SYSTEMIC OXIDANT STRESS AND ITS EFFECTS ON HEPATOTOXICITY

A thesis submitted for
the degree of

DOCTOR OF PHILOSOPHY

in

The Department of Clinical & Experimental Pharmacology
The University of Adelaide, South Australia.

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August 1988

ABSTRACT

Experimental work which concerned certain aspects of oxyradical formation *in vitro* by a cell type involved in systemic oxidant stress, and the effect of inflammation-induced systemic oxidant stress on hepatotoxicity *in vivo*, produced the results detailed below.

1. A sensitive and specific HPLC-amperometric assay for hydroxylated salicylate was developed for the detection of hydroxyl radicals (OH·). The sensitivity of the assay was attributable to the optimisation of extraction and storage procedures, and the use of electrochemical detection of the reaction products. Both specificity and sensitivity compared favourably with the [^{14}C]-benzoate decarboxylation assay in the ferrous-EDTA autoxidation and xanthine oxidase-hypoxanthine-ferric-EDTA systems. This assay was sufficiently sensitive to measure OH· formation in isolated cellular systems.

2. Human neutrophils (PMN) activated by either serum treated zymosan (STZ), phorbol myristate acetate (PMA), formyl-methionyl-leucyl-phenylalanine (with cytochalasin B) or A23187, produce a partially reduced oxygen species capable of hydroxylating salicylate to form dihydroxybenzoates (DHB), as determined by HPLC/amperometric detection. Irrespective of the stimulus, the hydroxylation was markedly inhibited by superoxide dismutase but not catalase. Azide selectively inhibited DHB formation in PMN

activated with STZ (a potent degranulating agent which stimulates myeloperoxidase release), but had less effect on PMA-stimulated hydroxylation. Desferrioxamine failed to inhibit DHB production suggesting that it is independent of the Fenton reaction. Taken together with the lack of inhibitory effect of chelating agents, the data suggest that salicylate is hydroxylated by PMN *in vitro* by a highly reactive species, probably the $OH^{\cdot}$. This hydroxylating species is superoxide- and myeloperoxidase-dependent and is formed by a Fenton reaction-independent mechanism. Therefore two phagocytic functions - the respiratory burst and degranulation, may interact to produce a toxic species in addition to the hypochlorite ion.

3. Systemic oxidant stress is a condition in which an excess of oxyradical production can lead to altered cellular antioxidant levels and other responses. It was induced in LACA Swiss mice, albino Porton and hooded Wistar rats, by the tail-base injection of the acute inflammogen, oleyl alcohol (OA), or the chronic inflammogen, adjuvant (ADJ, *Mycobacterium tuberculosis* in squalane). The resultant effects included the inhibition of the hepatic microsomal mixed function oxidase (MMFO) profiles in a temporally phasic manner. Hepatic MMFO activity was assessed *in vivo* by pentobarbital sleeping times (PST) and zoxazolamine paralysis times; and *in vitro* by ethoxycoumarin O-deethylation and diphenyloxazole hydroxylation activities.

The injection of inflammogens also resulted in decreased in body weight and fluctuations in hepatic glutathione levels.

Paracetamol-induced hepatotoxicity was ameliorated by the presence of inflammation, however hepatotoxicity induced by bromobenzene was unaffected (assessed by serum alanine aminotransferase). The effects induced by the arthritogenic ADJ treatment were more prolonged than those caused by the short-term inflammogen OA (particularly in the hooded rat). The differential effect of inflammation on two oxidative-microsomally activated hepatotoxins precludes the assumption that all MMFO-activated compounds would be affected in a similar manner by the presence of systemic oxidant stress.

4. Concomitant piroxicam administration repeatedly and specifically attenuated OA-mediated effects on the liver - daily dosage reduced OA-induced PST prolongation (Swiss mice and hooded rats) and OA-mediated ceruloplasmin synthesis (hooded rats); and restored PC-induced hepatotoxicity in OA-inflamed Swiss mice. Clozic administration reduced the inflammation-mediated effects on PC-induced hepatotoxicity in both OA and ADJ treated Swiss mice. The immunological status of Swiss mice and C3H/HeJ mice during OA and/or piroxicam treatment, which was assessed by *ex vivo* lymphocyte proliferation, suggested the presence of high levels of PGE_2 and functionally equivalent levels of IL-1; whereas ADJ treatment caused the production of mainly IL-1.

From these results it may be concluded that: (a) inflammation induced by OA is accompanied by large increases in PGE_2 and IL-1 levels which stimulates the liver (via another cytokine, IL-6) to produce Acute Phase Reactants, turn down certain MMFO systems, and cause fluctuations in hepatic glutathione levels; (b) ADJ-induced inflammation is accompanied by increases in IL-1 predominantly which promotes the same hepatic changes seen with OA treatment (also via IL-6), but in a piroxicam-insensitive (and therefore PGE_2-insensitive) manner. (c) Clozic interacts with presently unknown cytokines common to both pathways. (d) Piroxicam exhibits immunostimulatory action by inducing lymphocyte proliferation in OA-treated mice.

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