Biochemical Pharmacology, Vol. 32, No. 22, pp. 3355–3358, 1983.
Printed in Great Britain.

0006-2952/83 $3.00 + 0.00

THE USE OF CORTICOSTEROIDS ENCAPSULATED IN ERYTHROCYTES IN THE TREATMENT OF ADJUVANT INDUCED ARTHRITIS IN THE RAT

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(*Received* 14 *March* 1983; *accepted* 13 *June* 1983)

Abstract—Corticosteroid esters have been encapsulated into intact erythrocytes and used as an intravenous treatment for adjuvant induced arthritis in the rat. The treatment consisted of injections of the encapsulated steroids with the effects monitored for up to 14 days. On an equivalent weight basis both encapsulated cortisol-21-phosphate and prednisolone-21-sodium hemisuccinate proved superior to the free steroid esters administered in solution by injection.

We have described a method where drugs including corticosteroid esters can be encapsulated into intact erythrocytes [1] and that the cells survive *in vivo* when returned to the circulation. One possible application of this preparation is in the treatment of chronic rheumatoid disease where patients require long term drug therapy. Anti-inflammatory steroids are well known for their adverse side-effects. Encapsulated steroids may offer advantages in that in a slow release preparation *in vivo* fluctuations in blood levels might be avoided. The usual form of administration of steroids is by tablets and by avoiding the oral route it is possible that gastro-intestinal lesions associated with steroid therapy might be avoided.

An animal model extensively used for screening drugs for the treatment of arthritis is adjuvant induced arthritis in the rat [2]. The model is a chronic model and the arthritis is progressive. The disease is not identical to the human disease but it has certain similarities [3]. The histopathology of the synovium in rat adjuvant arthritis has been reported to be identical to inflammatory synovitis in man [3]. According to Pearson [4, 5] adjuvant arthritis is similar to both rheumatoid arthritis and Reiter's syndrone in humans. Other workers [6] have described the rat model as the best available model for studying human rheumatoid arthritis. It was as a model for the human disease that adjuvant induced arthritis was chosen to test our encapsulation system.

MATERIALS AND METHODS

Adjuvant induced arthritis in the rat. Adjuvant arthritis was induced in the rat by the method of Newbould [2]. The adjuvant agent was heat-killed human strains of C, DT and N tubercle bacilli (kindly supplied by the Ministry of Agriculture Veterinary Laboratories, Weybridge, Surrey, England). It was finely ground in liquid paraffin to a final concentration of 5 mg/ml. A portion (0.05 ml) of the suspension was injected into the planter tissue of the left hind foot of the rat. Control animals were injected with saline. Inflammation was measured as volume changes in the injected foot (primary inflammation) and the non-injected hind foot (secondary inflammation). Foot volumes were measured immediately before the injection of adjuvant and every two or three days during the experiment. The foot volume was measured by immersing each foot to the hair line in a mercury manometer. The mercury displaced was measured by linking it to a pressure transducer coupled with a oscillograph recorder (Searle Bioscience Ltd., Kent, England). The output was adjusted so that the displacement of 1 ml of mercury produced a movement of 1 cm on the oscillograph.

Drugs and assay systems. The source and assay methods have been described [1].

Comparison of the anti-inflammatory action of encapsulated cortisol-21-phosphate, free cortisol-21-phosphate and sham encapsulating erythrocytes on adjuvant induced arthritis in the rat. Male Wistar strain rats (body weight 200 g) were used. For experimental purposes they were divided into groups of seven. Arthritis was induced as described and cortisol-21-phosphate was encapsulated in rat erythrocytes by our standard procedure [1]. Sham encapsulation cells were cells that were subjected to the encapsulating procedure but no drug was added. Treatments commenced on Day 0 when the arthritis was induced. The following treatments were given

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to groups of rats. *Group 1* (Control C group) was injected daily s.c. with 0.5 ml saline B.P. for 20 days; *Group 2* (sham encapsulation cells group, EO) was injected with 0.5 ml of packed sham encapsulation cells on day 0 and day 10; *Group 3* (free cortisol-21-phosphate group CP) was injected daily s.c. with 0.268 mg cortisol-21-phosphate dissolved in 0.5 ml saline B.P. for 20 days. This dose is equivalent to 0.200 mg cortisol at a dose of 1 mg/kg body weight; *Group 4* (encapsulated cortisol-21-phosphate group ECP) was injected on day 0 and day 10 with 0.5 ml of packed cells containing 2.68 mg of cortisol-21-phosphate. The packed cells were diluted with 0.5 ml of saline B.P. and injected into the femoral vein. This encapsulated dose was equivalent to 2 mg of cortisol and represented the equivalent of ten daily single doses of 1 mg/kg body weight; *Group 5* (free cortisol-21-phosphate CPiv group) was injected on day 0 and 10 via the femoral vein with 2.68 mg cortisol-21-phosphate dissolved in 1 ml saline B.P. (i.e. equivalent to 2 mg cortisol). This group was included as it would represent the maximum dose that the rats would receive if all the cells containing encapsulated steroid lysed immediately on injection into the circulation.

Comparison of the effect of encapsulated cortisol-21-phosphate and cortisol-21-phosphate incubated with erythrocytes on adjuvant induced arthritis. The object of this experiment was to see if cortisol-21-phosphate incubated with erythrocytes in eutonic K^+-Hanks solution would be taken up by the cells and that these cells would be anti-inflammatory against the adjuvant arthritic rat. The same experimental design was used as in the previous experiment except that cortisol-21-phosphate was encapsulated at nominal 5 and 10 day doses, i.e. containing the equivalent of 1 mg and 2 mg cortisol. At the same time that the encapsulated preparation was being prepared, steroid ester at the same concentration as used for encapsulation was incubated with 1 ml of packed erythrocytes in eutonic K^+-Hanks solution. The rats were given a single injection of the preparations on day 0.

Comparison of free and encapsulated cortisol-21-phosphate administered at the early (acute) and late (chronic) phase of adjuvant arthritis. In this experiment 7 day doses of cortisol-21-phosphate were encapsulated in erythrocytes (i.e. equivalent to 1.4 mg cortisol). The same experimental design was used as in the previous experiments except that one group were injected with the encapsulated preparation on day 0 (ECPa) and a second group were given daily s.c. injections of free cortisol-21-phosphate (equivalent to 1 mg/kg cortisol) for seven days only (CPa). In another two groups the arthritis was allowed to develop untreated for seven days but on day 7 one of these groups was injected with the encapsulated preparation (ECPc) and daily free steroid treatment was commenced in the other group (CPc).

The effect of encapsulated prednisolone sodium succinate with free drug on adjuvant induced arthritis in the rat. Prednisolone is about five times more anti-inflammatory in man on an equivalent weight basis than cortisol. Therefore for experimental purposes a daily effective dose was taken to be 0.3 mg/kg body weight (P). Prednisolone-21-sodium hemisuccinate was encapsulated at a nominal 10 day dose level (EP-10) and erythrocytes were also incubated in eutonic K^+-Hanks solution with prednisolone-21-sodium hemisuccinate for the same time and at the same concentration as the steroid esters used in the encapsulation procedure (RBC + P-10). All preparations were administered on day 0.

RESULTS

Encapsulated cortisol-21-phosphate compared with free cortisol-21-phosphate. The results in Fig. 1 show that encapsulated cortisol-21-phosphate was considerably more anti-inflammatory than free steroid administered daily. The effect was not due to a counter irritant action [7] by the empty cells since they were not anti-inflammatory. Neither was the anti-inflammatory action due to a massive initial leakage of steroid since single i.v. injections of steroid were not anti-inflammatory. Presumably this was due to rapid metabolism. Figure 2 shows the effect of the various treatments on the secondary inflammation in the non-injected hindfoot. These results were similar to those for the injected foot.

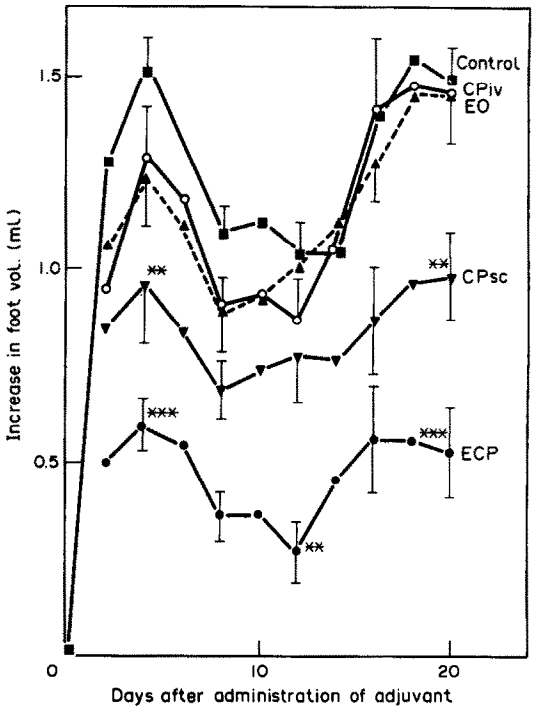

Fig. 1. The effect of encapsulated cortisol-21-phosphate compared with free steroid ester on the injected feet of arthritic rats. Only two groups ECP-encapsulated steroid, and CPsc-free daily steroid, showed anti-inflammatory activity when compared to the untreated controls. The anti-inflammatory activity in the ECP group was higher than in the CPsc group. Significance levels in this paper are $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$. Results are presented as means ± S.E.M. Full experimental details are given in the text.

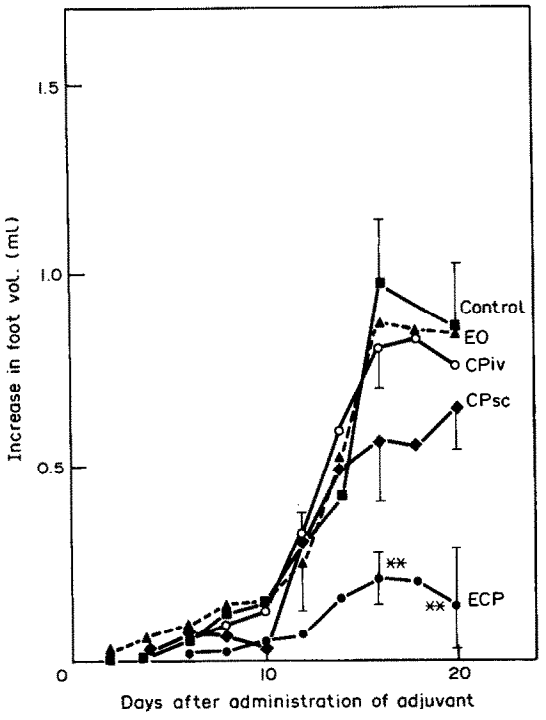

Fig. 2. The effect of encapsulated cortisol-21-phosphate compared with that of free steroid ester on the non-injected right hind feet of arthritic rats. The results for the injected feet are in Fig. 1 together with the details of the experiment.

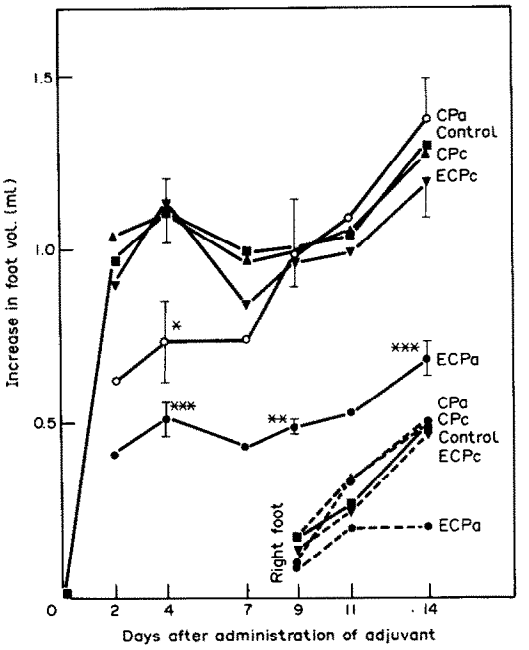

Fig. 4. The effect of steroid treaments on acute and chronic inflammation in the arthritic rat. Only the encapsulated steroid (ECPa) administered at the start of the experiment was anti-inflammatory throughout the experiment. Daily free steroid treatment (CPa) for seven days from the start of the experiment was also anti-inflammatory but the effect disappeared when the treatment was stopped. Other treatments (see text) were not anti-inflammatory. The results for the non-injected right hind foot are included on the graph.

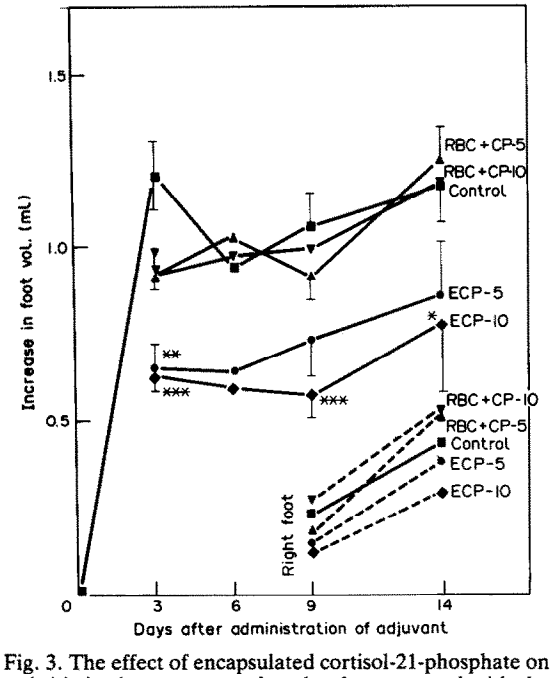

Fig. 3. The effect of encapsulated cortisol-21-phosphate on arthritis in the rat at two dose levels, compared with the effect of cells incubated with the steroid esters at two dose concentrations. The incubated cells (RBC + CP-5 or CP-10) were not anti-inflammatory compared to the encapsulating cells (ECP-5 and ECP-10). For comparative purposes the results for the non-injected right hind foot are included on the graph.

Encapsulated cortisol-21-phosphate compared with cells incubated with the steroid. These results are plotted in Fig. 3. Both the five and ten day encapsulated doses were anti-inflammatory although there was no dose response. Cells incubated with steroids did not possess anti-inflammatory activity and this agrees with the analytical findings in our examination of the encapsulation procedure [1].

The effect of encapsulated cortisol-21-phosphate on the acute and chronic stages of adjuvant induced arthritis. The results in Fig. 4 show that the only effective treatment for the arthritis was the administration of the encapsulated preparation on day 0. Over the first 7 days the free steroid treatment was also effective although not as effective as the encapsulated preparation. When the free steroid treatment was discontinued after seven days the inflammation rapidly increased to reach control levels. Free and encapsulated steroid administered after the arthritis had been allowed to develop for 7 days was ineffective. It may be that a longer period would be necessary to study the effects of steroid treatment on established arthritis but the experiment was discontinued after 14 days to avoid further suffering to the animals.

The effect of encapsulated prednisolone-21-sodium hemisuccinate on adjuvant induced arthritis. The encapsulated prednisolone-21-sodium succinate at the 10 day dose was considerably more anti-inflammatory than the single daily dose of free steroid (Fig.

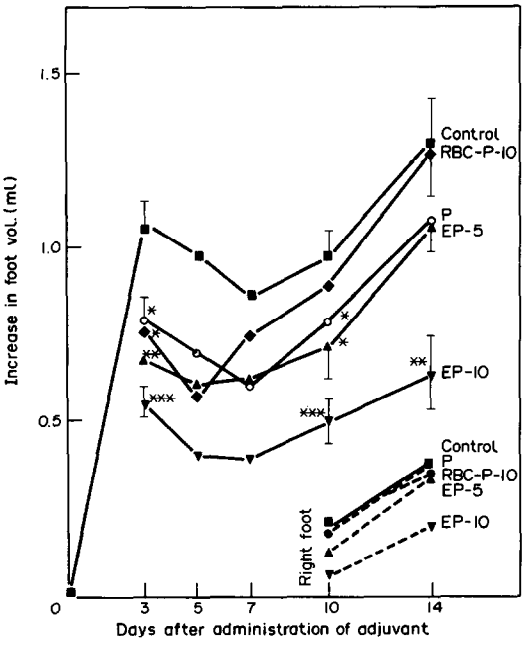

Fig. 5. The effect of encapsulated prednisolone-21-hemisuccinate compared with the effect of cells incubated with the steroid ester on arthritis in the rat. The encapsulated preparation (EP-10) was significantly anti-inflammatory throughout the experiment but the free daily dose (P) was less effective although anti-inflammatory on day ten. When the amount of steroid encapsulated was reduced by half (EP-5) the preparation was anti-inflammatory in the early part of the experiment (to day 10) when its activity was similiar to that of the free steroid (P). However the total free steroid administered over ten days was twice the amount encapsulated in the EP-5 preparation. Cells incubated with the steroid ester were not anti-inflammatory (RBC + P-10). For comparative purposes the results for the non-injected right hind foot are included on the graph.

5). The free steroid was only as effective as the encapsulated 5 day dose. Cells incubated with the steroid were not anti-inflammatory. When the cells containing the encapsulated steroid were haemolysed *in vitro* and examined by tlc [1] free prednisolone was detected. This suggests that the cells possessed some esterase activity.

DISCUSSION

The encapsulation technique developed in our laboratories has clearly demonstrated its potential in treating adjuvant arthritis in the rat. The probable mode of action of the preparation was by acting as a slow release system *in vivo*. Our analytical data for cortisol blood levels tend to support this suggestion [1]. The half-life of intravenously injected cortisol (400 mg) in man is only 110 min [8]. It is unlikely that it has a much longer retention time in the rat and our observations that single large intravenous doses of steroid were ineffective against the induced inflammation are consistent with a short half-life. The results were achieved with water-soluble esters that were probably hydrolysed within the cell. It may well be that non-esterified drugs may leave the cell at a faster rate and this may alter their therapeutic action. Unfortunately we cannot encapsulate free corticosteroids because of their low solubility in aqueous media. It may be possible to encapsulate steroids bound to proteins.

The effect of a sustained elevated steroid blood level may have important therapeutic consequences. It is known that at least part of their anti-inflammatory action is due to their ability to stimulate the synthesis of a protein inhibitor against phospholipase-A_2 [9].

It may be that steroids control endogenous mechanisms which are anti-inflammatory and the effect of steroid levels on the synthesis of such factors would appear to be worthy of further investigation.

Acknowledgements—This work was funded by the Medical Research Council and later by the Wellcome Trust.

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