reductase in *Escherichia coli*. *J. Biol. Chem.* **255**:6706–6712 (1980).
9. Ochiai, E. I., G. J. Mann, A. Gräslund, and L. Thelander. Tyrosyl free radical formation in the small subunit of mouse ribonucleotide reductase. *J. Biol. Chem.* **256**:15758–15761 (1990).
10. Nordlund, P., B. M. Sjöberg, and H. Eklund. Three-dimensional structure of the free radical protein of ribonucleotide reductase. *Nature (Lond.)* **345**:593–598 (1990).
11. Eriksson, S., A. Gräslund, S. Skog, L. Thelander, and B. Tribukait. Cell cycle-dependent regulation of mammalian ribonucleotide reductase. *J. Biol. Chem.* **259**:11695–11700 (1984).
12. Cory, J. G., and A. H. Cory, eds. *Inhibitors of Ribonucleoside Diphosphate Reductase Activity*. Pergamon Press, New York (1989).
13. Liermann, B., G. Lassmann, and P. Langen. Quenching of tyrosine radicals of M2 subunit from ribonucleotide reductase in tumor cells by different antitumor agents: an EPR study. *Free Radicals Biol. Med.* **9**:1–4 (1990).
14. Schaper, K. J., J. K. Seydel, M. Rosenfeld, and J. Kazda. Development of inhibitors of mycobacterial ribonucleotide reductase. *Lepr. Rev.* **57**:254–264 (1986).
15. Dewey, D. L., F. W. Butcher, and A. R. Galpine. Hydroxyanisole-induced regression of the Harding-Passey melanoma in mice. *J. Pathol.* **122**:117–126 (1977).
16. Morgan, B. D. G. Recent results of a clinical pilot study of intra-arterial 4-HA chemotherapy in malignant melanoma, in *Hydroxyanisole: Recent Advances in Anti-melanoma Therapy* (P. A. Riley, ed.). IRL Press, Oxford, UK, 233–241 (1984).
17. Riley, P. A. Hydroxyanisole: the current status, in *Hydroxyanisole: Recent*

Advances in Anti-melanoma Therapy (P. A. Riley, ed.). IRL Press, Oxford, UK, 1–10 (1984).
18. Elford, H. L. The effect of hydroxyanisoles on mammalian ribonucleotide reductase, in *Hydroxyanisole: Recent Advances in Anti-melanoma Therapy.* (P. A. Riley, ed.). IRL Press, Oxford, UK, 71–75 (1984).
19. Lassmann, G., B. Liermann, W. Arnold, and K. Schwabe. Ribonucleotide reductase in melanoma tissue: EPR detection in human amelanotic melanoma and quenching of the tyrosine radical by 4-hydroxyanisole. *J. Cancer Res. Clin. Oncol.* **117**:91–95 (1991).
20. Belcher, H. J., M. Nizam, and T. J. O'Neill. Intra-arterial 4-hydroxyanisole chemotherapy for locally recurrent malignant melanoma: a re-appraisal. *Br. J. Plast. Surg.* **45**:208–210 (1992).
21. Rustin, G. J., M. R. Stratford, A. Lamont, N. Bleehen, P. A. Philip, N. Howells, R. R. Watfa, and J. A. Slack. Phase I study of intravenous 4-hydroxyanisole. *Eur. J. Cancer* **28**:1362–1364 (1992).
22. Naish-Byfield, S., C. J. Cooksey, A. M. Latter, C. I. Johnson, and P. A. Riley. *In vitro* assessment of the structure-activity relationship of tyrosinase-dependent cytotoxicity of a series of substituted phenols. *Melanoma Res.* **1**:273–287 (1991).
23. Smit, N. P., K. Peters, W. Menko, S. Pavel, and P. A. Riley. Cytotoxicity of a selected series of substituted phenols towards cultured melanoma cells. *Melanoma Res.* **2**:295–304 (1992).
24. Schwabe, K., J. Redslob, D. Breitfeld, R. Zeisig, B. Tschiersch, W. Wohlrab, K. D. Wozniak, C. Beyer, C. Nowak, A. Projan, and W. Krüger. Verfahren zur Herstellung von Hydrochinonmonoalkylethern. German patent DD 287 482 A5 (1991).
25. Sjöberg, B. M., S. Hahne, M. Karlsson, H. Jörnvall, M. Göransson, and E. Uhlin. Overproduction and purification of the B2 subunit of ribonucleotide reductase from *Escherichia coli*. *J. Biol. Chem.* **261**:5658–5662 (1986).
26. Mann, G. J., A. Gräslund, E. I. Ochiai, R. Ingemarson, and L. Thelander. Purification and characterization of recombinant mouse and herpes simplex virus ribonucleotide reductase R2 subunit. *Biochemistry* **30**:1939–1947 (1991).
27. Kulkarni, G. A., and L. Nathanson. Specifity of growth inhibition of melanoma by 4-hydroxyanisole. *Pigment Cell Res.* **2**:40–43 (1989).
28. Krakoff, I. H. Clinical and pharmacological effects of hydroxyurea, in *Antineoplastic and Immunosuppressive Agents* (A. C. Sartorelli and D. G. Johns, eds.), Part II. Springer-Verlag, New York, 789–792 (1975).
29. Gao, W. Y., A. Cara, R. C. Gallo, and F. Lori. Low levels of deoxyribonucleotides in peripheral blood lymphocytes: a strategy to inhibit human immunodeficiency virus type 1 replication. *Proc. Natl. Acad. Sci. USA* **90**:8925–8928 (1993).
30. Lassmann, G., A. Gräslund, and L. Thelander. EPR stopped flow studies of the reaction of the tyrosyl radical of protein R2 from ribonucleotide reductase with hydroxyurea. *Biochem. Biophys. Res. Commun.* **188**:879–887 (1992).
31. Kjøller-Larsen, I., B. M. Sjöberg, and L. Thelander. Characterization of the active site of ribonucleotide reductase of *Escherichia coli*, bacteriophage T4 and mammalian cells by inhibition studies with hydroxyurea analogues. *Eur. J. Biochem.* **125**:75–81 (1982).

Send reprint requests to: Günter Lassmann, Max Delbrück Center of Molecular Medicine, Robert-Rössle-Str. 10, D-13122 Berlin, Germany.