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A COMPARISON OF THE FREE RADICAL SCAVENGING ACTIVITY OF LEUKOTRIENES AND PROSTAGLANDINS

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The relative reactivity of leukotrienes and prostaglandins to free radicals and the superoxide ion generated by the photo oxidation of dianisidine have been assessed *in vitro*. Leukotrienes are effective general radical scavengers rather than superoxide scavengers, with the order of effectiveness $LTD_4 > LTC_4 > LTB_4$. Prostaglandins are relatively unreactive. It is suggested that this susceptibility to free radical attack may be one reason for the formation of chemotactic agents from leukotrienes rather than prostaglandins and this may be one part of the mode of action of these compounds in chronic inflammation.

KEY WORDS: Leukotrienes, prostaglandins, superoxide.

INTRODUCTION

Mast cells and secondary cells of inflammation such as neutrophils, mononuclear cells and macrophages release substances such as leukotrienes, lipid chemotactic factor, and platelet activation factor as well as free radicals on activation by immunological and non immunological means.[1-3] However, in spite of reports of the existence of a free radical pathology in inflammation[4-6] and of the importance of arachadonic acid products in inflammation, little attention has been paid to the oxidation of these products by free radicals.

Since such products are unsaturated compounds, reactions with free radicals produced simultaneously at loci in close proximity make *in vivo* reactions between them likely. In this paper we assess the relative scavenging activity of a selection of leukotrienes and prostaglandins in an *in vitro* assay developed by Misra and Fridovich[7] for the estimation of superoxide dismutase activity. With this assay it is possible to differentiate between free radical scavenging activity and specific superoxide dismutase scavenging activity. Thus, as well as defining the relative reactivity of the compounds studied, the relative efficiency of their reaction with the superoxide ion and other radicals is assessed.

MATERIALS AND METHODS

Leukotrienes were supplied by Merck Frost Inc and prostoglandins were supplied by Upjohn Limited. Bovine superoxide dismutase (SOD) and glutathione (GSH) were obtained from the Sigma Chemical Company. o-dianisidine and riboflavin were

TABLE I

	Volume Riboflavin Solution (ml)	Volume O-dianisidine Solution (ml)	Volume Test Solution (ml)
Test	2.88	0.06	0.06
Blank	2.94	0.06	-

obtained from BDH Limited. All reagents were of the highest purity available and were used without further purification.

Illumination for the photochemical reaction was provided by a pair of parallel 10 watt Phillips Fluorescent tubes mounted 6 inches apart in an aluminium foil-lined open-ended box (base area 648 cm^2 × 38 cm in height). Stock solutions of riboflavin (1.3×10^{-5} M) in 0.01 M potassium phosphate buffer at pH7.5 and o-dianisidine (10^{-2} M) in ethanol were prepared fresh for each set of experiments. The assay required the preparation of test solutions and a blank as shown in Table I.

The absorbance of aliquots of the sample and blank in quartz cuvettes were measured at 460 mm on Beckman Acta MIV double beam spectrometer. The cuvettes were then transferred to the light box, illuminated for 4 minutes, and the absorbance remeasured at 460 mm. From this, the change in absorbance at 460 mm after 4 minutes illumination was calculated. Sample absorbances were compared with blank absorbances and a percentage inhibition or augmentation was calculated. Each measurement was repeated from four to five times and experiments were repeated either using the same concentration of sample in the solution or after changing the final concentration of sample depending on the availability.

RESULTS

Leukotrienes when added to the assay produced an inhibitary effect on the photo-oxidation of o-dianisidine (Table II). The concentration of the leukotrienes in the final reaction mixture was 4 $\mu g\ ml^{-1}$ and the order of the inhibitory effect on the assay was $LTD_4 > LTC_4 > LTB_4$. The extent of inhibition by leukotrienes was however much reduced in the presence of SOD in the reaction mixture. [Table III]. Leukotrienes appear to have a similar inhibitory effect on the assay as has been reported for GSH previously.[8] This assay can be used to classify compounds as SOD-like or GSH-like

TABLE II
Effect of Leukotrienes on the Photo-Oxidation of o-Dianisidine n – number of measurements taken

	Conc. of Leukotrienes in the final solution	Abs after 4 mins illumination Mean ± S.D. (n)	Percentage Inhibition Mean ± S.D. (n)
	$\mu g\ ml^{-1}$		
Blank	0.0	0.69	
LTB_4	4.0	0.57 ± 0.001 (4)	16.9 ± 2.7 (4)
LTC_4	4.0	0.46 ± 0.003 (5)	32.4 ± 4.6 (5)
LTD_4	4.0	0.32 ± 0.001 (3)	52.6 ± 2.1 (3)

TABLE III
Effect of Leukotrienes on Photo-Oxidation of o-Dianisidine in the Presence of SOD

	Conc. of Leukotrienes in final solution	Conc. of SOD in final solution	Abs after 4 mins illumination Mean ± SD (*n*)	% Age Inhibition on SOD Activity Mean ± SD (*n*)
	$\mu g\,ml^{-1}$	$\mu g\,ml^{-1}$		
	0.0	4.0	0.192 ± 0.008 (3)	
LTB_4	4.0	4.0	0.179 ± 0.004 (4)	6.4 ± 2.4 (4)
LTC_4	8.0	4.0	0.162 ± 0.000 (3)	15.6 ± 2.1 (3)
LTD_4	8.0	4.0	0.149 ± 0.003 (3)	22.3 ± 1.8 (3)

TABLE IV
a) Effect of SOD on Photo-Oxidation of O-dianisidine

	Concentration of SOD in final solution	Abs at 460 nm After 4 minutes illumination Mean ± S.D. (*n*)
	$\mu g\,ml^{-1}$	
Blank	0.0	0.59 ± 0.002 (3)
Test Solution	2.0	0.160 ± 0.003 (3)
	4.0	0.192 ± 0.008 (3)

TABLE IV
b) Effect of GSH on Photo-Oxidation of o-dianisidine

	Conc. of GSH in Final Solution	Abs after 4 mins illumination Mean ± S.D. (*n*)	% Age Inhibition Mean ± S.D. (*n*)
	$\mu g\,ml^{-1}$		
Blank	0.0	0.59 ± 0.002 (6)	
Test Solution	2.0	0.35 ± 0.002 (6)	40.6 ± 3.5 (6)
	4.0	0.28 ± 0.001 (7)	52.7 ± 3.1 (7)
	8.0	0.23 ± 0.001 (7)	61.2 ± 1.7 (7)

because SOD-like compounds produce an augmentation and GSH like compounds an inhibition (Table IV).

Prostaglandins when added to the assay failed to exhibit a significant effect. Very little inhibitory effect was exhibited by synthetic prostacyclin and even at concentrations much higher than those used for leukotrienes, the effect is relatively small [Table V].

DISCUSSION

The assay used in this study was developed by Misra and Fridovich,[7] and is based on the photo-oxidation of o-dianisidine sensitized by riboflavin. The photo-oxidation of o-dianisidine involves a complex series of free radical chain reactions involving the superoxide ion as a chain propagating species. Misra and Fridovich proposed that the mechanism of photo-oxidation of o-dianisidine involved the following reactions:-

TABLE V
Effect of Prostaglandins on Photo-Oxidation of o-dianisidine

Compound	Concentration of the Sample in Final Solution	% Age Inhibition on the assay Mean ± S.D. (*n*)
	$\mu g\,ml^{-1}$	
PGE_1	20	No effect
PGE_2	40	No effect
PGF_2	80	No effect
Synthetic Prostacyclin	20	9.8 ± 2.1 (10)

$$Rb + hv \longrightarrow Rb^* \quad (a)$$

$$Rb^* + DH_2 \longrightarrow RbH^0 + DH^0 \quad (b)$$

$$RbH^0 + O_2 \longrightarrow Rb + O_2^- + H^+ \quad (c)$$

$$DH^0 + O_2^- + H^+ \longrightarrow DH_2 + O_2 \quad (d)$$

$$DH^0 + DH^0 \longrightarrow D + DH_2 \quad (e)$$

$$O_2^- + O_2^- + 2H^+ \xrightarrow{SOD} H_2O_2 + O_29 \quad (f)$$

DH_2 is o-dianisidine
Rb is riboflavin
D is the product measured at 460 nm
In reaction (a), the riboflavin absorbs a photon and becomes electronically excited. In reaction (b) the excited riboflavin oxidises dianisidine, yielding a flavin semiquinone and a dianisidine radical which, in the absence of competing reactions would dismute as in reaction (e) to yield oxidised dianisidine (*D*) which absorbs at 460 nm. However, the flavin semiquinone produced in reaction (b) can reduce O_2 to O_2^- (reaction c) and the O_2^- can, in turn, reduce the dianisidine radical (reaction d). SOD which scavenges the O_2^- ion (reaction f) prevents the reaction (d) relative to reaction (e). In the presence of SOD an increase in absorbance due to *D* will be observed. On the other hand any radical scavenging species acting on reaction (b) would reduce the formation of *D* in reaction (e) and a decrease in absorbance would be observed.

A general radical scavenging action therefore causes an inhibition of the coloured product resulting in a reduction in signal works. Leukotrienes (Table II) and GSH (Table IV) both act on reaction (b). The inhibitory effect exhibited by leukotrienes varied from compound to compound giving an order of effectiveness $LTD_4 > LTC_4 > LTB_4$. The larger inhibitory effect exhibited by LTC_4 and LTD_4 may in part be attributed to the presence of a glutathione moiety in these molecules. Leukotrienes therefore, seem to be reactive towards free radicals in a general way rather than to the superoxide ion specifically. The chemotactic activity exhibited by leukotrienes in inflammation could be attributed to this reactivity towards free radicals, particularly since there is evidence that other less specific radicals than superoxide including hydroxyl and singlet oxygen can convert arachadonic acid and leukotrienes to potent chemo-attractant products.[9-13]

Prostaglandins unlike leukotrienes were found to be fairly stable towards free

radicals in the assay used in this study [Table V]. There are some reports suggesting that prostaglandins inhibit leucocyte chemotaxis,[14] adherence to various substrates including endothelium,[15-16] phagocytosis and generation of oxygen derived free radicals.[17,18] Hence part of the inflammatory effect of prostaglandins may be due to their stability towards free radical attack.

In conclusion the assay used in this study can be effectively used to determine if a particular compound is superoxide ion specific or a general free radical scavenger. Leukotrienes are shown by this assay to be reactive towards free radicals but unlike SOD are not a specific scavenger of superoxide ions. Prostaglandins on the other hand, although unsaturated compounds, are less reactive, possibly explaining why leukotrienes and not prostaglandins are chemotactic and cause damage in chronic inflammatory processes.

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