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SHORT COMMUNICATIONS

Some biochemical effects of anti-rheumatic drugs

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Anti-rheumatic drugs act through multiple and varied mechanisms [1–4]. Domenjoz [1] reported that anti-rheumatic drugs have a cytostatic effect in cell systems *in vitro*. However, more information may be gained through assessment of alterations brought about by anti-rheumatic agents in the metabolism of tissues *in vivo*. We have studied the deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) content of edematous tissue in two different experimental models of inflammation *in vivo* in rats, namely carrageenin hind-paw edema and cotton pellet granuloma, and also in the presence of non-steroidal anti-inflamatory drugs. Changes in the polynucleotide content may reflect a cytostatic effect, if any, of the anti-rheumatic drug systems *in vivo*

Male albino rats (120–140 g) of the Haffkine strain were used. The rats were given pellet food (Hindustan Lever feed) and kept in an animal house on a 12-hr light–dark cycle at constant temperature (22–24°).

The anti-rheumatic drugs, aspirin, oxyphenylbutazone and indomethacin, were prepared as suspensions in 1% (w/v) carboxy methyl cellulose (CMC).

Carrageenin edema. Edema was produced acutely by the injection of carrageenin into the plantar region of the hind-paws of the rat, according to the method of Winter *et al.* [5].

Oxyphenylbutazone (200 mg/kg), aspirin (300 mg/kg) and indomethacin (10 mg/kg) were given orally, each to a group of ten rats, in a volume of 1.0 ml/100 g body weight, followed by 4.0 ml of distilled water. The control animals (40 rats) received the vehicle only. After exactly 1 hr, 0.1 ml of 1% (w/v) carrageenin was injected into the plantar region of each hind-paw. Groups of ten rats were sacrificed at 1, 3, 6 and 24 hr after carrageenin treatment in order to study the time course effect. Animals treated with drugs (ten rats/group) were sacrificed 3 hr after the carrageenin treatment. The edematous tissue was quickly removed from the hind-paws for biochemical determinations.

Cotton pellet granuloma. Cotton pellet granuloma was produced in rats by the method of Winter and Portar [6] with slight modifications described elsewhere [7]. Oxyphenylbutazone (100 mg/kg), aspirin (200 mg/kg) and indomethacin (5 mg/kg) were given orally to rats (ten/group) in a volume of 1.0 ml/100 g body weight, daily for 7 days. A control group received the vehicle for the same number of days. On day 8, the animals were sacrificed and the granuloma tissues were separated from the implanted pellet for biochemical determinations.

Biochemical determination. The inflamed tissue was homogenized in 10% (w/v) trichloroacetic acid (TCA) and extracted by the method of Schneider [8]; DNA and RNA contents were determined in this extract by the methods of Burton [9] and Mejbaum [10] respectively.

In carrageenin-induced inflammation, the RNA content of the tissues was decreased whereas the DNA content increased (Table 1). The increase in DNA content was maximum at 3 hr and remained constant up to 6 hr. RNA content decreased significantly 1 hr after carrageenin treatment and this decrease was evident up to 6 hr. After 24 hr the increased DNA content of edematous tissues declined, whereas their RNA content showed an increasing trend.

Pretreatment orally with aspirin (300 mg/kg), oxyphenylbutazone (200 mg/kg) and indomethacin (10 mg/kg) antagonized the increase in DNA content and also restored the decrease of RNA content of the inflamed tissues (Table 2). Surprisingly, the RNA content after drug treatment was slightly but significantly higher than the normal level. Pretreatment of normal rats with drugs alone significantly decreased their tissue DNA content but the RNA content remained unaltered.

The DNA and RNA content of cotton pellet granuloma was higher in comparison to the normal skeletal muscle content. Further, in this granuloma, pretreatment with the anti-rheumatic drugs did not alter the DNA content of this tissue, although the RNA content decreased, when compared to untreated controls (Table 3).

Anti-rheumatic drugs prevented the increase in DNA levels of carrageenin-induced edematous tissue. This may be attributed to the inhibition of cell proliferation, a possibility which is supported by the results of our earlier study in which anti-rheumatic drugs reduced the hydroxyproline, hexosamine and sialic acid contents of edematous tissue [11]. Another possibility is that anti-rheumatic drugs stabilize the lysosomal membrane [12, 13] and thereby prevent RNA degradation by lysosomal catheptic enzymes.

A general depression by the anti-rheumatic drugs [14, 15] of the synthesis of DNA, proteins and acid mucopolysaccharides has been shown in granulomatous tissues. Acute treatment of normal rats with anti-rheumatic drugs decreased DNA content without affecting the RNA levels. It is probable that the anti-rheumatic drugs, which are also cytostatic [11] decrease DNA content without adversely affecting DNA-dependent RNA synthesis.

In chronic inflammation (cotton-pellet granuloma), pretreatment with anti-rheumatic drugs reduced only the RNA content without affecting the DNA levels in granula-

Table 1. DNA and RNA content of inflamed rat paw after carrageenin treatment*

Tissue	DNA (mg/g)				RNA (mg/g)			
	1 hr	3 hr	6 hr	24 hr	1 hr	3 hr	6 hr	24 hr
Normal	1.19 ± 0.02	1.27 ± 0.36	1.24 ± 0.03	1.2 ± 0.1	2.6 ± 0.1	2.6 ± 0.2	2.5 ± 0.1	2.4 ± 0.1
Inflamed	1.8 ± 0.14	2.1 ± 0.21	2.4 ± 0.2	1.5 ± 0.1	1.6 ± 0.1	1.0 ± 0.1	0.8 ± 0.1	2.0 ± 0.2
	($P < 0.01$)	($P < 0.01$)	($P < 0.001$)		($P < 0.001$)	($P < 0.001$)	($P < 0.001$)	

* Ten rats/group were used for both normal and inflammation experiments at each interval.
Values are expressed as mean ± S.E.

Table 2. Effect of anti-rheumatic drugs on DNA and RNA content of carrageenin edema tissue*

	Drug treatment and dose	No. of rats	DNA (mg/g)	RNA (mg/g)
Normal	Vehicle (1 ml/100 g)	10	1.27 ± 0.036	2.6 ± 0.15
	Oxyphenylbutazone (200 mg/kg)	10	0.90 ± 0.1 (P < 0.04)	2.2 ± 0.2
	Indomethacin (10 mg/kg)	10	0.94 ± 0.20 (P < 0.05)	2.4 ± 0.4
	Aspirin (300 mg/kg)	10	0.85 ± 0.24	2.2 ± 0.5
Inflamed	Vehicle (1 ml/100 g)	10	2.1 ± 0.2	1.0 ± 0.1
	Oxyphenylbutazone (200 mg/kg)	10	1.2 ± 0.12 (P < 0.01)	3.5 ± 0.1 (P < 0.001)
	Indomethacin (10 mg/kg)	10	1.54 ± 0.14 (P < 0.05)	3.2 ± 0.2 (P < 0.001)
	Aspirin (300 mg/kg)	10	1.5 ± 0.1 (P < 0.05)	3.4 ± 0.2 (P < 0.001)

* Values are expressed as mean ±S.E.

Table 3. Effect of anti-rheumatic drugs on DNA and RNA content in cotton pellet granuloma tissue*

Treatment and dose	No. of rats	DNA (mg/g)	RNA (mg/g)
Vehicle (1 ml/100 g)	10	1.86 ± 0.35 (P < 0.01)†	4.8 ± 0.6 (P < 0.001)†
Oxyphenylbutazone (100 mg/kg)	10	1.75 ± 0.4	3.0 ± 0.3 (P < 0.01)
Aspirin (200 mg/kg)	10	1.82 ± 0.5	2.9 ± 0.4 (P < 0.01)
Indomethacin (5 mg/kg)	10	1.7 ± 0.4	2.8 ± 0.5 (P < 0.01)
Skeletal muscle	10	1.2 ± 0.2	2.5 ± 0.3

* Values are expressed as mean ± S.E.
† Compared with skeletal muscle of ventral side.

tion tissue. This effect of anti-rheumatic drugs may be correlated with inhibition of protein synthesis which is also reported by Domenjoz [1].

The DNA and RNA content of cotton pellet granuloma was found to be higher relative to the normal skeletal muscle levels. The increase in the levels of nucleic acids in such tissues probably reflects the net result of a general cellular proliferation during the course of 7 days.

The differences between the polynucleotide contents in the two types of experimental inflammation studied may depend upon the different characteristics of the inflamatory agents employed, namely carrageenin (a sulfated polysaccharide) and cotton pellet (a non-sulfated polysaccharide).

The cytostatic action of anti-rheumatic drugs in cell culture systems *in vitro* has been confirmed in inflamatory systems *in vivo* in our study, and we believe that this action contributes to the anti-inflamatory effects of these drugs.

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Superoxide anion production by liver microsomes from phenobarbital treated rat

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It is generally agreed that drug hydroxylation is mediated by the NADPH-dependent cytochrome P-450 systems in liver microsomes [1]. The flavoprotein NADPH-cytochrome *c* reductase (EC 1.6.2.4) of the NADPH-specific microsomal electron transport chain, reduces various acceptors among which are ferricytochrome *c* [2], nitroblue tetrazolium [3], and cytochrome P-450 [4] its natural electron acceptor. Cytochrome P-450, once reduced and oxygenated, catalyses the xenobiotic hydroxylation reactions [1]. It has been suggested that, in addition to acting as cytochrome P-450 reductase, NADPH cytochrome *c* reductase may generate superoxide anion (O_2^-).The O_2^- produced could then be the "active oxygen form" which reduces cytochrome P-450 thus permitting the fixation of molecular oxygen (O_2) and further drug hydroxylation [5–8]. Interpretation of these findings have been complicated by the use of the oxidation of epinephrine into adrenochrome as a measure of O_2^- production, since O_2^- may be produced by the direct reaction of epinephrine semiquinone with O_2, as reported by Misra and Fridovich [9].

The aim of the present study was to reinvestigate the rate and mechanism of O_2^- production by liver microsomes from control and phenobarbital treated rats. The effect of drugs added in the incubation medium, on this O_2^- production was also studied. The importance of studying the generation of O_2^- in various conditions is obvious since it has been shown that O_2^- can potentially be harmful in a number of tissues [10–13] and also may participate in the hydroxylation reaction [14].

MATERIALS AND METHODS

Adult Sprague–Dawley female rats, weighing 180–250 g, received daily intraperitoneal injections of phenobarbital (40 mg/kg) dissolved into 0.15 M NaCl for 5 days. Control animals received 0.15 M NaCl alone. The animals were decapitated 48 hr after the last injection, and the livers were immediately removed, weighed, washed into cold 0.15 M NaCl, and stored at −80° for 24 hr. After thawing, 5 g of liver were homogenized in cold 0.34 M sucrose containing 0.04 M Tris–HCl pH 7.5 (Suc-Tris Buffer), with a Potter–Elvejhem type homogenizer. The microsomal fraction was prepared by differential centrifugation according to Shenkman *et al.* [15]. The microsomal pellet of the last centrifugation was resuspended in appropriate volumes of Suc-Tris Buffer for addition to the assay cuvettes. Protein was determined by the method of Lowry *et al.* [16] and cytochrome P-450 according to Omura and Sato [17]. Aniline hydroxylase activity was measured according to Imai and Sato [18] in the presence of 1.25 mM of NADPH and 6.25 mM of aniline. Reduction of cytochrome *c* was measured according to Williams and Kamin [19], with minor modifications. Nitroblue tetrazolium (NBT) reduction was measured as in cytochrome *c* reduction but using NBT instead of ferricytochrome *c*. NADPH-dependent oxygen uptake by liver microsomes, in the absence of exogenous electron acceptors, was measured by the polarographic conventional method using a Gilson oxygraph equipped with a Yellow Spring electrode. NADPH oxidation was measured fluorimetrically by the amount of NADP produced, according to Lowry *et al.* [20] as previously described [21]. The experimental conditions were identical to those used for the measurement of the NADPH-dependent oxygen uptake. O_2^- production by microsomes from control and phenobarbital treated rats, was calculated after determination of superoxide dismutase (SOD) inhibitable reduction of ferricytochrome *c* (or NBT) according to McCord and Fridovich [22]. O_2^- was also measured after determination of SOD apparent inhibitable O_2 consumption. This technique relies on the ability of SOD to dismutate O_2^- according to the following reaction: $2\,O_2^- + 2\,H^+ \rightarrow H_2O_2 + O_2$ [22] and can be used if this reaction does not occur spontaneously (in the absence of SOD). This latter condition was verified in the incubation medium used: more than 95 per cent of the O_2^- generated by the xanthine–xanthine oxidase system was recovered in the medium containing the microsomes. Oxygen uptake and O_2^- production were also measured in the presence of drugs added to the incubation medium. Details of the different procedures used are indicated under each table of results. All measurements were made in triplicate for each animal studied. Statistical significance was determined by Student's *t*-test [23]. Reagents: SOD and NBT were purchased from Sigma Chemical Co., glucose-6-phosphate, glucose-6-phosphate dehydrogenase, NADP, NADPH and horse heart cytochrome *c*, from Boehringer Mannheim. Other reagents were the best grade commercially available.

RESULTS AND DISCUSSION

Table 1 presents the results (1) of the total and O_2^- dependent reduction rate of cytochrome *c* and NBT by liver microsomes, and (2) O_2 uptake and associated O_2^-