flow cell" and back to the conical flask with a flow rate of 11 drops/min at 37 ± 1 °C temperature. At specified intervals of time, 2 ml of the sample solution was withdrawn from the conical flask and replaced with fresh buffer solution. The samples were analyzed after necessary dilutions by colorimeter.

*3.2.3. Determination of average weight and thickness*

The average weight was determined by randomly selecting 10 samples from the batch. They were weighed individually and the value was reported. The thickness of the ocular films was determined by microscopy [9].

*3.2.4. IR and TLC studies*

IR spectra of the pure drug and formulations were determined using a Shimadzu Fourier Transform IR Spectrophotometer by the KBr disc method, TLC studies were carried out using chloroform/methanol/toluene/diethylamine/water (40:40:20:14:8) as the developing solvent and observing under UV radiation at 254 nm.

*3.2.5. In vivo studies*

Six albino rabbits of age 6–8 months weighing between 2.5–3.0 kg with normal diet were used for the study. The studies were set up using a single blind randomized cross-over design with a wash out period of 2 weeks. One film was placed below the upper eye lid of each eye of the rabbit [10]. At specific time intervals, the films were carefully removed and analyzed for the remaining drug content. The drug content obtained was subtracted from the initial drug content in the ocular film which gave the amount of drug released in the rabbit's eye at that particular time. The animals were also observed for any ocular irritation through out the period of study.

*3.2.6. Sterilization and sterility testing*

Sterilization was carried out by gamma radiation at doses of 0.5, 1.0, 1.5 and 2.0 Mrads and sterility testing was carried out according to the literature [11].

*3.2.7. Stability studies*

The formulations were stored at temperatures of 25 °C, 37 °C, 45 °C, 60 °C and 37 °C/75% RH for a period of 2 months [12]. The samples were assayed for drug content and observed for any physical changes in the film.

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# Loteprednol etabonate, a new soft steroid is effective in a rabbit acute experimental model for arthritis

L. F. Buris[1], N. Bodor[3] and L. Buris[2]

Loteprednol etabonate, a new soft steroid designed for use as a local therapeutic, was compared to dexamethasone in rabbit experimental model for arthritis. Methods: Joint inflammation was induced by local injection of antigen into the patellofemoral articulation in sensitized rabbits. Co-administration of either dexamethasone or loteprednol etabonate directly into the joint effectively blocked the inflammatory response. Both the synovial fluid cellular content and synovium histology were examined. The steroid treatments prevented the adverse inflammatory effects of antigen action. These results, together with previous studies showing decreased systemic activity of the soft steroid, indicate that the loteprednol etabonate could provide a therapeutic advantage over currently used intra-articular steroids for alleviating rheumatoid arthritis.

## 1. Introduction

Arthritis has plagued mankind since the beginning of time and the pailful, debililtating condition can affect any joint. However, the associated stresses from our upright posture make the leg and back articulations particularly vulnerable to damage from inflammation. Unchecked, the condition can progress and result in partial or complete disability. Statistics gathered in the United States show that osteoarthritis followed cardiovascular disease as the leading cause of disability in individuals over 50 years of age [1]. Rheumatoid arthritis is an inflammation condition that often precedes the more severe osteoarthritis. The pathogenesis involves an immunological response with initial synovial membrane inflammation contributing to fibrinoid degeneration and white blood cell infiltration into the synovial fluid. Continued irritation can progress from slight morning stiffness to total joint failure from severe cartilage destruction [1, 2].

Current therapies for rheumatoid arthritis are designed to control inflammation. Nonsteroidal anti-inflammatory drugs are first choice therapeutic options to control the disease. More advanced cases can be treated with immune

response modulators such as gold, penicillamine, and hydroxychloroquine, or immunosuppressive agents like methotrexate, cyclophosphamide and azathioprine. The systemic immunomodulators have a slow onset and unpredictable or mixed response profiles. One of the more effective treatments to improve specific joint function in severe forms of rheumatoid arthritis involves intra-articular corticosteroid injections [2]. Peripheral effects of these agents as well as increased risks associated with the injection technique limit this treatment approach [2, 3].

Loteprednol etabonate (LE) was designed as a soft drug following the "inactive metabolite" approach for retrometabolic drug design [4–6]. The inactive hydrocortisone metabolite, $\Delta^1$-cortienic acid, was chemically modified to form an active corticosteroid that could be metabolized to the inactive 17α-carbonate and then back to $\Delta^1$-cortienic acid. A fundamental aspect of design involved the predicted metabolism of the 17β-chloromethyl ester to the inactive $\Delta^1$-cortienic acid derivative. This predictable and facile metabolic inactivation was designed to optimize local efficacy and reduce the systematic activity of corticosteroids that are associated with dose limiting effects.

Accordingly, studies on local anti-inflammatory activity as reflected by cotton pellet granuloma vs. systemic side effects measured by thymus involution resulted therapeutic indices of 20–200 for the soft steroids, as compared to around 1 for all major conventional steroids, like betamethasone valerate, hydrocortisone butyrate, clobetasol propionate or dexamethasone. Thus, the selected LE has a therapeutic index of 24, while its intrinsic activity as reflected by receptor binding activity is about five times higher than that of dexamethasone [10, 11] (relative binding activity, RBA to rat lung corticosteroid receptor is 490 for LE, while 100 for dexamethasone).

While several animal models are available to examine anti-inflammatory drug action, the rabbit antigen sensitized model is commonly used to evaluate treatments for rheumatoid arthritis. The site of experimental arthritis can be controlled [7] and the acute inflammatory response to intra-articular antigen injection is quite predictable in this animal model [7–9]. Although the rabbit is not bipedal, the joint pathology occurring in the experimental model has several similarities with clinical manifestations of rheumatoid arthritis [7].

## 2. Investigations, results and discussion

Three days following intra-articular injection, rabbits given bovine serum albumin (BSA) alone displayed the expected evidence of joint inflammation. Mobility was decreased in the injected joint and surrounding soft tissue was swollen. Typical pain responses were pronounced when gentile articular manipulation was used to access mobility. Synovial fluid contained macroscopic evidence of cellular infiltration with a pink, viscous appearance. The surface of the dissected pallatofemoral joint was hyperemic and covered with a fibrinlike material in BSA injected rabbits.

In contrast, no pain response was displayed during joint mobility assessment in groups treated with dexamettha-sone or LE. Macroscopically, both the dissected joints and the collected synovial fluids appeared normal in steroid-treated rabbits. Appearance did not differ from HPCD vehicle treated tissues. No fibrin deposits or hyperemia were evident.

Differential cell counts in synovial fluids are shown in the Table. Leukocytes, eosinophils and macrophages were elevated in fluid collected from the BSA-only injected group. Some cellular infiltration was detected in synovial fluid from both of the steroid treated groups. However, cell counts did not differ significantly from vehicle-only-treated joint fluids. Synovial fluid from the dexamethasone treated group contained some cells that could not be identified due to swollen nuclei and vacuoles. The minimal cellular infiltration observed in vehicle treated joints is consistent with injection trauma. Eosinophils were not consistently detectable in synovial fluids from steroid treated or vehicle treated groups.

The histological appearance of BSA-only injected tissue was consistent with acute inflammation. As shown in Fig. 1, ependymal cells lining the synovia showed proliferation. Cells were organized into 2–3 layers after BSA compared to the single cell layer in vehicle injected tissues. Erythrocyte extravasation and some vascularization was evident in BSA treated tissue. Paravascular fibrinoid deposits as well as necrotic cells were found in this treatment group. The histologic pattern of cellular infiltration is consistent with acute immunogenic synovial inflammation.

The steroid treated groups were histologically very similar to the vehicle treated group. Joints were essentially normal with minimal cellular infiltration, fibrinoid reaction or vascularization (Fig. 2). Fig. 3 shows no inflammation, or fibrinoid deposits.

The present study demonstrated that local application of loteprednol etabonate (LE) is effective in blocking the acute antigen-induced local inflammatory response in large joints. The results were consistent among all measured parameters and evidenced by behavior, macroscopic and microscopic analysis of the treated animals and tissues. The anti-inflammatory action of the soft steroid could not be distinguished from intraarticular dexamethasone action which is consistent with the potent corticosteroid receptor interaction of LE [10]. However, considering the large separation of activity-toxicity for both systemic [5] and local application [16, 17] between LE and dexamethasone indicating a therapeutic index of 24 h and lack of IOP elevating activity [16], LE should be a much safer drug in the treatment of arthritis.

The antigen-induced acute synovial inflammation model is a useful method for evaluating the therapeutic potential of new drug agents and formulations to treat rheumatoid arthritis *in vivo*. The method closely resembles the actual clinical application of steroid suspensions that are used to control joint degeneration in advanced rheumatoid arthritis. The clinical utility of intra-articular steroid injection in

**Table: Cellular components in synovial fluid from sensitized rabbits treatment**

| Cell Type | BSA | Loteprednol 0.1% | Dexamethasone 0.1% | HPCD |
|---|---|---|---|---|
| Leukocytes | 20.68 ± 3.34 | 5.75 ± 1.55 | 7.16 ± 2.40 | 4.82 ± 1.39 |
| Eosinophils | 13.68 ± 2.73 | 0.70 ± 0.72 | 0.91 ± 0.93 | 0.48 ± 0.25 |
| Macrophages | 4.92 ± 1.55 | 1.08 ± 0.87 | 1.85 ± 1.25 | 0.67 ± 0.17 |
| Unidentified | 2.84 ± 1.66 | 0.86 ± 0.84 | 5.02 ± 2.23 | 0.53 ± 0.17 |

Values group averages ± S.D. (n = 7) for cells counted in 50–50 slide fields

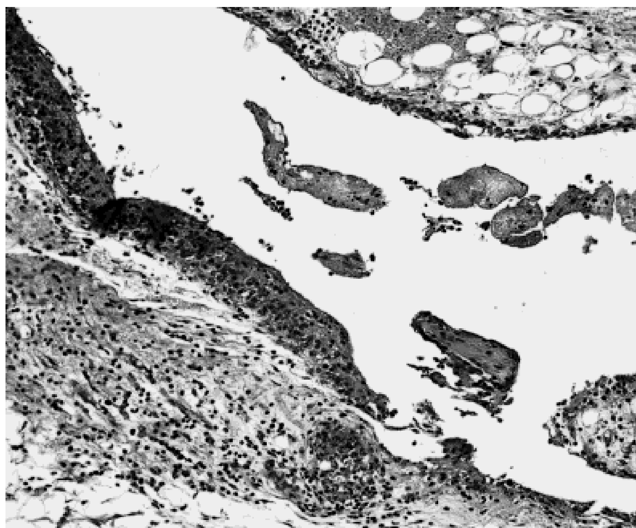

Fig. 1: Photomicrograph of the patellofemoral synovia region 3 days after BSA injection n a sensitized rabbit. Typical acute synovitis with fibrin deposits (F), diffuse inflammatory appearance (S), perivascular fibinoid deposits and synovial ependymal cell proliferation is present (P)

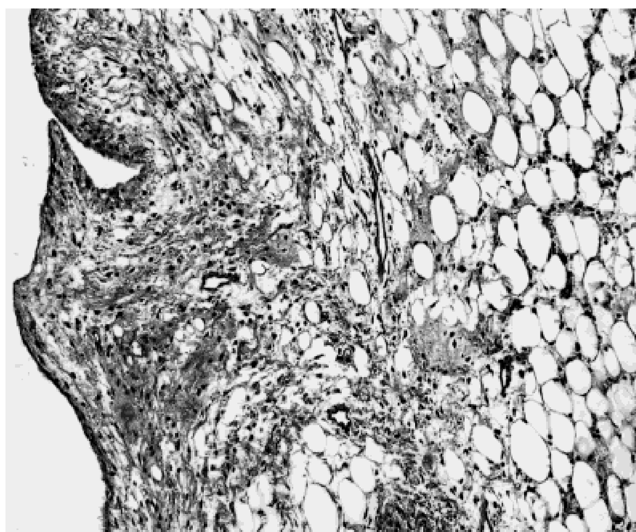

Fig. 2: Photomicrograph co-administration with BSA. Note the minimal inflammatory response (Fibrinoid deposits)

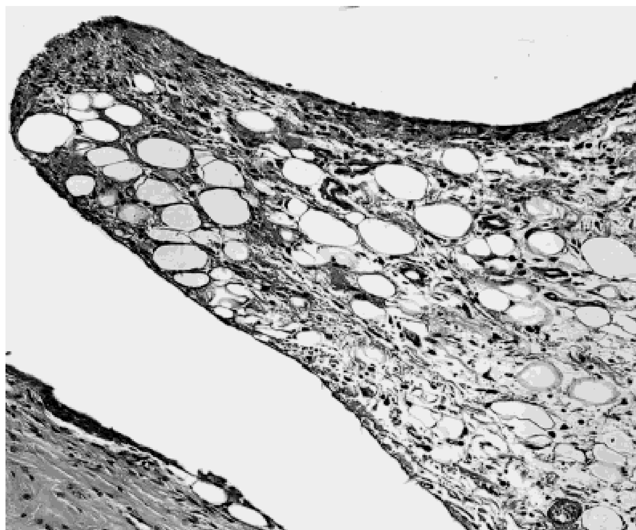

Fig. 3: Photomicrograph co-administration with BSA

managing the disease is a topic often discussed because definitive clinical trials are not available [12]. This study demonstrated the therapeutic potential of the new soft steroid LE for controlling intra-articular inflammation.

Several therapeutic options are available for treating rheumatoid arthritis [1–3]. The most common approach for managing the progressive and often debilitating disease uses nonsteroidal anti-inflammatory agents. Drugs are selected on the basis of cost and tolerance with more expensive agents chosen to improve tolerance. More advanced disease cases often cannot be managed with non-steroidal anti-inflammatory agents alone. Immunomodulators and immunosuppressants are sometimes effective in controlling the rate of disease progression. Adverse effects are considerable for these agents and the therapeutic benefit is often difficult to evaluate. Clinical improvement requires several weeks while dose limiting effects can appear quite suddenly. In contrast, clinical improvement after intra-articular steroid injection is rapid and pronounced. Limitations are due to systemic steroid toxicity and trauma associated with the injection technique. Crystal precipitation from the suspension formulation may also limit efficacy [2]. Intraarticular-arterial steroids are also reported to inhibit proteoglycan and protein synthesis which may contribute to articular cartilage degeneration; independent from arthritis [7, 8, 13].

LE was designed to circumvent the common limitations of currently used steroids and minimize systemic action. The systemic effects are diminished due to the facile metabolism of loteprednol etabonate to an inactive metabolite. Previous work with the compound demonstrated that the potent corticosteroid is rapidly metabolized and inactivated after systemic administration. Pharmacokinetic parameters measured in dogs, rats and rabbits were consistent with soft drug action [14, 15]. The therapeutic potential for the drug in controlling ophthalmic inflammation was proven in extensive clinical trials. These clinical trials demonstrated a potent anti-inflammatory action was achieved without the common steroid-mediated undesired adverse effect of increased intraocular pressure and confirmed preclinical findings [16].

The compelling clinical evidence from ophthalmic trials that LE functions as a potent local, but systemically inactive, corticosteroid stimulated interest in rheumatoid arthritis. An *in vivo* animal model was selected to evaluate acute antiinflammatory of local intra-articular dosing because the approach most closely mimicked a clinical paradigm. The vehicle included modified cyclodextrin, HPCD, as an excipient in order to limit the potential for steroid precipitation from the vehicle. Previous experience with HPCD demonstrated that the excipient could both stabilize protein formulations [19] and was effective in solubilizing dexamethasone [18]. Studies in dogs demonstrated that the HPCD did not inhibit bioavailability of dexamethasone after i.v. dosing.

This preliminary study indicates that LE has potent antiinflammatory effects in the acute antigen-induced rabbit model for rheumatoid arthritis. While LE offers the potential to eliminate systemic steroid toxicity, additional preclinical studies are required to evaluate its therapeutic potential in treating joint inflammation. Studies should evaluate longer term drug effects on joint function.

## 3. Experimental

Random bred New Zealand rabbits (2.0–2.5 kg) were sensitized to bovine serum albumin (BSA) following the procedure described by Gall and Gall [7]. For the first exposure, an emulsion containing a 1:1 mix of sterile filtered BSA (50 mg/ml, Sigma Chemical, St. Louis MO) and complete

Freund's adjuvant (Sigma Chemical) was injected into the dorsal neck muscle and foot pads. Each rabbit used in the study was initially sensitized with 3 ml of the antigen emulsion. Two weeks later, rabbits were given a booster injection (1–1.5 ml), in the neck muscle that contained BSA and Freund's incomplete adjuvant (1:1 emulsion). On the 20th day following initial BSA exposure, rabbit serum samples were collected for BSA antibody determination using ELISA techniques.
Twenty-eight days following initial immunization, acute local inflammatory responses were stimulated by intra-articular administration of BSA antigen. Antigen was injected into a patellofemoral joint in a vehicle (0.5 ml) containing 20% aqueous hydroxypropyl-cyclodextrin (HPCD) (Pharmos Corp., Alachua, Florida) Groups of rabbits (n = 7) were given one of the following treatments: 30% BSA, 30% BSA with 0.1% LE or 30% BSA with 0.1% dexamethasone, HPCD vehicle. Three days after intra-articular-arterial injection, animals were examined and sacrificed. Synovial fluid was removed for white blood cell evaluation, using 50–50 field counting methods, and joint were fixed in 10% neutral formalin. Synovial tissues were embedded in paraffin, sectioned, stained with hematoxylin and eosin or trichrome for fibrin and fibrinoid. Histologic sections were examined for inflammatory alterations including: synovial cell hyperplasia, inflammatory, infiltration and fibrin deposits.

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# The new antiarrhythmic substance AWD 23-111 inhibits the delayed rectifier potassium current (IK) in guinea pig ventricular myocytes


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The effects of *N*-(dicyclohexyl-carbamoylmethyl)-*N*-(3-diethylamino-propyl)-4-nitro-benzamide hydrochloride (AWD 23-111), a novel antiarrhythmic compound, were studied in isolated cardiomyocytes of guinea pigs. Using whole-cell configuration of the patch-clamp technique AWD 23-111 was tested for its ability to block the delayed rectifier potassium channel (IK). In guinea pig ventricular myocytes the current is composed of two components: $IK_r$, a rapidly activating current and $IK_s$, a slowly activating component which were discriminated by their different activation and deactivation behaviour. In this preparation AWD 23-111 displayed concentration dependent inhibitory effects on $IK_r$ as well as on $IK_s$ in the tested concentration range between 1 and 100 μmol/l. This blocking effect was independent of the stimulation frequency (0.2, 1 and 2 Hz). There was no influence of AWD 23-111 on the amplitude of L-type calcium whole-cell currents. The compound significantly prolonged action potential duration (APD) at a stimulation frequency of 2 Hz (1 and 10 μmol/l). At 0.2 Hz there was no effect on APD. Our results suggest that AWD 23-111 blocks both components of IK without a reverse use-dependent effect on APD which limits the therapeutic potential of most other class III agents.


## 1. Introduction

*N*-(Dicyclohexyl-carbamoylmethyl)-*N*-(3-diethylamino-propyl)-4-nitro-benzamide hydrochloride (AWD 23-111) is a newly synthesised aminocarbonacide amide derivative structurally related to detajmium, a compound with sodium channel antagonistic properties [1]. In preliminary experiments it was shown that AWD 23-111 exhibits antiarrhythmic actions. AWD 23-11 prolongs the action potential duration (APD) and the effective refractory period (ERP) in papillary muscles of guinea pigs dose dependently between 0.3 and 1 μmol/l [2]. In conscious dogs the drug increases the cardiac repolarisation time in AV-node and ventricular tissue [3]. These effects were frequency independent. Because of its action potential prolonging characteristics AWD 23-111 has been classified as a novel class III antiarrhythmic substance according to Vaughan Williams [4].
Antiarrhythmic actions that selectivity prolong APD and ERP are known to suppress or prevent atrial and ventricular arrhythmia in animal models and in humans [5–7]. APD prolongation by antiarrhythmic drugs is a result of either an increase in inward currents or a decrease in outward currents. Several types of outward currents have been identified in mammalian tissues including time independent currents such as $IK_1$, ICl [8] and $IK_p$ [9], inactivating currents such as $I_{to}$ [10] and the delayed rectifier