

CONNECTIVE-TISSUE EFFECTS OF DRUGS CAUSING LUPOID SYNDROME

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Abstract. Three inducers of the lupoid syndrome have been tried for their effect on the biosynthesis of essential connective tissue components in 10-day-old chicken embryo tibiae. Hydralazine, procainamide and chlorpromazine decreased the incorporation of (14 C)-D-glucosamine into glyco- and/or mucoproteins. An inhibition of the biosynthesis of collagen also resulted. Under the influence of hydralazine an underhydroxylated collagen was synthesized. The fact that three collagen synthesis inhibiting drugs on clinical trial as therapeutics for scleroderma can produce an l.e.-like syndrome is discussed.

The development of a syndrome with clinical and laboratory characteristics of systemic lupus erythematosus has been reported to be caused by various drugs (1, 12, 13, 14, 16, 20, 23, 24, 26, 29, 30, 31, 32, 33). The pathogenesis is so far unknown. Three drugs of the group, i.e. procainamide, hydralazine and chlorpromazine have been tried for a possible effect on the incorporation of (14 C)-L-proline and (14 C)-L-lysine and the hydroxylation of these amino acids to hydroxyproline and hydroxylysine by 10-day-old chicken embryo tibiae. Glycosylation of hydroxylysine and incorporation of (14 C)-D-glucosamine into undialysable material of connective tissue were also studied. The biosynthesis of (14 C)-Hypro and (14 C)-Hylys is a parameter of collagen formation, while the incorporation of labelled glucosamine is indicative of gluco- and/or mucoprotein biosynthesis.

Abbreviations

Pr = proline
Lys = lysine
Hypro = hydroxyproline
Hylys = hydroxylysine

MATERIAL AND METHODS

Procainamide, hydralazine and chlorpromazine were products of E. R. Squibb & Sons, Ltd., England, E. Merck, Darmstadt, and Nordisk Droge, Copenhagen, respectively.

Uniformly labelled (14 C)-L-Pr (specific activity 180 μ Ci/ μ mole), (14 C)-L-Lys (spec. act. 223 μ Ci/ μ mole) and (14 C)-D-glucosamine (spec. act. 5-10 μ Ci/ μ mole) were obtained from New England Nuclear Corp., Boston.

Two tibiae per tube out of a pool from twelve 10-day-old chicken embryos were preincubated in a medium of salts, buffer and glucose (28) at 37°C for 30 minutes. The preincubation was continued for 30 additional minutes in the presence of increasing doses of procainamide, hydralazine or chlorpromazine. Controls were run without addition of the drugs. At the end of the preincubation period, incubation with 5 μ Ci of (14 C)-Lys, (14 C)-Pr or (14 C)-glucosamine per tube was performed for 2 hours at 37°C under aerobic conditions. The tissues were then homogenized and dialysed against running tap-water overnight. Tissues labelled with (14 C)-Pr were hydrolysed with 6 N HCl at 110°C for 16 hours while samples labelled with (14 C)-glucosamine were hydrolysed with 4 N HCl at 100°C for 4 hours. The material labelled with (14 C)-Lys was divided into two aliquots. One of them was hydrolysed with 6 N HCl as indicated for the (14 C)-Pr labelled material, while the other was analysed without hydrolysis. After hydrolysis, HCl was evaporated under vacuum at 65°C. The samples were then dissolved in distilled water. A 0.1 ml aliquot of each sample was used for determination of the total (14 C) uptake which was expressed as (14 C)-Pr, (14 C)-Lys and (14 C)-glucosamine incorporated into the undialysable material synthesized. (14 C)-Hypro was assayed on the (14 C)-Pr labelled material according to Juva & Prockop (18). Total and unglycosylated (14 C)-Hylys were assayed on the hydrolysed and unhydrolysed (14 C)-Lys labelled aliquots according to Blumenkrantz & Prockop (2). The value of glycosylated (14 C)-Hylys was obtained as the difference between total and unglycosylated (14 C)-Hylys. The results were calculated as dpm per bone per μ Ci per hour of incubation (dpm/b/ μ Ci/h) and expressed as a percentage of controls considered as 100%.

Table I. Biosynthesis of proteins and glyco- and/or mucoproteins by 10-day-old chicken embryo tibiae under the influence of three drugs known to cause lupoid syndrome

Substances tested	Conc. in medium (mM)	Total (^{14}C) uptake, % of control		
		(^{14}C) L-proline	(^{14}C) L-lysine	(^{14}C) D-glucosamine
Procainamide	2.4	58	79	84
	4.8	53	66	78
	12.0	35	51	40
Hydralazine	2.4	67	66	84
	4.8	52	39	66
	7.2	39	25	58
	12.0	5.4	10	27.8
Chlorpromazine	0.018	92	80	90.28
	0.055	41	37	79.35
	0.27	2.3	1.32	2.83

RESULTS

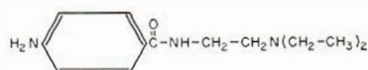
A decreased biosynthesis of collagen and glyco- and/or mucoprotein was demonstrated under the effect of increasing concentrations of the three drugs assayed (Table I). Procainamide and chlorpromazine decreased the (^{14}C)Pr and (^{14}C)Lys incorporation and, to a similar degree, restricted the hydroxylation of both precursors of the amino acids characteristic of collagen, i.e. hydroxyproline and hydroxylysine. Consequently a decreased collagen biosynthesis resulted.

Table II. Effect of lupoid syndrome inducing drugs on the biosynthesis of collagen by 10-day-old chicken embryo tibiae

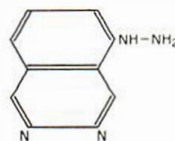
The synthesis of collagen was measured by formation of hydroxyproline, hydroxylysine and glycosylated hydroxylysine. Values indicate % of control

Substances tested	Conc. in medium (mM)	(^{14}C) Hypro	(^{14}C) Hyls	
			Total	Glycosylated
Procainamide	2.4	55.8	76	70
	4.8	55.4	71	71
	12.0	35	48	47
Hydralazine	2.4	7.1	7.0	5.6
	4.8	5.9	2.2	1.3
	7.2	2.4	2.0	0.9
	12.0	2.2	1.6	0.3
Chlorpromazine	0.018	82	101	96
	0.055	33	30	28
	0.27	4	3	0

PROCAINAMIDE



HYDRALAZINE



CHLORPROMAZINE

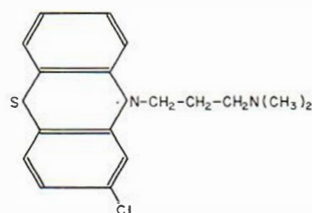


Fig. 1. Chemical structure of three inducers of lupoid syndrome.

Hydralazine inhibited the hydroxylation of the precursor amino acids to a greater degree than their incorporation. A decreased glyco- and/or mucoprotein synthesis was also observed with similar doses of the three drugs.

DISCUSSION

A diminished biosynthesis of an underhydroxylated collagen (procollagen) is a possibility, which can explain the hydralazine effect. Fe^{++} ions are co-factors required for the hydroxylation of proline and lysine to hydroxyproline and hydroxylysine (3, 7, 8, 15, 17, 31), while Mn^{++} ions are co-factors for the glycosylation of hydroxylysine (5). By chelating Fe^{++} and Mn^{++} (9, 10, 26) and by combining with α -ketoglutarate (26), hydralazine eliminates some important co-factors required for the hydroxylation of procollagen, a polypeptide rich in proline and lysine, as well as for the glycosylation of the already hydroxylated procollagen. Considering the fact that Mn^{++} ions are required in the biosynthesis of glycosaminoglycans (21, 22), the decreased incorporation of (^{14}C)glucosamine into glyco- and/or mucoproteins may also be related to the Mn^{++} -chelating effect of hydralazine (9, 10). It is interesting that hydralazine may cause manganese

deficiency in chicken (10), as Comens (9) stressed the advantage of administering manganese ions to patients with hydralazine-induced lupoid syndrome. The same author also observed a Mn^{++} induced reversal of renal lesions in dogs fed hydralazine. A slow turnover of manganese in rheumatoid arthritis was reported by Cotzias et al. (11).

The decreased biosynthesis of normally hydroxylated collagen observed under the effect of chlorpromazine, may be related to the decreased protein biosynthesis, which has been observed under the effect of this drug as a possible result of the interaction of chlorpromazine with nucleic acids (6, 19, 25, 27).

The inhibition of glyco- and/or mucoprotein biosynthesis under the effect of chlorpromazine may be related to its chelating effect on Mn^{++} ions (4). Procainamide may act in a similar way considering its chemical similarity to the other two substances studied (Fig. 1).

In view of the fact that the pathology of lupus erythematosus is characterized by acute exudative inflammation and scleroderma by chronic fibrotic "healing" phenomena, it is noteworthy that three substances which can produce an i.e.-like syndrome can inhibit collagen synthesis. In this clinic, the same substances are now on clinical trial as therapeutics for scleroderma.

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