THE DISTRIBUTION OF AN OIL-BASED COR-TICOSTEROID FOLLOWING INTRALYM-PHATIC INFUSION

N. C. M. Fyfe M.B., F.R.C.S., W. A. Webb, Ph.D., and J. B. Kinmonth M.S., F.R.C.S., Hon. F.A.C.S.*

Departments of Surgery and Chemical Pathology, St. Thomas' Hospital Medical School, London

ABSTRACT

Following infusion of tritium labeled clobetasol propionate in Ultrafluid Lipidol (UFL) into a right hind limb lymphatic of rabbits, the radioactivity levels in various tissues at intervals up to 28 days were determined by liquid scintillation counting. There was a rapid decline in activity in the right popliteal node over the first three days due to early bloodstream absorption. From three to 28 days radioactivity levels were consistently higher in the right popliteal node and lung than in other tissues sampled. This distribution suggests that there is an affinity between clobetasol and the lipidol vehicle which retards (but does not prevent) free diffusion of this agent out of lymphatic tissues. Thus, while permitting generalized perfusion of tissues by clobetasol propionate, intralymphatic infusion maximizes its initial concentration and duration of activity within specific node groups and, therefore, may be useful in certain patients with primary lymphedema where lymph nodes affected by fibrosis constitute a major site of obstruction.

Intralymphatic corticosteroid therapy has been introduced as a new alternative in management of primary lymphedema (1,2) based upon the observations that draining lymph nodes in this disorder often show considerably greater abnormality than peripheral lymph vessels (3), with substantial hilar and medullary fibrosis (4-6). Such nodes impede flow of lymph, and this stagnant lymph, by virtue of high protein content, promotes chronic inflammation within surrounding tissues (7), which in turn retards free flow of materials within the interstitial space. Treatment designed to modify these latter processes is likely to improve the clinical state.

In this context, Mannheimer and Pfleger (8) reported good long-term results with an oral corticosteroid (prednisolone) and a diuretic drug. We preferred to administer a steroid by intralymphatic infusion in order to localize the focus of treatment upon draining lymph nodes—the presumed critical site of obstruction. To prolong the local action, a lipophilic steroid, Clobetasol propionate (Glaxo group research, Ltd., Greenford, Middlesex) has been combined with Ultrafluid Lipiodol (UFL). If the lymph nodes are indeed the main site of hindrance to lymph flow, then having clobetasol suspended in UFL would advantageously concentrate this agent within the nodes. Alternatively, if the interstitial space changes are of greater importance, then widespread permeation of the drug throughout the tissues would be desirable. The present experimental study was designed to provide data on the fate of intralymphatic clobetasol propionate; first to determine the duration of retention of clobetasol propionate within the popliteal lymph node of the rabbit after administration by intralymphatic infusion with UFL, and, second, to determine the distribution of this steroid throughout other tissues at varying intervals after administration.

MATERIALS AND METHODS

Six New Zealand white rabbits (R1-R6) of both sexes and weighing between 2.4 and 2.9 Kg. were used. A solution of

*deceased

Clobetasol propionate (Fig. 1) containing a tracer dose of 3H-Clobetasol propionate was prepared as follows. The labeled compound which was supplied as 1mCi clobetasol propionate in 1 ml of benzene:ethanol (94:6 v/v) having a specific activity of 119 mCi/mg, was evaporated to dryness. 20 mg of micronized (unlabeled) clobetasol propionate powder was added. Dissolution was achieved in a 50μl of acetone and 2ml of UFL then added. Thus 0.2ml of the solution (the volume to be infused) contained 2mg of clobetasol propionate and 100 μCi of [3H]-clobetasol propionate.

The materials and technique of rabbit lymphatic perfusion have been described elsewhere (9). In brief, it involves isolation of a superficial lymphatic which has previously been rendered visible by a subcutaneous injection of Patent Blue Violet into the foot and insertion of an appropriately designed cannula. The infusion is driven by a constant rate pump set at 4ml per hour.

Following sedation by an intramuscular injection of a Fentanyl/Fluanisone mixture, one lymphatic of the right hind limb of each rabbit in turn was cannulated and 0.2ml of the [3H]-clobetasol propionate in UFL solution was infused. The wounds were closed with catgut.
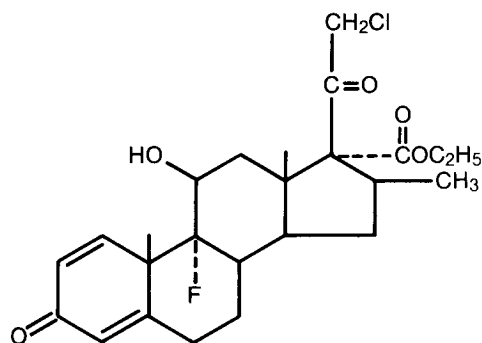

Fig. 1: The structure of Clobetasol-17-propionate.

The last rabbit treated (R6) was killed by a bolus of Pentobarbitone into a marginal ear vein immediately after infusion. The abdomen and chest were opened and small pieces of lung and liver were excised. Then the left popliteal node and finally the right popliteal node were excised. Separate gloves and instruments were used in the collection of each specimen to avoid cross-contamination by radioactive material. The specimens were individually washed with saline, dried, weighed, and deep frozen in separate containers. The remaining five rabbits (R5-R1) were killed and the same tissue sampling carried out at 1,3,7,14, and 28 days respectively after clobetasol infusion.

Tissue samples were dissolved in 2ml of a tissue-digesting fluid ("Lumasolve", LKB Instruments Ltd., Croydon, Surrey) at room temperature over five days. Ten ml of a xylene based scintillation fluid ("lipoluma", LKB Instruments Ltd.) was then added and the radioactivity of each sample estimated by liquid scintillation counting. 50 μl of the labeled infusate, diluted 1:100 with UFL, was also counted in order to calculate the actual dose of radioactivity infused. Counts obtained were corrected for background radiation and samples corrected for quenching using the internal standard method.

RESULTS

The measured dose of radioactivity given to each animal was 97.6 μCi. Table 1 shows the weight standardized radioactivity content of each tissue sample. As anticipated, the levels in the right popliteal node immediately after the infusion was substantially higher than elsewhere although that level represented only 14% of the total dose given. The level fell by 94% over the first day and by a further 86% over the next two days. Thereafter the fall-off was relatively gradual until at 28 days only 0.04% of the original dose remained. In the right node, the overall reduction in radioactivity over the period was by a fac-

Table 1
Weight standardized radioactivity of each sample (nCi/0.2g)

Days	0(R6)	1(R5)	3(R4)	7(R3)	14(R2)	28(R1)
R. Node	11,020	620	82.7	88.9	54.2	31.9
(% of total dose)	14.1	0.76	0.07	0.12	0.09	0.04
L. Node	16.4	1.5	4.5	0.05	0.02	0.004
Lung	13.8	11.0	10.8	4.0	2.9	2.1
Liver	12.3	36.4	3.5	1.6	0.54	0.12

tor of 10^2. By contrast, the decline in activity of the left node was by a factor of 10^4.

The samples of left popliteal node, lung, and liver showed initial levels of a similar magnitude. However, the lung showed a considerably slower rate of loss of radioactivity than the other tissues, such that at 28 days, its level (2.1 nCi/0.2g) ranked highest of all tissues except the right popliteal node.

The total amount of radioactivity in lungs and liver was calculated using reference values for the percentage of body weight of those organs (10) (Table 2). These results showed that lung activity, starting from a lower base, declined at a rate slowe and ended at a level higher than liver.

DISCUSSION

The very low percentage of the administered dose of radioactivity in the right popliteal node immediately after infusion may be accounted for in two ways. First,

the size and internal architecture of the node impose a physical constraint on its capacity to retain UFL. When that capacity is exceeded, any further infusion of UFL results in filling of the efferent lymphatic. Whereas 0.2ml consistently fills the efferent lymphatic it is insufficient to delineate iliac pathways. Thus, in part, the low initial activity within the right node reflects simply the small capacity of the node (~0.2ml).Second, there is likely prompt leakage of clobetasol out of the lymph system, as demonstrated by substantial radioactivity in anatomical sites far distant from the site of injection (Table 1, R6). Because the labeled steroid only reached these sites by hematogenous spread it must have gained entry to the bloodstream by direct diffusion out of the lymph system into the interstitial space.

The radioactivity levels in the right popliteal node remained substantially higher than those in the contralateral node

Table 2
Total radioactivity (nCi) in lungs and liver

Days	0(R6)	1(R5)	3(R4)	7(R3)	14(R2)	28(R1)
Rabbit Wt. (Kg)	2.6	2.8	2.9	2.8	2.5	2.4
Activity (nCi):-						
Lungs	897	770	810	280	189	126
Liver	4,243	13,468	1,348	592	181	38

Weight of rabbit organs per 100g body weight: lungs = 0.5g, liver = 2.66g. (10)

throughout the whole period of observation. This finding is unlikely if unrestricted diffusion of clobetasol out of the right node into the bloodstream had occurred. In that event, the levels in both nodes would have tended to equalize with passage of time. That they did not equalize is strong evidence that absorption into the bloodstream from the right popliteal node was retarded. Such retardation may have been the consequence of UFL, a lipid solution in which clobetasol propionate is preferentially solubilized.

Three days after infusion, the rate of decline of activity was slowest in the right popliteal node and lung and the ultimate levels at these two sites were higher than any other. These findings were in keeping with expected distribution of UFL which was infused via a peripheral lymphatic in the right popliteal node and had delayed spill-over into the lungs from the thoracic duct. Findings of highest radioactivity levels at these two sites of expected accumulation of UFL also suggested that clobetasol propionate and UFL existed in combination.

[3H]-atoms on the clobetasol propionate molecule are not at positions which are usually changed by steroid metabolism (11). The radioactivity measured in these experiments therefore reflects either the amount of unchanged clobetasol propionate or its metabolites and not [3H]-atoms dissociated from the original steroid molecule. The liver is regarded as the major site of steroid metabolism, with some catabolism occurring in peripheral tissues. Lymphoid tissue, however, is not a catabolic site and therefore the steroid remaining in the right popliteal node after administration is likely unchanged clobetasol propionate. This arrangement contrasts with the situation in the left popliteal node, where clobetasol propionate necessarily had to traverse the liver, and thereby may have been metabolically altered.

In summary, these experiments indicate that following intralymphatic infusion of clobetasol propionate in UFL, most of the drug is rapidly absorbed into the bloodstream. In the lymph node directly perfused, there is a notable initial decline in nodal steroidal concentration over the first two days. Thereafter, the decline is gradual probably because UFL retards free diffusion of lipid soluble clobetasol. Although the amount of this steroid remaining within the node is extremely small, its concentration is high relative to other tissues. Because clobetasol propionate is among the most potent of the antiinflammatory steroids (12), its administration by intralymphatic infusion in patients with lymphedema is favored as it fulfills the objective of delivering the agent directly to regional nodes thereby maximizing its concentration and duration there, while at the same time permitting generalized diffusion via the blood circulation.

REFERENCES

1. Fyfe, NCM, DL Rutt, JM Edwards, JB Kinmonth: Intralymphatic steroid therapy for lymphoedema: preliminary studies. Lymphology 15 (1982), 23.
2. Fyfe, NCM, DL Rutt, JM Edwards, JB Kinmonth: Intralymphatic clobetasol propionate in the treatment of primary lymphoedema. In "Advances in lymphology" eds. V Bartos, JW Davidson. Prague. Avicenum Medical Press. (1982).
3. Kinmonth, JB, PW Eustace: Lymph nodes and vessels in primary lymphoedema. Ann. Roy. Coll. Surg. Engl. 58 (1976), 378.
4. Kinmonth, JB, JH Wolfe: Fibrosis in the lymph nodes in primary lymphoedema. Ann. Roy. Coll. Surg. Engl. 62 (1980), 344.
5. Rada, IO, N Tudose, R Bibscu Roxin: Lymphonodal fibrosclerosis in primary lymphoedema. Part One. Lymphology 16 (1983), 217.
6. Rada, IO, N Tudose, R Bibscu Roxin: Lymphonodal fibrosclerosis in primary lymphoedema. Part Two. Lymphology 16 (1983), 223.
7. Casley-Smith, JR, RM Gaffney: Excess plasma protein as a cause of chronic inflammation and lymphoedema. Quantitative electron microscopy. J. Path. 133 (1981), 243.
8. Mannheimer, E, L Pfleger: Perphere Lymphandiopathien — 15 Jahre Follow-Up. Acta. med. Austr. 3 (1976), 128.
9. Guiney, EJ, MH Gough, JB Kinmonth: Lymphography with fat-soluble contrast media. J. Cardiovasc. Surg. 5 (1964), 346.
10. Humphry, GF In: Biochemists' handbook. Long, C, EJ King, WM Sperry (eds.). E and FM Spong Ltd. (1961), p. 639.

11. Dorfman, RI, F Ungar. In: Metabolism of steroid hormones. London, NY Acad. Press, (1965), p. 289.

12. Phillips, GH: Locally active corticosteroid: structure activity relationships. In: Wilson, L, R Marks (eds.): Mechanisms of topical corticosteroid activity. Glaxo symposium. London, NY. Churchill Livingstone. (1976), p. 1-18

ACKNOWLEDGEMENTS