[Chem. Pharm. Bull. 25(11)3107—3110(1977)]

UDC 615.276.015.45.076.9 : 577.159.04.05

Modification of Drug-Metabolizing Enzyme Activity of Rats in Carrageenin-Induced Inflammation

Morio Fukuhara and Eigo Takabatake

The Institute of Public Health[1]

(Received March 25, 1977)

The activity of drug-metabolizing enzymes in hepatic microsomes of rats with inflammatory lesions was determined. In the Donryu-rats carrying carrageenin-induced granuloma, the cytochrome P-450 and b_5 levels and the activity of aniline hydroxylase in hepatic microsomes were depressed markedly at day 6 after *s.c.* injection of carrageenin when the granuloma formation and exudate accumulation occur rapidly. However, at day 15 after carrageenin injection when the granuloma is in regression, the activity of these enzymes was decreased slightly but without significant differences.

Keywords——carrageenin; granuloma; inflammation; drug-metabolizing enzyme; cytochrome P-450

The importance of evaluating the drug-metabolizing activity in patients is recently emphasized in clinical field in respect of therapeutic use. The activity of drug metabolism in individuals is known to be dependent on variuos factors[2] including sex, age, race, nutrition, *etc.* It also depends on the pathological state of individuals. However, few studies have been done on the changes of the activity in patients with various diseases in which drugs are therapeutically employed. Several workers have reported the variations of drug metabolism in the animals with experimentally induced hepatic lesions,[3] such as hepatoma, hepatitis, obstructive jaundice, and hepatic injury, with alloxan diabetis,[4] and with adjuvant-induced arthritis.[5]

We have examined the activity of drug-metabolizing enzymes of animals with inflammatory lesions. As a model for inflammation, carragenin-induced granuloma was employed, which was shown to represent the responses of clinically observed inflammatory diseases such as wound healing and rheumatoid arthritis.[6]

Experimental

Carrageenin granulomas were induced in 6 week-old male and female rats of Donryu strain (Nippon Rat Co. Ltd., Tokyo) by injecting 4 ml of 2% (w/v) carrageenin (Marine Colloid Inc., New Jersey, U.S.A.) solution into the air sac previously formed on the dorsum of rats by *s.c.* injection of air.[6] The activity of the drug-metabolizing enzymes was determined in two different phases of the inflammatory process; first in the early phase of inflammatory process (6 day after carrageenin injection) where the formation of granuloma and the accumulation of exudate occur rapidly and secondly, in the later phase of inflammatory process (15 days after carrageenin injection) where the preformed granuloma and accumulated exudate are in gradual regression.

1) Location: *6–1, Shirokanedai, 4-chome, Minato-ku, Tokyo, 108, Japan.*
2) J.R. Gillette, "Ciba Foundation Symposium on Drug Responses in Man," ed. by E.W. Wolstenholme and R.J. Porter, J. & A. Churchill Ltd., London, 1967, pp. 24—49.
3) J.R. Fouts, "Advances in Enzyme Regulation," Vol. 1. ed. by G. Weber, Pergamon Press, London, 1963, pp. 225—233.
4) R.L. Dixon, L.C. Hart, and J.R. Fouts, *J. Pharmacol. Exp. Ther.*, **133**, 7 (1961).
5) *a*) A. Quevauviller, M.A. Chalchat, H. Brouihet, and F. Delbarre, *C.R. Soc. Biol.*, **162**, 618 (1968); *b*) D.M. Morton and D.H. Chatfield, *Biochem. Pharmacol.*, **19**, 473 (1970); *c*) M.A. Cawthorne, E.D. Palmer, and J. Green, *Biochem. Pharmacol.*, **25**, 2683 (1976).
6) M. Fukuhara and S. Tsurufuji, *Biochem. Pharmacol.*, **18**, 475 (1969).

TABLE I. Activity of Hepatic Microsomal Drug-Metabolizing Enzymes in Carrageenin Granuloma-Bearing Rats

Phase of inflammation	Groups		Body wt. (g)	Liver wt. (g/100 g body wt.)	Microsomal protein (mg/g-liver)	Cytochromes (nmol/mg-protein)		Enzyme activity[a] (nmol/min/mg-protein)			
						P-450	b_5	NADPH-cyt.c reductase	Aminopyrine demethylase	Aniline hydroxylase	*p*-Nitroanisol demethylase
6 day-old granuloma	Male										
	Control	(6)[b]	168±3[c]	4.74 ±0.05	28.3 ±0.8	0.589 ±0.022	0.267 ±0.017	53.7 ±1.9	2.47 ±0.22	0.429 ±0.034	1.86 ±0.08
	Granuloma	(6)	163±6	4.73 ±0.09	28.1 ±0.4	0.395 ±0.037[d]	0.195 ±0.010[d]	47.7 ±2.4	2.15 ±0.08	0.339 ±0.020[e]	1.65 ±0.11
	Female										
	Control	(6)	150±5	4.61 ±0.16	27.5 ±0.4	0.517 ±0.022	0.238 ±0.014		1.64 ±0.07	0.242 ±0.024	1.04 ±0.12
	Granuloma	(6)	160±6	4.62 ±0.14	26.9 ±0.6	0.418 ±0.022[e]	0.189 ±0.010[d]		1.64 ±0.06	0.156 ±0.010[d]	0.95 ±0.09
15 day-old granuloma	Female										
	Control	(6)	159±10	4.76 ±0.33	25.4 ±1.1	0.503 ±0.045	0.225 ±0.014	33.7 ±2.4	1.52 ±0.07	0.381 ±0.052	
	Granuloma	(6)	155±5	5.02 ±0.42	24.7 ±0.4	0.466 ±0.051	0.187 ±0.010	30.1 ±2.0	1.45 ±0.10	0.297 ±0.051	

a) Enzyme activities are expressed as nmoles of reduced cytochrome c, nmoles of formaldehyde released from aminopyrine, nmoles of *p*-aminophenol formed from aniline and nmoles of *p*-nitrophenol formed from *p*-nitroanisol in min per mg of protein for NADPH-cytochrome c reductase, aminopyrine N-demethylase, aniline hydroxylase and *p*-nitroanisol O-demethylase, respectively.
b) Number of animals per group.
c) Mean ± standard errors.
d) Significantly different from controls at $p<0.01$.
e) Significantly different from controls at $p<0.05$.

The livers were excised after the decapitation of the animals, immediately minced with scissors and homogenized in 4 volumes of 0.25 M sucrose solution (pH 7.4) and the homogenates were subjected to the isolation of microsomes according to the method of Kamath and Rubin.[7] The microsomal precipitates were suspended in a 0.15 M KCl-Tris buffer solution (pH 7.4) and the contents of protein[8] and cytochrome[9] P-450 and b_5 in this microsomal fraction were determined. The activities of NADPH-cytochrome c reductase, aminopyrine N-demethylase, aniline hydroxylase and *p*-nitroanisol O-demethylase were also determined.[10]

Results

The results are presented in Table I. No significant differences were obtained in the body weight gain, the liver weight and the microsomal protein content of the liver between the controls and the granuloma-bearing rats. The activity of the drug-metabolizing enzymes expressed as per mg of microsomal protein, cytochrome P-450 and b_5 levels were reduced markedly in the early phase of granuloma formation (*i.e.* in the 6 day-old granuloma rats) and the reduction was more pronounced in male than in females. The activity of aniline hydroxylase was also reduced, while those of NADPH-cytochrome c reductase, aminopyrine N-demethlase and *p*-nitroanisol O-demethylase were not changed so markedly in granuloma bearing rats.

In the later phase of inflammation (*i.e.* in the 15 day-old granuloma rats), the cytochromes levels and the enzymes activities were reduced slightly but without significant differences.

Discussion

The present study has shown that in the animals with inflammatory lesions, the levels of cytochrome P-450 in hepatic microsomes is reduced and that the reduction is related more to the early phase which represents acute inflammation than to the later phase which represents chronic inflammation.[6] These observations are in good agreement with those of the other workers[5b,c] who reported reduced cytochrome P-450 levels in rats with adjuvant arthritis which has also certain similarities to human arthritis. Reduced activity of drug-metabolizing enzymes has been demonstrated by the prolongation of sleeping time by barbitals in adjuvant-induced arthritic rats.[5a,b] The mechanism of the reduction of the cytochrome P-450 levels in inflammatory state is, at present, unknown. Cawthorne, *et al.*[5c] suggested a failure in haem biosynthesis and/or an accelerated breakdown of existing haem in arthritic rats. They also discussed the possibility of the influence of the body weight loss in arthritic rats on the cytochrome P-450 levels but it might be excluded in case of carrageenin-granuloma rats which showed a similar growth gain as control animals.

In the present study, the decrease in the levels of cytochrome P-450 and b_5 and in the activity of aniline hydroxylase was significant but not in the activities of the other enzymes. Though some drugs are known to reduce in a differential way the activities of the drug-metabolizing enzymes,[11a,b] it is not known whether the differential reduction is specific to the carrageenin granuloma rats or is due to some factors involving the experimental conditions employed.

The clinical studies on the modification of drug metabolism in rheumatic diseases have not been done but the results of the present study indicate that a defect in drug metabolism may be present in patients with inflammatory diseases. This must also be taken into con-

7) S.A. Kamath and E. Rubin, *Biochem. Biophys. Res. Commun.*, **49**, 52 (1972).
8) G.L. Miller, *Anal. Chem.*, **31**, 964 (1961).
9) T. Omura and R. Sato, *J. Biol. Chem.*, **239**, 2370 (1964).
10) P. Mazel, "Fundamentals of Drug Metabolism and Drug Disposition," ed. by B.N. LaDu, H.G. Mandel, and E.L. Way, Williams and Wilkins Co., Baltimore, 1972, pp. 546—582.
11) *a*) M.G. Parkki, J. Marniemi, and H. Vainio, *Toxicol. Appl. Pharmacol.*, **38**, 59 (1976); *b*) M. Vorne and J. Idänpään-Heikkilä, *Experientia*, **31**, 962 (1975).

sideration in the screening of anti-inflammatory drugs using these kinds of experimental system, especially in evaluating the toxicity or anti-inflammatory activity of drugs that might be converted to an active metabolite like phenylbutazone.

[Chem. Pharm. Bull. 25(11)3110—3114(1977)]

UDC 547.753.04 : 547.539.3.04

Arylindoles. I. Synthesis of Some N-Arylindoles[1]

Misbahul Ain Khan and Emely Kazan Rocha

Seção de Quimica, Instituto Militar de Engenharia[2]

(Received April 4, 1977)

Indole, 2-phenylindole, indole-2-carboxylic acid, indole-3-carboxaldehyde, 3-acetylindole and gramine were condensed with bromobenzene and halonitrobenzenes under the Ullmann conditions (copper(II) oxide catalyst and N,N-dimethylformamide as the solvent). The corresponding N-arylindoles were isolated in good yields with the exception of the gramine due to its possible decomposition under these reaction conditions. Spectral data (infrared and proton magnetic resonance) for the new compounds is presented.

Keywords——N-arylation; Ullmann reaction; N-arylindoles synthesis; N-nitrophenylindole-3-carboxaldehydes; N-nitrophenylindole-2-carboxylic acids; N-arylindoles; 3-acetyl-N-arylindoles; copper-catalyzed condensations

Although C-, and N-alkylindoles as well as C-arylindoles may readily be obtained by means of a variety of methods, N-arylindoles are not easily accessible by these synthetic routes.[3] Indoles containing aryl substituents at the nitrogen have, however, been obtained in the Ninetzescu synthesis[4] but this method is also limited in its applications as it invariably leads to the 5-hydroxy derivatives containing other substituents in the 2 or 3 position of the indole ring. Another method used in the preparation of N-phenylindole–dehydrogenation of N-phenylindoline[5]–may also have its set back when applied as a general method for the dehydrogenations of arylindolines containing sensitive groups.

Recently Ullmann's reaction (copper-catalyzed condensation using aryl halides) has proved to be an efficient method for the syntheses of N-arylazoles[6] and was used with success for the N-arylation of indole.[7] Since the N-aryl analogs of the indoles that are biologically active may also present interesting pharmacological properties, in the present work we have tried to apply the Ullmann reaction for the arylation of indoles containing different substituents in the 2 and 3 position.

1) Taken in part from the Masters thesis of Emely Kazan Rocha, Instituto Militar de Engenharia, 1976.
2) Location; *Urca, Rio de Janeiro, R.J. Brasil.*
3) W.C. Sumpter and F.M. Miller, "Heterocyclic Compounds with Indole and Carbazole Systems," The Chemistry of Heterocyclic Compounds, ed. by A.W. Weissberger, Interscience, New York, 1954; W.J. Houlihan, "Indoles," Parts I and II, The Chemistry of Heterocyclic Compounds, ed. by A. Weissberger and E.C. Taylor, Wiley-Interscience, New York, 1972.
4) C.D. Ninetzescu, *Bull. Soc. Chim. Romania*, **11**, 37 (1929).
5) C.R. Ganellin and H.F. Ridley, *J. Chem. Soc.* (*C*), **1969**, 1537.
6) M.A. Khan, *Rec. Chem. Progr.*, **31**, 43 (1970).
7) M.A. Khan and J.B, Polya, *J. Chem. Soc.* (*C*), **1970**, 85.