Effects of catalase, peroxidase, superoxide dismutase and 10 scavengers of oxygen radicals in carrageenin edema and in adjuvant arthritis of rats

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Summary. Catalase, peroxidase and superoxide dismutase were found to inhibit significantly carrageenin edema and the primary phase of adjuvant arthritis in rats after i.v. injection. Heat-inactivated enzymes were as effective as the native enzymes. None of 10 scavengers of oxygen radicals inhibited the adjuvant arthritis at any time. Accordingly, no evidence for a participation of oxygen radicals in the secondary arthritis phase could be found, whereas a role of oxygen radicals in the primary arthritis phase and in carrageenin edema cannot be ruled out.

Superoxide anions are thought to be involved in inflammatory reactions since they are produced by phagocytosing cells[1], and superoxide dismutase was found to exert anti-inflammatory/antirheumatic activity[2]. The formation of the hydroxyl radical[3] and singlet oxygen[4] by phagocytosing cells are discussed. They might mediate cytotoxic reactions, too. Furthermore, inhibition of oxygen radical formation and scavenging of oxygen radicals, respectively, has been found in vitro for non-steroidal anti-inflammatory agents[5-7]. The participation of oxygen radicals in pathological processes, however, is questioned[8,9]. Most conclusions supporting the involvement of oxygen radicals in inflammatory reactions are based on in vitro findings. Results from experiments in vivo are difficult to interprete. For example, it is at least doubtful whether the radical-scavenger is sufficiently concentrated at the site of inflammation. Moreover, we cannot differentiate with certainty between enzymatic activity and further biological effects of an enzyme protein. Remembering these difficulties we investigated, nevertheless, the influence of catalase, peroxidase and superoxide dismutase and of 5 non-enzyme proteins on both phases of rat adjuvant arthritis and on carrageenin rat paw edema. 10 scavengers of oxygen radicals were included in the experiments. Indomethacin was used as a standard non-steroidal anti-inflammatory agent. Part of its anti-inflammatory activity has been suggested to be mediated by its ability to react with singlet oxygen[10].

Materials and methods. Enzymes: Superoxide dismutase from bovine erythrocytes (superoxide: superoxide oxidoreductase, EC 1.15.1.1, 1600 U/mg, Worthington, Freehold); catalase from bovine liver (hydrogen peroxide oxidoreductase, EC 1.11.1.6, 50000 U/mg, Reanal, Budapest); peroxidase from horse-radish (donor: hydrogen peroxide oxidoreductase, EC 1.11.1.7, 50 U/mg, Boehringer, Mannheim). Proteins: histone sulfate from calf thymus (Ferak, Berlin West); haemoglobin from horse erythrocytes (Reanal, Budapest); human serum albumin (Institut für Impfstoffe, Dessau); protamine sulfate (VEB Berlin-Chemie, Berlin); trypsin inhibitor from soybean (Reanal, Budapest). Further agents used are substances according to the pharmocopoeia of the GDR (AB 2-DDR) or such as could be purchased from commercial sources.

Table 1. Influence on paw swelling in carrageenin edema. The proteins were administered in the native and in the heat-denaturated form (h.) as well. Chlorpromazine, propyl gallate and indomethacin were given p.o., all other substances were administered by the i.v. route, simultaneously with carrageenin injection. POD = peroxidase; CAT = catalase; SOD = superoxide dismutase; SBTI = soybean trypsin inhibitor; HIST = histone sulfate; PROT = protamine sulfate; HB = haemoglobin; HSA = human serum albumin

Substance	mg/kg	Inhibition (%) 2 h	3 h	5 h	n
POD	10	57+	69+	58+	8
POD h.	10	53+	62+	43+	8
CAT	10	21(+)	38+	31+	16
CAT h.	10	26(+)	35+	29+	16
SOD	5	35+	30(+)	21(+)	16
SOD h.	5	30(+)	31+	23(+)	8
SBTI	2.5	53+	46+	21(+)	8
SBTI h.	2.5	42+	35(+)	25	8
HIST	10	7	35+	28+	8
HIST h.	10	0	6	20(+)	8
PROT	50	40+	52+	41+	8
PROT h.	50	51+	52+	42+	8
HB	20	16	13	11	8
HB h.	20	31	9	12	8
HSA	20	18	8	7	10
HSA h.	20	0	0	0	10
Cysteamine	50	32+	38+	25+	15
$MnCl_2$	20	2	24	35+	10
Mannitol	2×200	23	18	18	8
Chlorpromazine	20	48+	36+	14	10
Propyl gallate	250	n.d.	39+	19	10
Indomethacin	1	35+	41+	34+	10

+; (+) $p < 0.01$; 0.05 according to Student's t-test. The following substances were found ineffective: L-cys (100 mg/kg i.v.), D-penicillamine (100 mg/kg p.o.), promethazine (10 mg/kg p.o.), vitamin E (500 mg/kg p.o.), L-his (500 mg/kg p.o.). n.d. = not determined.

Table 2. Influence on paw swelling in primary and secondary phase of rat adjuvant arthritis. Substances were administered from day 1 until day 3 and, in other animals, from day 12 until day 14, respectively. Repeated injections were performed at intervals of about 7 h, namely 08.00–09.00 h and 15.00–16.00 h. Negative data indicate increase of paw swelling. For abbreviations see legend of table 1. Route of administration as indicated in table 1

Substance	mg/kg	Inhibition (%) 2 d	4 d	n	14 d	16 d	n
POD	2× 5	32+	50+	10	5	11	12
POD h.	2× 5	23+	41+	10	5	0	8
CAT	2× 5	25+	39+	12	8	10	12
CAT h.	2× 5	27+	37+	12	5	8	8
SOD	2× 2.5	20	21	6	0	0	6
SOD h.	2× 2.5	25(+)	23	6	0	0	6
SBTI	2× 5	17(+)	23+	10	12	15	10
SBTI h.	2× 5	22+	26+	10	10	5	10
HIST	2× 10	25+	15	10	11	13	15
HIST h.	2× 10	12	0	10	8	5	8
PROT	2× 25	5	9	10	7	10	12
PROT h.	2× 25	10	0	10	0	2	8
HB	2× 20	0	0	8	0	5	8
HB h.	2× 20	0	0	8	4	7	8
Cysteamine	2× 25	0	−21(+)	10	0	3	10
$MnCl_2$	2× 20	0	10	10	−7	−19	10
Mannitol	2×200	8	0	12	0	0	12
Chlorpromazine	10	0	5	10	0	3	10
Propyl gallate	250	2	6	10	6	0	10
Indomethacin	1	25(+)	42+	10	43+	14+	12

+; (+) $p < 0.01$; 0.05 according to Student's t-test. The following substances were found to be ineffective: L-cys (100 mg/kg p.o.), GSH (100 mg/kg i.v.), D-penicillamine (100 mg/kg p.o.), promethazine (10 mg/kg p.o.), L-his (500 mg/kg p.o.).

The carrageenin edema was induced by injecting 1 mg Viscarin® (Marine Coll. Inc., Springfield) in 0.1 ml distilled water into the pad of the left hind paw of female Wistar rats, b.wt 100–150 g. Local administration was performed by injecting the substances together with carrageenin. The adjuvant arthritis was produced by injection of 0.1 ml Freund's complete adjuvant (5 mg Mycobact. tubercul., strain C, heat-killed, Forschungsinstitut für Impfstoffe Dessau, suspended in 1 ml paraffinum perliquidum) into the pad of the left hind paw of female Wistar rats, b.wt 125–200 g. Heat denaturation of the proteins was performed by a 10-min incubation in a 100 °C water bath. Coagulated proteins were redispersed by sonication.

Results and discussion. As can be seen from tables 1 and 2 a significant inhibition of carrageenin edema and of the primary phase of adjuvant arthritis by the enzymes could be found. The results with catalase and peroxidase confirm our previous findings[11]. McCord and Wong[12] did not find any anti-inflammatory activity of catalase in carrageenin edema and in the reverse passive Arthus reaction. This difference might be explained by the different doses used. Heat-inactivated enzymes were, strikingly, almost as effective as the active enzymes. Unspecific inhibition of the inflammatory reaction by the heated proteins cannot be excluded; however, anti-inflammatory activity is apparently not a general property of heated proteins, as can be seen from the inactivity of haemoglobin and of human serum albumin. Furthermore, the heated proteins in the doses used were as well tolerated as the native proteins, and there was no difference in the irritating potency after local injection of both native and heated catalase (table 3). The ineffectiveness of haemoglobin also demonstrates that haemoproteins do not generally exert anti-inflammatory activity. The carrageenin edema was inhibited by cysteamine, manganese chloride, chlorpromazine and propyl gallate. No inhibition of adjuvant arthritis by any of the radical-scavengers could be found (table 2). As to the inactivity of D-penicillamine in edema and arthritis; a D-penicillamine copper complex is formed in vivo, and this complex has been shown to exert superoxide dismutase activity in vitro[13] in concentrations that may be achieved in vivo in the connective tissue and skin[14]. Since the half-life of D-penicillamine is long[15], the superoxide detoxifying Cu-penicillamine could be expected to be at the inflamed site in a sufficient concentration during a sufficient time.

After local injection of the substances, only indomethacin and the trypsin inhibitor acted inhibitorily (table 4). Differences of pH (table 4) and osmolarity apparently did not significantly influence the paw swelling. For instance, neither a hypertonic (1 osmol/l) nor a hypotonic (0.05 osmol/l) solution of sodium chloride caused significant edema formation (data not shown).

Altogether, no relation could be found between enzyme activity and anti-inflammatory effects of the enzymes. Somewhat similar results with superoxide dismutase have been reported by Huber et al.[2] which found, in addition, no close correlation between blood levels of the enzyme and anti-inflammatory effects[16]. In contrast to these data McCord and Wong[12] found anti-inflammatory activity of superoxide dismutase only when its half-life was essentially prolonged by derivatization.

There is no unequivocal opinion in the literature on the role of oxygen radicals in pathological processes. As to the present results, there is no reference to a role of oxygen radicals in the secondary phase of adjuvant arthritis. For the primary phase of adjuvant arthritis and mainly in carrageenin edema a participation of oxygen radicals cannot be ruled out. However, these inflammatory reactions are relatively susceptible to unspecific effects.

Table 3. Paw swelling in rats after subplantar injection of 0.5 mg native or heated catalase (10 min, 100 °C) in 0.1 ml distilled water. n = 8 per group

Group	Paw swelling, $\bar{x} \pm SD$ (ml)			
	1 h	3 h	5 h	24 h
Native catalase	0.13 ± 0.04	0.19 ± 0.04	0.15 ± 0.05	0.11 ± 0.04
Heated catalase	0.14 ± 0.05	0.19 ± 0.05	0.19 ± 0.05	0.17 ± 0.06*

* $p < 0.05$ according to Student's t-test.

Table 4. Inhibition of carrageenin paw edema after local injection of the substances together with carrageenin. Negative data indicate increase of paw swelling. For abbreviations see legend of table 1. pH = pH value of the mixture of carrageenin plus investigated substance in distilled water

Substance	mg/paw	pH	Inhibition (%)			n
			2 h	3 h	5 h	
POD	0.1	7.4	−42+	−17	0	10
CAT	0.5	6.7	−30+	−36+	−4	8
SOD	0.3	n.d.	11	9	13	8
HB	0.5	7.6	−31	6	15	8
SBTI	0.3	7.5	74+	0	−21	8
Cysteamine · HCl	1	6.3	−29(+)	−8	−19	10
$MnCl_2$	0.2	7.4	−4	−19	−40(+)	6
Mannitol	10	7.5	8	17	14	6
Propyl gallate	1	6.3	0	20	9	6
L-histidine	5	8.1	−37(+)	−26	−34	8
Indomethacin	0.1	6.3	38+	49+	45+	15

+; (+) $p < 0.01$; 0.05 according to Student's t-test.

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