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INHIBITION BY RIFAMPIN OF ELASTASE AND LYSOZYME SECRETION IN MOUSE PERITONEAL MACROPHAGES

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The secretion of neutral proteinases by activated macrophages is biologically important in tissue injury and repair (1). Since the neutral proteinase content of macrophages in tissue culture is small, such secretion must depend on continuous protein synthesis (2). We therefore examined the effects of rifampin, a drug which can inhibit protein synthesis (3), on the secretion of one neutral proteinase, elastase. To assess cell viability, the releases of a lysosomal enzyme, β-glucuronidase, and a cytosol enzyme, lactate dehydrogenase (LDH), were measured. The effects of rifampin on lysozyme secretion were also examined. This study demonstrates that rifampin, at subtoxic concentrations, diminishes elastase and lysozyme secretion.

MATERIALS AND METHODS

Rifampin was a gift from the late Dr. Hans Heymann of Ciba-Geigy Corp. (Summit, NJ). Lysozyme standard, Micrococcus lysodeikticus (lysozyme substrate) and thioglycollate broth were purchased from Difco Laboratories (Detroit, MI). Porcine pancreatic elastase, elastin, pyruvic acid, NADH and phenolphtalein glucuronic acid were purchased from Sigma Chemical Co. (St. Louis, MO). Tissue culture media, serum and balanced salt solution were obtained from Gibco Laboratories (Grand Island, NY). [^{14}C] Formaldehyde (10 mCi/nmole) and aquasol were obtained from New England Nuclear (Boston, MA).

Mouse peritoneal macrophages were harvested 48 hr following the interperitoneal injection of thioglycollate (2). The cells were washed once in Hanks' balanced salt solution and cultured for 24 hr in Medium 199 containing 10% acid-treated fetal calf serum. After allowing 24 hr for cell adherence, the medium was changed to serum-free Newman-Tytell medium (containing lactalbumin hydrolysate) for up to 7 days. On day 5, fresh Newman-Tytell medium was added, together with rifampin. Medium and cells were separately harvested after 48 hr. Cellular and medium lysozyme, LDH and β-glucuronidase were assayed on the same day without further processing, apart from sonication of the cell pellet. Culture medium was prepared for elastase assay by dialyzing the 4 ml aliquots against 10 mM Tris-HCl with 1 mM $CaCl_2$ (pH

7.8) for 24 hr. The material was subsequently lyophilized.

Lysozyme (4), LDH (5) and β-glucuronidase (6) were assayed by standard techniques. Protein was assessed by the Folin procedure.

Elastase activity was measured by the method of Bielefeld *et al.* (7). Briefly, bovine ligamentum nuchae elastin was purchased from Worthington Co. (Freehold, NJ) and labeled with [^{14}C] formaldehyde (7). The specific activity of the product was approximately 200,000 cpm/mg protein. For each assay we used approximately 0.1 mg of [^{14}C] elastin suspended in 200 µl of 0.2 M Tris-HCl (pH 7.8) and 0.05 M $CaCl_2$, together with 100 µl of either cell lysate suspension or concentrated tissue culture medium or pancreatic elastase standards. Substrate and samples were incubated for 18-24 hr at 37°. After centrifugation at 16,000 g for 30 min, solubilized counts were measured in aquasol in a Beckman liquid scintillation spectrophotometer. Approximately 0.1 µg of pancreatic elastase could be detected with this method.

No effects of rifampin (25-50 µg/ml) on the enzyme assays *per se* were detected. The effect of rifampin on elastase activity in the culture medium was also examined in mixing experiments. Cell-free aliquots of the culture media from control and rifampin-treated macrophages were pooled. This pooled medium and separate cell-free aliquots of media from both control and rifampin-treated macrophages were incubated for 12 hr under tissue culture conditions. There were no differences between the elastase activities of the pooled medium and the sum of the elastase activities of separately incubated aliquots of medium from control and rifampin-treated cells.

Data are presented as mean ± one standard deviation; significance of difference was determined using Student's t-test for paired variables (control vs drug).

RESULTS AND DISCUSSION

The data in Table 1 demonstrate the following features. First, 100% of the elastase activity was present in the medium, while 86% and 23% of the total lysozyme and β-glucuronidase activities were respectively released into the medium. Thus, all the elastase and the majority of the lysozyme were secreted while much smaller amounts of β-glucuronidase were released. Second, rifampin produced a concentration-dependent diminution (expressed as % of control) in both the total elastase and lysozyme activities but did not affect their distribution between cell and medium. Third, rifampin (25-50 µg/ml) had no effect on β-glucuronidase activities either in cell or medium. Fourth, while there was a slight increase in % LDH release at 50 µg/ml rifampin, there was little change in cell viability as judged by the release of this cytosol enzyme.

In combination, the data indicate that these concentrations of rifampin inhibited the production of both elastase and lysozyme without affecting cell viability as judged by

either LDH release or a lysosomal granule enzyme (β-glucuronidase) release. Other observations indicate: (a) rifampin (25-50 μg/ml) did not affect the total protein in the system; and (b) rifampin (> 62.5 μg/ml) produced falls in total protein and sharp increases in the % release of LDH so indicating cytotoxicity.

Table 1. Effects of Rifampin*

	Control		Rifampin			
			25 μg/ml		50 μg/ml	
	Total Activity (units)	Percent Activity in medium	Total Activity (% control)	Percent Activity in medium	Total Activity (% control)	Percent Activity in medium
Elastase	1.2 ± 0.40	100	80.0 ± 15.0†	100	44.1 ± 10.4††	100
Lysozyme	97 ± 7.8	86 ± 4	82.0 ± 6.1†	83 ± 3	62.1 ± 7.5††	88 ± 4
β-Glucuronidase	86.1 ± 15.2	23 ± 3	97.1 ± 4.1	24 ± 5	94.1 ± 4.6	24 ± 3
LDH % Release		8.3 ± 1.2		9.2 ± 1.9		12 ± 2

*Control data (8 experiments) for elastase, lysozyme and β-glucuronidase are total cell + medium enzyme activities expressed per mg cell protein in 48 hour cultures. The units are, respectively, 1 μg porcine pancreatic elastase equivalent, 1 μg egg white lysozyme equivalent, and Δ O.D. units, where 1 unit activity releases 1 μg phenolphthalein/hr. Note: in order to compare directly the effects of rifampin on elastase, lysozyme and β-glucuronidase with the appropriate control experiments, the rifampin data are expressed as the % of the matched control experiments. Data (mean ± 1 S.D.) for rifampin at 25 and 50 μg/ml were respectively derived from 5 and 6 separate experiments performed in triplicate.

† $P < 0.05$.

†† $P < 0.01$.

While we have not specifically measured general protein synthesis nor determined the mode of action of rifampin in this system, the simplest and most likely explanation of these observations is that rifampin predominantly inhibits the formation of the vigorously synthesized proteins, specifically lysozyme and elastase. This explanation is supported by the following arguments. First, the protein synthesis inhibitor cycloheximide (0.5-1.0 μg/ml) inhibits elastase production by such cultured macrophages (2). Second, there is no evidence in this cell system for the existence of inactive "pro-elastase;" thus, elastase activity in the medium must directly reflect elastase synthesis. Third, our interpretation is consistent with the effects of rifampin on protein synthesis by human liver and rat lymphocyte microsomes (3) and its effects on bacteria (8). Impaired synthesis necessarily limits the secretion of these enzymes. Additionally, the failure of rifampin at these concentrations to affect β-glucuronidase release suggests that the drug does not affect the secretory process *per se*.

The potential therapeutic significance of these observations requires further investigation. However, our experimental concentrations of rifampin can be compared with tissue concentrations of rifampin in normal experimental animals. Doses of 10 mg/kg body weight yield tissue concentrations in the range of 64 and 9 µg/ml in liver and lung, respectively (9,10). In man, conventional daily doses of rifampin are 600 mg (i.e. 7.5 mg/kg in 80 kg man). Thus, modest impairment of the tissue injuring proteinase, elastase, could conceivably occur at conventional doses of rifampin. Additionally, conventional therapeutic doses of rifampin do impair some immune mechanisms in man (11) but the site(s) of action is not known.

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REFERENCES

1. A.J. Barrett (Ed.), *Proteinases in Mammalian Cells and Tissues*, Elsevier/North-Holland Inc., New York (1977).
2. Z. Werb and S. Gordon, *J. Exp. Med.* 142, 361 (1975).
3. W.C. Buss, R. Morgan, J. Guttmann, T. Barela and K. Stalter, *Science* 200, 432 (1978).
4. G. Litwack, *Proc. Soc. Exp. Biol. Med.* 89, 401 (1955).
5. F. Stolzenbach, in *Methods in Enzymology* (Ed. S. Colowick), Vol. 9, p. 278-288, Academic Press, New York (1966).
6. *Worthington Enzyme Manuel*, p. 88, Millipore Corp., Bedford, MA (1978).
7. D. Bielefeld, R. Senior and S. Yu, *Biochem. Biophys. Res. Comm.* 67, 1553 (1975).
8. L. Weinstein, in *The Pharmacological Basis of Therapeutics* (Ed. L.S. Goodman and A. Gilman), 5th edition, p. 1208-1210, MacMillan Publishing Co., Inc., New York (1975).
9. G. Binda, E. Domenishini, A. Gottardi, B. Orlandi, E. Ortelli, B. Pacini and G. Fowst, *Arzneim. Forsch. (Drug Res.)* 21, 1907 (1971).
10. H.G. Meyer-Brunot, K. Schmid and H. Keberle, *5th International Congress of Chemotherapy*, Wien, Austria, 1, 763 (1967).
11. P. Mukerjee, S. Schuldt and J.E. Kasik, *Antimicrob. Agents Chemother.* 4, 607 (1973).