Biochemical Pharmacology, Vol. 20, pp. 2821–2824. Pergamon Press, 1971. Printed in Great Britain

SLOWED LYSOSOMAL ENZYME RELEASE AND ITS NORMALIZATION BY DRUGS IN ADJUVANT-INDUCED POLYARTHRITIS

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(*Received* 12 *November* 1970; *accepted* 24 *March* 1971)

Abstract—In adjuvant-induced polyarthritis, rat liver lysosomes release acid phosphatase and β-glucuronidase more slowly than normal. The effect appears on the first day and persists for at least 2 weeks. Slowed release seems to reflect altered membrane stability rather than decreased enzyme synthesis, since there are insignificant differences between enzyme activities of detergent-treated lysosomes from normal and adjuvant-induced arthritic rats. Oral administration of either cycloleucine or aspirin partially normalizes the enzyme release. These results indicate that membrane labilization may be a factor in diminishing the expression of tissue injury.

ONE OF the widely held ideas about inflammation is that it involves excessive release and increased activity of lysosomal hydrolases. This concept of increased lysosome fragility leading to pathological damage rests largely on three lines of strong but circumstantial evidence: the stabilization *in vitro* of lysosomal membranes by anti-inflammatory steroids and chloroquine;[1,2] the induction of chronic inflammation by repeated administration of streptolysin S, an enzyme capable of rupturing lysosomal membranes;[3] and the mediation of cartilage damage by cathepsin D, a lysosomal enzyme.[4]

Development of adjuvant-induced polyarthritis[5] permits a direct investigation of the release of lysosomal enzymes in an experimental model of chronic, systemic inflammation. This paper reports the results of such a study, using rat liver lysosomes and two marker enzymes, acid phosphatase and β-glucuronidase.

METHODS

Adjuvant arthritis was induced in male Lewis rats weighing 185–210 g, and its course was observed over the experimental period.[6] We followed the detailed procedures of Tanaka and Iizuka[7] for preparing the whole lysosomal fraction and for the subsequent pre-incubation of the lysosomal suspension at 37° and pH 7·4 with shaking. (Two differences were the use of a Sorvall SS-34 rotor with minimum and maximum radii of 5·7 and 10·8 cm, respectively, and final reconstitution to give 1·0 g of liver equivalent per ml.) Yields averaged about 27 per cent (calculated as acid phosphatase), and comparison of sp. act., based on protein, in Triton X-100-treated homogenates and lysosomal fractions showed 5-fold enrichment in the lysosomes, about that of de Duve *et al.*[8] Rates of enzyme release were determined by assaying, at 30 min intervals (0–2 hr), acid phosphatase action on sodium α-naphthyl phosphate[9] and β-glucuronidase action on phenolphthalein mono-β-glucuronic acid.[10] Initial (zero-time) release values reflected little damage in preparation: 26 control rats showed average

zero-time releases of 4·9 ± 0·2% of their lysosomal acid phosphatase and 13·4 ± 0·8% of their β-glucuronidase; corresponding percentages for adjuvant arthritic rats (at day 15) were 3·8 ± 0·1 and 11·1 ± 0·6.

Drugs were administered orally daily or on alternate days as described in Table 1. Cycloleucine was synthesized in these Laboratories; phenylbutazone was kindly supplied by Geigy Laboratories; aspirin was a recrystallized U.S.P. preparation.

TABLE 1. RATES OF ENZYME ACTIVITY RELEASE FROM LIVER LYSOSOMES OF NORMAL, ADJUVANT ARTHRITIC, AND DRUG-TREATED RATS AFTER 15 days

Experimental group	Linear regression coefficient*	
	Acid phosphatase	β-glucuronidase
Normal rats (26)†	198·5 ± 10·96	150·2 ± 6·1
Untreated arthritic rats (26)	63·5 ± 4·5‡	106·4 ± 6·9‡
AA rats (6)	50·8 ± 5·2	99·9 ± 8·2
AA + cycloleucine (6)§	95·8 ± 8·4‡	127·3 ± 16·0‖
AA rats (8)	76·2 ± 9·9	113·6 ± 10·7
AA + phenylbutazone (8)	86·7 ± 9·3	114·0 ± 10·5
AA rats (8)	70·6 ± 9·4	134·0 ± 13·3
AA + aspirin (8)	198·1 ± 16·9‡	156·1 ± 10·9

* The values are optical density (absorbancy) increments ($\times 10^3$) per 30-min interval ± S.E. Tests of significance for linear and curvilinear trends showed linear trends to be dominant for both enzymes.

† Number of rats in each group given in parenthesis.

‡ Significant at $P < 0.01$.

§ Drug schedules and percentage inhibition of swelling of injected and non-injected paws on day 15 were: cycloleucine, 35 mg/kg alternate days, 53 and 92%; phenylbutazone, 75 mg/kg daily, 55 and 81%; aspirin, 200 mg/kg daily, 58 and 65%.

‖ Significant at $P < 0.05$.

RESULTS AND DISCUSSION

Tables 1–3 show that in adjuvant-induced polyarthritis, liver lysosomes release acid phosphatase and β-glucuronidase more slowly than normal. Slower release became evident soon after induction of the polyarthritis, and it persisted throughout the experimental period.

Differences in stability of released enzymes would give similar results, but comparisons made after disruption of the lysosomal membranes with Triton X-100 (0·1%), followed by incubation at 37°, showed no differences in either enzyme between normal and adjuvant-treated rats. Moreover, the total activities of the Triton X-100-treated lysosomes in the two groups did not differ significantly, indicating that the enzyme *content* did not govern release rates in systems not treated with the detergent. This points to increased membrane stability, rather than enzyme depletion or lowered synthesis, in lysosomes of the arthritic rats.

Table 1 indicates that oral administration of cycloleucine, which is a potent immunosuppressive agent,[11,12] or of aspirin produces an accelerated enzyme release from the lysosomes of adjuvant arthritic rats, i.e. a tendency toward normalization. In control experiments, using normal rats treated with the drugs in exactly the same way as were

TABLE 2. LYSOSOME LABILIZATION AT VARIOUS STAGES AFTER INDUCTION OF ADJUVANT ARTHRITIS—RELEASE OF ACID PHOSPHATASE ACTIVITY

Day		Percentage activity release at 30-min assay intervals*				
		0	30	60	90	120
1	N	5 ± 0·5	17 ± 1·1	21 ± 1·4	23 ± 1·3	32 ± 0·8
	AA	8 ± 0·7	16 ± 0·9	18 ± 1·2	19 ± 0·9	27 ± 2·5
3	N	4 ± 0·2	20 ± 0·8	25 ± 1·3	34 ± 1·8	49 ± 1·8
	AA	5 ± 0·5	14 ± 0·4†	16 ± 0·4†	19 ± 0·5†	27 ± 0·6†
7	N	6 ± 0·5	18 ± 1·1	26 ± 2·2	30 ± 2·2	44 ± 3·1
	AA	4 ± 0·4	13 ± 0·6‡	15 ± 0·9†	18 ± 1·2†	27 ± 1·9†
11	N	3 ± 0·1	10 ± 0·8	20 ± 2·3	26 ± 2·1	36 ± 2·3
	AA	3 ± 0·2	8 ± 0·5‡	10 ± 1·6‡	14 ± 2·5‡	20 ± 3·5†
15	N	4 ± 0·3	18 ± 1·3	38 ± 3·2	60 ± 4·5	77 ± 2·2
	AA	4 ± 0·5	8 ± 0·4†	17 ± 1·2†	20 ± 1·2†	29 ± 2·1†
18	N	6 ± 0·5	21 ± 2·1	28 ± 2·1	39 ± 2·3	51 ± 2·3
	AA	4 ± 0·1	10 ± 0·3†	14 ± 0·7†	11 ± 0·3†	15 ± 0·5†

* Percentage release is calculated from the mean absorbancy of 20 systems treated with 0·1% Triton X-100. Each figure in a column represents the mean and S.E. from four normal (N) or four adjuvant-arthritic (AA) rats.
† Significant at $P < 0·01$.
‡ Significant at $P < 0·05$.

TABLE 3. LYSOSOME LABILIZATION AT VARIOUS STAGES AFTER INDUCTION OF ADJUVANT ARTHRITIS—RELEASE OF β-GLUCURONIDASE ACTIVITY

Day		Percentage activity release at 30-min assay intervals*				
		0	30	60	90	120
1	N	11 ± 1·0	32 ± 1·3	49 ± 3·2	57 ± 3·4	69 ± 1·6
	AA	16 ± 1·2	22 ± 0·9†	36 ± 1·6†	50 ± 2·3	63 ± 3·0
3	N	9 ± 0·6	48 ± 3·5	64 ± 5·8	78 ± 4·0	83 ± 5·6
	AA	11 ± 1·5	33 ± 4·2‡	42 ± 5·4‡	59 ± 2·2†	70 ± 2·6
7	N	13 ± 1·4	31 ± 3·1	55 ± 3·4	61 ± 3·5	73 ± 6·7
	AA	11 ± 2·8	23 ± 0·9‡	35 ± 3·2†	53 ± 3·3	65 ± 5·9
11	N	15 ± 2·3	46 ± 6·2	80 ± 4·3	101 ± 3·5	132 ± 2·9
	AA	20 ± 3·4	31 ± 2·9	56 ± 6·9‡	73 ± 8·7‡	92 ± 12·6‡
15	N	12 ± 2·3	40 ± 1·2	73 ± 2·2	99 ± 3·2	102 ± 3·2
	AA	11 ± 1·1	27 ± 4·0‡	53 ± 4·6†	79 ± 3·9†	87 ± 5·6
18	N	15 ± 3·2	36 ± 2·7	59 ± 5·2	71 ± 2·7	80 ± 8·4
	AA	8 ± 0·8	21 ± 1·5†	39 ± 2·0‡	40 ± 1·4†	53 ± 3·3‡

* See Table 2.
† Significant at $P < 0·01$.
‡ Significant at $P < 0·05$.

the arthritic rats, no accelerated enzyme release was found; in only one case was there any significant enzyme stabilization, that of acid phosphatase after phenylbutazone treatment (linear regression coefficients 165·6 ± 23·9 and 124·0 ± 18·1 respectively).

The results of this study suggest that suppression by some drugs of the inflammatory process induced by a delayed hypersensitivity reaction is associated with *release* of lysosomal hydrolases, rather than their retention. Weissmann *et al.*[13,14] made the related finding of a diminished delayed hypersensitivity to immune challenges following hypervitaminosis A and heightened liver lysosome fragility.

Lysosomes may play disparate roles in acute and chronic inflammatory states. While the extrusion of cathepsin D and polysaccharidases could contribute to increased catabolic activity and tissue erosion, other lysosome components, such as the tissue activator of plasminogen,[15–17] may quench the expression of chronic inflammation by eliminating antigenic debris and materials which evoke hyperplasia.

Acknowledgement—The authors wish to thank Mrs. Catherine Kryzanauskas, Mrs. Theresa M. Tobin and Mr. L. Datko for their competent technical assistance.

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