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The effect of steroidal and non-steroidal anti-inflammatory drugs on chronic muscle inflammation

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Zymosan (400 μg) and thioglycollate medium (0·1 ml of 2·4% w/v) have each been shown to induce a chronic inflammation when injected into the hamstring muscle of the mouse. The inflammation is characterized by an increase in muscle weight and *N*-acetylglucosaminidase concentrations. The response is not inhibited by oral treatment with hydrocortisone, dexamethasone or naproxen, but is inhibited by the local injection of methylprednisolone. The relevance of this model to polymyositis and fibrositis is discussed.

Myositis, a soft tissue rheumatism and secondary fibrositis which accompanies rheumatoid arthritis are common and painful conditions which are difficult to treat with drugs. Treatment usually consists of analgesia and rest (Traut 1968) or corticosteroids either in high doses orally (Hudgson 1976; Erbsloch 1972) or given directly into the muscle (Galli 1974; Traut 1968).

Chronic inflammation is characterized by macrophage infiltration (Spector 1974) which has been demonstrated histologically in biopsy samples from patients with myositis (Martinez et al 1974; Mastaglia & Currie 1971; Hughes & Esiri 1975) and secondary fibrositis (Traut 1968). Schorlemmer et al (1977) have reported that zymosan induced macrophage infiltration when injected into the hamstring muscle of mice. Macrophage infiltration was accompanied by the release of acid hydrolases into the muscle. We have, therefore, examined this response as a model of chronic muscle inflammation. The effects of steroidal anti-inflammatory drugs and the non-steroidal anti-inflammatory drug naproxen have been studied in this model.

METHODS

Animals

Female T.O. mice (25 ± 3 g) were used in most of this work. Some experiments used female C.F.L.P. mice. No strain difference in the response was observed.

Materials

Naproxen was a gift from Syntex Labs, Palo Alto, California. The depot preparation of methylprednisolone used was Depo Medrone (Upjohn, Crawley, Sussex). Thioglycollate (Difco Labs, Detroit, U.S.A.) was made up to 2·4% w/v (single strength) or 4·8% w/v (double strength). Other chemicals were purchased from Sigma, London. Zymosan particles were boiled for 30 min in 0·9% w/v NaCl (saline), washed three times with saline and finally resuspended in sterile saline.

Treatment of animals

Sterile saline (0·1 ml) was injected into the left hamstring muscle and the inflammatory stimulant (in 0·1 ml) into the right hamstring muscle. Enzyme concentrations in left and right hamstrings were compared using a paired *t*-test. Control mice showed no difference between enzyme concentrations in left and right hamstrings. Groups of 8 mice were used.

Measurement of N-*acetylglucosaminidase activity* (EC 3, 2, 1,30)

N-Acetylglucosaminidase activity in the muscle was measured basically according to Schorlemmer et al (1977). Briefly, the hamstring muscle was removed, weighed and homogenized in 5 ml 0·1M-phosphate buffered saline, pH 7·4 containing 0·1% w/v Triton X 100. The homogenate was centrifuged at 2000 rev min^{-1} for 20 min at 4 °C and *N*-acetylglucosaminidase activity determined on the supernatant according to Woolen et al (1961) using *p*-nitrophenyl-2-acetamido-2β-D-glucopyranoside as substrate. Blanks were included in which the substrate was added at the end of the incubation.

RESULTS

Schorlemmer et al (1977) reported that maximum inflammation was produced by 40 μg zymosan per muscle seven days after injection. While we also found that seven days was the optimum time, in our

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mice 40 μg produced only a weak inflammatory response, 400 μg being required to produce a large, consistent inflammation (Table 1a). The inflammation was characterized by an increase in muscle weight, total *N*-acetylglucosaminidase activity and enzyme activity per unit wet weight of muscle.

As thioglycollate broth is commonly used to induce macrophage accumulation and activation in the peritoneal cavity of mice (see for example Ringrose et al 1975), its effects in the muscle were examined. Table 1b compares the effects of 0·1 ml of normal and double strength broth. The increase in

In view of the slight effect of hydrocortisone in the thioglycollate model, the effects of dexamethasone, a more potent steroidal anti-inflammatory drug, and the non-steroidal drug naproxen were compared in this model. The results are in Table 3. Neither drug at the doses used inhibited the response.

It seemed possible that the inflammation produced in these experiments was an intense local response and that drugs administered orally did not reach the site of the inflammation in sufficient concentrations to inhibit the response. To increase the local drug concentration an intramuscular injection of a depot

Table 1. Effect of zymosan and thioglycollate on muscle inflammation. The left hamstring was dosed with saline and the right hamstring with the inflammatory stimulant in a 0·1 ml dose volume. Enzyme concentrations were determined seven days later. Left and right sides were compared using a paired *t*-test.

	Left hamstring			Right hamstring		
Treatment	Wet wt (mg)	*N*-Acetylglucosaminidase (n mol h^{-1} per muscle)	Enzyme activity mg^{-1} wt	Wet wt (mg)	*N*-Acetylglucosaminidase (n mol h^{-1} per muscle)	Enzyme activity mg^{-1} wt
(a) Zymosan 40 μg	113 ± 13	1614 ± 183	14·5 ± 0·8	197 ± 15 (+73%*)	3786 ± 559 (+135%*)	18·0 ± 2·0 (+24%)
400 μg	119 ± 7	1706 ± 230	14·5 ± 1·0	223 ± 17 (+88%*)	5869 ± 1076 (+244%*)	29·0 ± 2·9 (+102%*)
(b) Thioglycollate (single strength)	127 ± 16	1568 ± 325	12·0 ± 1·0	187 ± 19 (+47%)	4862 ± 489 (+210%*)	26·3 ± 1·7 (+119%*)
(double strength)	119 ± 7	1634 ± 135	11·9 ± 1·2	142 ± 19 (+19%)	4685 ± 556 (+187%*)	28·5 ± 3·6 (+140%*)

* $P < 0·05$.

N-acetylglucosaminidase activity per unit wet weight was similar to that produced by 400 μg zymosan, and there was no difference between the two doses of thioglycollate. It was confirmed histologically that the enzyme release induced by these stimuli was due to the infiltration of inflammatory cells.

We have used the response produced by these inflammatory stimuli to look at the effects of anti-inflammatory drugs. In Table 2 the effects of hydrocortisone are compared on the inflammation produced by both zymosan and thioglycollate and at the high dose of 20 mg kg^{-1} orally it produced, at most, a slight inhibition of the inflammatory response. Only with total *N*-acetylglucosaminidase activity in the thioglycollate-treated mice was the effect of hydrocortisone significant on a grouped *t*-test comparison.

form of methyl prednisolone (0·8 mg in 20 μl) was given into each hamstring muscle of one group of mice 24 h before thioglycollate or zymosan was injected into the right hamstring. A control group received saline i.m. The results are in Table 4.

Methyl prednisolone did not inhibit the inflammation produced by thioglycollate broth but muscle weight was reduced in both legs. However, a group *t*-comparison showed that the steroid did reduce muscle weight and total *N*-acetylglucosaminidase activity in zymosan-treated muscles. Enzyme activity per unit weight was slightly, but not significantly, reduced.

DISCUSSION

We have confirmed the observation by Schorlemmer et al (1977) that an injection of zymosan into the hamstring muscle of the mouse induced an

Table 2. Effect of hydrocortisone on the muscle inflammation produced by zymosan or thioglycollate. Mice were dosed with hydrocortisone (20 mg kg^{-1} orally) for 30 min before zymosan (400 μg) or thioglycollate medium (0·1 ml normal strength) was injected into the right hamstring and saline (0·1 ml) into the left. Thereafter the mice were dosed daily with hydrocortisone until they were killed 7 days later. Control mice received 0·7% methyl cellulose orally daily.

		Left hamstring			Right hamstring		
	Treatment	Wet wt (mg)	*N*-Acetylglucosaminidase (n mol h^{-1} per muscle)	Enzyme activity mg^{-1} wt	Wet wt (mg)	*N*-Acetylglucosaminidase (n mol h^{-1} per muscle)	Enzyme activity mg^{-1} wt
(a)	Zymosan control	141 ± 11	2019 ± 269	14·4 ± 1·4	205 ± 17 (+45%*)	5310 ± 689 (+163%*)	26·2 ± 3·2 (+82%*)
	Hydrocortisone	134 ± 13	1983 ± 213	14·9 ± 1·1	184 ± 11 (+37%*) (−10%[1])	3848 ± 440 (+94%*) (−28%[1])	21·0 ± 2·4 (+41%*) (−20%[1])
(b)	Thioglycollate control	146 ± 11	2139 ± 161	15·0 ± 1·3	167 ± 12 (+14%)	4547 ± 516 (+113%*)	27·1 ± 2·2 (+81%*)
	Hydrocortisone	154 ± 12	1958 ± 143	13·0 ± 0·9	148 ± 6 (−4%) (−11%[1])	3263 ± 255 (+67%*) (−28%[2])	22·3 ± 1·9 (+72%*) (−18%[1])

*$P < 0·05$ paired *t*-test—left compared to right
[1] $P > 0·05$ grouped *t*-test } hydrocortisone compared to control
[2] $P < 0·05$ grouped *t*-test }

increase in the content of the lysosomal acid hydrolase *N*-acetyl-glucosaminidase seven days after injection. In addition we have shown that thioglycollate medium produced a similar response. The increase in enzyme release was shown by Schorlemmer et al (1977) to be due to macrophage infiltration. The involment of macrophages in chronic muscle inflammation is well documented (Mastaglia & Currie 1971; Martinez et al 1974; Hughes & Esiri 1975) and, therefore, the response to zymosan and thioglycollate may represent an animal model of chronic muscle inflammation, and be of use in finding drugs for the treatment of diseases such as polymyositis and secondary fibrositis.

The inflammation produced in the hamstring muscle of the mouse by zymosan or thioglycollate medium was difficult to inhibit. High oral doses of the steroidal anti-inflammatory agents hydrocorti-

Table 3. Effect of dexamethasone (50 μg kg^{-1} day^{-1} oral) and naproxen (20 mg kg^{-1} day^{-1} oral) on the muscle inflammation produced by thioglycollate medium. The dosing schedule and experimental conditions were as described in the legend to Table 2.

		Left hamstring			Right hamstring		
	Treatment	Wet wt (mg)	*N*-Acetylglucosaminidase (n mol h^{-1} per muscle)	Enzyme activity mg^{-1} wt	Wet wt (mg)	*N*-Acetylglucosaminidase (n mol h^{-1} per muscle)	Enzyme activity mg^{-1} wt
(a)	Control	146 ± 9	2112 ± 169	14·5 ± 0·6	194 ± 17 (+33%*)	3745 ± 430 (+77%*)	19·2 ± 1·4 (+32%*)
	Dexamethasone	171 ± 7	2321 ± 267	13·6 ± 1·4	182 ± 13 (+6%) (−6%[1])	3481 ± 329 (+50%) (−7%[1])	19·0 ± 0·8 (+40%*) (−1%[1])
(b)	Control	149 ± 10	3791 ± 185	26·0 ± 1·7	206 ± 6 (+38%*)	6810 ± 558 (+80%*)	32·9 ± 2·1 (+27%*)
	Naproxen	146 ± 11	4020 ± 406	27·8 ± 1·8	188 ± 6 (+35%*) (−2%[1])	6243 ± 492 (+68%*) (+2%[1])	33·8 ± 3·4 (+26%*) (+5%[1])

*$P < 0·05$ paired *t*-test—left compared to right
[1] $P > 0·05$ grouped *t*-test } drug compared to control
[2] $P < 0·05$ grouped *t*-test }

Table 4. Effect of intramuscular depot methyl prednisolone (Depot Medrone) on the inflammation produced by zymosan (400 μg) and thioglycollate broth (0·1 ml). 0·8 mg of methyl prednisolone in 20 μl volume was injected into each hamstring of one group of mice 24 h before the inflammatory stimulant was injected into the right hamstring. Control mice were dosed with 20 μl saline i.m. Seven days after the inflammatory stimulus was given muscle enzymes were measured.

	Left hamstring			Right hamstring		
Treatment	Wet wt (mg)	*N*-Acetylglucosaminidase (n mol h^{-1} per muscle)	Enzyme activity mg^{-1} wt	Wet wt (mg)	*N*-Acetylglucosaminidase (n mol h^{-1} per muscle)	Enzyme activity mg^{-1} wt
(a) Zymosan control	130 ± 7	2235 ± 136	17·5 ± 1·3	200 ● 8 (54%*)	5749 ± 797 (+157%*)	28·3 ± 3·0 (+62%*)
Methyl prednisolone	127 ± 13	2318 ± 215	19·7 ± 2·2	153 ± 15 (+20%*) (−24%[2])	3173 ± 324 (+37%*) (−45%[2])	23·6 ± 4·3 (+20%) (−17%[1])
(b) Thioglycollate control	140 ± 13	1678 ± 152	12·7 ± 1·5	204 ± 11 (+46%*)	3594 ± 324 (114%*)	17·5 ± 1·0 (+38%*)
Methyl prednisolone	125 ± 8	1844 ± 233	15·2 ± 2·2	164 ± 7 (+31%*) (−20%[1])	3281 ± 247 (78%*) (−9%[1])	20·4 ± 1·5 (+34%*) (+17%[1])

*$P < 0·05$ paired *t*-test—left compared to right
[1] $P > 0·05$ grouped *t*-test } methyl prednisolone compared to control
[2] $P < 0·05$ grouped *t*-test }

sone and dexamethasone and the non-steroidal anti-inflammatory drug naproxen failed to inhibit the reaction. Inhibition could only be obtained by giving methyl prednisolone directly into the muscle. It would seem that very high local concentrations of drug are required to inhibit what is an intense local inflammatory response. It is unusual to have such a marked difference between oral and local potency, since in other models of inflammation, for example the cotton pellet test, both orally and locally administered doses of steroids are equi-effective (unpublished results).

Hudgson (1976) has reported that oral steroids are effective in the treatment of polymyositis, but has stressed the need for high doses. Traut (1968) and Galli (1974) have both reported that local applications of steroids may be required in the treatment of fibrositis. Thus the animal model does reflect the difficulty of treating diseases such as polymyositis and fibrositis in the clinic and may be of use in testing for new drugs for these conditions.

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