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Arecoline-induced skin inflammation: irritant or allergic dermatitis?

Michael Y.L. Law, Lynn K. Pershing and Lee K. Roberts [1]

Department of Biochemical Pharmacology and Toxicology, College of Pharmacy, University of Utah, School of Medicine, Salt Lake City, UT 84112 (USA)

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Summary

A severe skin inflammatory reaction halted the development of a transdermal device to systemically deliver arecoline, a cholinergic agonist, for use in the management of a human neurological disorder This report demonstrates that the skin reaction is most likely an allergic contact dermatitis (ACD) exacerbated by inflammation, as characterized by classical immunological tests employing inbred mice abdominal skin sensitization followed by ear challenge and ear challenge of recipients of lymphoid tissues from previously sensitized donors. Chemical analysis of the parent compound in three vehicles was performed to determine the chemical stability of the prepared solutions and the antigen specificity of the murine ACD response Arecoline was hydrolyzed to the pharmacologically inactive metabolite, arecaidine, to the greatest extent in aqueous vehicles ACD reactions were greatest when mice were both topically sensitized and challenged with arecoline In contrast, arecaidine, the hydrolyzed product, did not have immunological activity, but was capable of producing local inflammation in a dose-dependent manner upon subcutaneous injection. These data support the mechanistic hypothesis that upon absorption into skin, arecoline induces a local inflammatory response and a systemic ACD response. Influx of lymphocytes to an arecoline-treated skin site likely mediate ACD through the combined inflammation produced by arecoline and arecaidine and recognition of arecoline as an immunogen

Introduction

Medicinal use of betel nut extracts containing the cholinergic agonist, *N*-methyl-1,2,5,6-tetrahydropyridine-3-carbonic acid methyl ester (arecoline) (Lewin, 1889) has been used extensively for treating various types of ailments in ancient Arabic and Chinese cultures (Jahn, 1888). The combined cholinergic and muscarinic actions of arecoline (AL) (Marmé, 1890) had not gained favorable acknowledgement in western human medicine, but had been used successfully in veterinarial medicine as a ruminatoric and teniacide (Wishart, 1979; Windholtz, 1983). Recent interest in the development of AL for human neurologi-

Correspondence to L K. Pershing, Dept of Internal Medicine (Dermatology), University of Utah, Salt Lake City, UT 84132, U.S.A

[1] *Present address.* Skin Biology Research, Schering-Plough Health Care Products, 3030 Jackson Avenue, PO Box 377, Memphis, TN 38151-0001, U.S A.

cal disorders was rekindled with reports of its use to enhance human serial learning (Sitaram et al., 1978), induce cholinergic REM sleep in patients with affective illness (Sitaram et al., 1980), and improve the cognitive memory disorders of Alzheimer presenile dementia (Christie et al., 1981). The nature of these neurological disorders, requires chronic continual oral AL dosing. Chronic oral dosing of a therapeutic drug with a short half life in blood, and probable low patient compliance, led to the development of a non-invasive sustained-release drug delivery device to administer AL. Application of the arecoline transdermal device on guinea pigs and human subjects however, demonstrated severe skin inflammatory reactions in toxicological studies (Kurihara-Bergstrom et al., 1991) characterized by severe erythema and edema. These data, together with a previous report on allergic contact dermatitis in humans caused by chronic exposure to AL (Wishart, 1979), suggest that the inflammatory reaction has an immunological origin.

Chemically, AL is subject to hydrolysis, which results in the formation of *N*-methyl-1,2,5,6-tetrahydropyridine-3-carbonic acid, arecaidine (AD), and other metabolites. Hydrolysis of AL to its pharmacologically inactive product, AD, is thought to be the predominant pathway of metabolism in skin (Boyland and Nery, 1969). This report addresses the mechanistic basis of the skin inflammatory reaction as an immune response and identifies AL or its metabolite, AD, as being responsible for the observed skin reaction.

Materials and Methods

Animals

6-week-old female BALB/cAnNCr1Br (Charles River Laboratories, Wilmington, DE) inbred mice were used in this study. These animals were obtained from a colony maintained in the Animal Resource Center at the University of Utah and housed according to the guidelines set forth by NIH. Mice were housed four or five per cage with sawdust bedding in a temperature (25°C) and humidity (60%) controlled room with 12 h light cycle. All animals had free access to food and water. All experiments had prior approval from the Institutional Animal Use and Care Committee at the University of Utah.

Chemicals

Arecoline hydrobromide, arecaidine hydrochloride, 2,4-dinitro-1-fluorobenzene (DNFB), and croton oil (CO) were purchased from Sigma Chemical Co. (St. Louis, MO). Distilled house water (pH 6.0) was used to prepare the vehicles and HPLC mobile phase. Sodium 1-heptanesulfonate (> 98%) and ethanol (denatured > 95%, Gold Label) were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI). Transdermal devices (TDD), both placebo vehicle placebo (TDD vehicle) and arecoline (AL-TDD), were generous gifts from Ciba-Geigy, Inc. (Ardsley, NY). All AL and AD solutions were prepared fresh in either 50% ethanol : olive oil (v/v) or in the TDD placebo.

Chemical purification and analysis

AL was purified by repeated extraction of aqueous arecoline hydrochloride (pH 10) solution with petroleum ether. The ether extract was evaporated under nitrogen gas, yielding a yellowish oily liquid. Arecaidine was extracted from arecaidine bromide using 50% methanol and water pH 10 (pH adjusted with ammonium hydroxide) elution and a Bond-Elut™ anion-exchange column (Analytichem International, Harbor, CA). The eluent was dried under nitrogen gas to recover a white powder.

A binary gradient high-performance liquid chromatograph (HPLC) was used to analyze the purity of both extracts (model 334, Beckman Instruments, Inc., San Ramon, CA) employing an anion-exchange Zorbax CN column (Dupont Co., Wilmington, DE) and a mobile phase of 5 mM 1-heptanesulfonate in methanol and water at pH 3.0 (30 : 70, v/v) at a flow rate of 1 ml/min. 20 μl of each sample was injected onto the HPLC and arecoline and arecaidine identified at 214 nm with a fixed UV detector (Beckman model 166). Chromatograms of AL (retention time = 5.8 min) and AD (retention time = 3.7 min) alone and in combination are shown in Fig. 1a–c, respectively. Trimethylsilyl derivatives of the AL and AD solu-

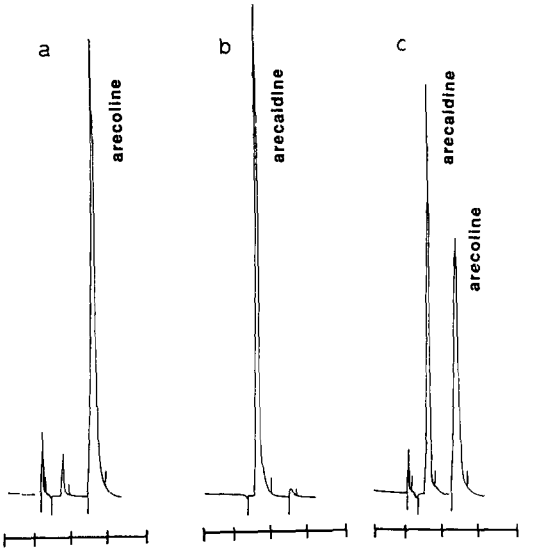

Fig 1. HPLC analysis of· (a) arecoline (AL), retention time, 5.6 min; (b) arecaidine (AD), retention time, 3 7 min and (c) equimolar solution of AL and AD HPLC procedures are described in Materials and Methods

tions were prepared and analyzed by gas chromatography/ion trap mass spectroscopy (GC/IT-MS) (Finnigan IT-20, San Jose, CA) using a DB1 column (0.25 mm i.d × 15 m; J & W Scientific, Cordova, CA) at 100°C with a hydrogen carrier gas (60 cm/s). AL and AD were separated with this procedure using a thermal gradient (20°C/min from 100 to 300°C). The injector temperature was 26°C and the interface temperature was 250°C. AL and AD solutions demonstrated a purity of 99% (results not shown).

Arecoline stability assay

Clear differentiation of the chemical species inducing the inflammation and/or contact allergic dermatitis response required that the parent compound be stable to hydrolysis in the vehicle solution at 37°C. The extent of AL hydrolysis to AD was evaluated in three potential vehicles: water (pH 6.0), phosphate-buffered saline (PBS) at pH 7.0, and 50% ethanol. Periodically over 54 h, samples of 100 μl volume were collected from each test solution and replaced with fresh solvent: once at day 0 for baseline measurement and twice daily, 6 h apart, thereafter for 54 h duration. Samples were stored at 4°C until analyzed by HPLC.

The stability of AL in three different vehicles in the presence of fresh, minced, split-thickness human abdominal skin or fresh, minced full-thickness mouse skin was also assessed. Human abdominal skin from elective abdominoplasties was donated to the Division of Dermatology and transported to our laboratory in a PBS solution (pH 7.2) containing streptomycin/fungizone (0.1 and 2.5 mM/l, respectively) within 2 h of surgical excision. An electric dermatome set to a thickness of 0.5 mm was used to obtain split-thickness skin from surgical skin specimens. These human skin specimens contained all of the stratum corneum, epidermis and approximately one third of the dermis. Six individual 6 mm diameter biopsies of the split-thickness (0.5 mm) human abdominal and fresh full-thickness mouse skin were finely minced, and incubated in the three solutions containing the same concentration of AL at 37°C for 54 h. Aliquots were collected from each incubation supernatant using the same time points as indicated above and submitted to HPLC analysis.

Induction and elicitation of skin allergic contact dermatitis in mice

The murine model used to evaluate the skin inflammatory reaction was a well-characterized abdomen sensitization and ear challenge test using DNFB as a positive control (Phanuphak et al., 1974). The mouse ear swelling assay was chosen for this study for two reasons. First, it provides quantitative measurements that allow for better statistical analysis of the data. Second, employing a well-characterized, inbred strain of mice allows the performance of syngeneic lymph node cell transfer experiments. Adoptive transfer experiments provide definitive evidence for cell-mediated immune responses. Thorne et al. (1991a,b) have recognized both of these as advantages with the mouse ear swelling assay in contrast to the guinea pig maximization test for these types of sensitization analyses. Briefly, for sensitization, 25 μl of the test solution was applied to the animal's shaved abdominal skin on days 0 and 1. On day 5, the mice were challenged by applying

10 μl of the test solution on the right pinnae. The thickness of the right treated and left untreated ears were measured 48 h later using an engineer's micrometer (Mitutoya no. 7326, Japan; sensitivity 0.0001 inch). The ear swelling difference between the animal's left and right ears was calculated and expressed as ear swelling units (0.0001 inch/ear swelling unit). Aqueous AL and AD solutions of different concentrations were prepared in a variety of vehicles immediately prior to use for sensitization and challenge to minimize hydrolysis of the solute. These included a 1:1 mixture (v/v) of 50% ethanol in PBS:olive oil, or as a suspension in the aqueous TDD vehicle, or in PBS pH 10 for topical application or PBS pH 7.2, for subcutaneous injection. DNFB was prepared as 0.5 and 0.1% solutions in ethanol:water:olive oil (4:4:1, by vol.) for topical sensitization and challenge doses, respectively.

Adoptive transfer of sensitized cells

100 μl of 3% AL, or 3% AD or PBS was injected subcutaneously into the abdomen of naive mice on days 0 and 1. These sensitized animals were killed on day 5 and their peripheral lymph nodes (axial, brachial, inguinal and cervical) excised. The lymphoid tissues from the donor mice were pooled and gently teased with forceps, to obtain a cell suspension. The cells were then washed by centrifugation and resuspended in PBS. 100 μl of the lymphoid cell suspension was then injected intravenously into syngeneic recipients via the tail vein, at a ratio of one donor per recipient. The recipients were subsequently challenged the same day with 10 μl of AL or AD (3%) solutions injected subcutaneously into the right pinnae. Ear swelling in the left and right pinnae was measured 1 and 2 days later. The ear swelling differences between the animals left and right ears were calculated and expressed as ear swelling units.

Results

Stability of AL in various vehicle solutions and skin

Identification of the chemical species responsible for the observed skin inflammatory reaction requires relatively pure solutions of AL and AD. HPLC analyses of the solutions presented in Fig. 1a–c were confirmed by GC/IT-MS (data not shown) and demonstrate a relative purity of > 99% of the prepared solutions used in the immunological studies. Before initiation of the immunological studies, however, stability studies of the parent drug in various vehicle solutions and the hydrolytic degradation of AL in skin were performed to identify an appropriate vehicle for use in the immunological studies.

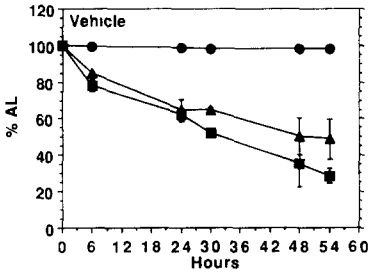

Fig. 2 Arecoline stability in water, PBS (pH 7.2) and 50% ethanol (mean ± SE for $n = 3$). (●) AL in 50% ethanol, (■) AL in water, (▲) AL in PBS, pH 7.2

AL was dissolved in water pH 6.0, PBS pH 7.2 and 50% ethanol at the same concentration. Hydrolysis of AL to AD in these solutions at 37°C over 54 h was quantitated by submitting samples of the solutions collected at various time points to HPLC analysis (Fig. 2). After 6 h of incubation, only 80% of AL remained in the original water and PBS solutions. At the end of 54 h, only 30 and 50% of the parent drug was still present in the water and PBS solutions, respectively. In contrast, AL is very stable in the 50% ethanol vehicle, as demonstrated by the minimal hydrolysis of AL over the same 54 h time period. These data demonstrate that AL stability is extremely sensitive to its vehicle environment, especially in aqueous solutions with a pH close to the drug pK_a of 6.84.

An experiment was conducted to determine whether additional significant hydrolysis of AL occurred in the presence of skin. Hydrolysis of AL by mouse and human abdominal skin was quantitated by incubating AL in the three different vehicle solutions with six fresh, minced full-thickness 6 mm diameter mouse skin biopsies or

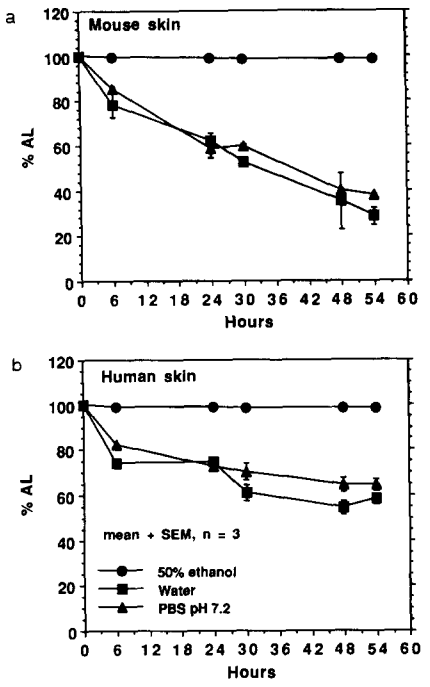

Fig. 3. Arecoline stability in three vehicles co-incubated with (a) mouse skin and (b) human skin in water, PBS (pH 7.2) and 50% ethanol (mean ± SE for $n = 3$). (●) AL in 50% ethanol; (■) AL in water; (▲) AL in PBS, pH 7.2.

six fresh, minced 6 mm diameter split-thickness human abdominal skin over 54 h. After 54 h, only 30 and 40% of the parent drug remained in the water and PBS vehicles, respectively (Fig. 3a). The extent of AL hydrolysis in these vehicles in the presence of mouse skin was not significantly different from that measured with vehicle alone (Fig. 2b). Addition of minced mouse skin to AL in 50% ethanol vehicle resulted in minimal hydrolysis of AL, which was similar to that measured with vehicle alone. Thus, the addition of mouse skin to the various AL solutions did not significantly alter the amount of drug hydrolysis.

Incubation of fresh split-thickness human abdominal skin with AL in the three different vehicles (Fig. 3b) did not significantly increase AL hydrolysis over time compared with the vehicles only (Fig. 2). Collectively, these data demonstrated that AL hydrolysis is greater in an aqueous environment than an organic environment and that this nonspecific hydrolysis dominates over the tissue hydrolysis of the parent compound. Thus, to ensure parent compound stability in subsequent experiments, AL was topically delivered to the skin in the ethanol vehicle or the supplied placebo aqueous TDD vehicle

Due to potent cholinergic activity of AL, various concentrations in the alcohol vehicle were tested topically on mice to determine the concentration of drug that was free from adverse neurological effects. Following high doses (10%) of topical AL in the alcohol vehicle, muscle spasms were noted in the treated mice, with an onset 5 min after topical application and a duration of 10 min. These observations are consistent with the known muscarinic/cholinergic action of AL and confirmed the systemic absorption of this topical drug. Animals topically treated with concentrations of AD up to 16% did not demonstrate this muscle response, consistent with its lack of cholinergic activity and poor skin penetrability. Subcutaneous injection of 10% AL in PBS (pH 7.2) resulted in immediate animal death. Subcutaneous injection of AD in PBS up to 16% did not result in any adverse neurological side effects in the animals. Subsequent immunological studies were therefore performed with AL concentrations of 4–10% and AD concentrations up to 16%.

Influence of vehicle on arecoline-induced murine ACD responses

To determine whether AL was capable of eliciting an ACD response in mice, animals were sensitized on the abdomen with a topical application of AL. Sensitizations were accomplished with either 10% AL prepared in ethanol : water : olive oil (4 : 4 : 1, by vol.), the placebo TDD vehicle, or the contents of the AL-TDD. Control animals were sensitized with the TDD vehicle alone. 5 days later, the right ear pinnae of sensitized animals were challenged by topical application of 10% AL in the ethanol : water : olive oil vehicle. Ear swelling responses were measured 48 h after challenge. Sensitization and challenge with the placebo TDD vehicle produced a negligible ear swelling response (< 5 units) (Fig. 4). An ear swelling response of approx. 15 ear swelling units was observed following topical challenge with 10%

AL in ethanol : water : olive oil to mice previously sensitized with the placebo TDD vehicle. This is an indication of the strong proinflammatory nature of AL. This level of ear swelling response, however, was significantly lower ($p < 0.05$) than the skin response following sensitization and challenge with AL or DNFB. Similar ear swelling responses to topical AL challenge were elicited in mice previously sensitized with either 10% AL prepared in the ethanol : water : olive oil vehicle or the AL-TDD. Overall, the ear swelling response produced by topical AL sensitization and challenge was comparable to that elicited with the known contact sensitizing agent, DNFB, suggesting an immunological origin. The results of this experiment indicated that AL is a strong contact sensitizing agent, especially when administered in the TDD vehicle.

Influence of AL and AD on the ear swelling response

The differential capabilities of AL, the parent compound, or AD, its hydrolysis product, to elicit an ACD response were investigated in mice topically sensitized with the 10% AD or 10% AL in ethanol : water : olive oil vehicle or the contents of

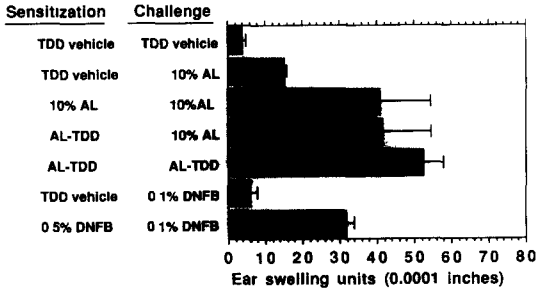

Fig 4. ACD elicited by topical application of AL. Mice were sensitized by applying 25 μl of 0.5% DNFB, TDD vehicle, 10% AL in ethanol . water . olive oil vehicle or AL-TDD to the abdomen on two consecutive days After 4 days, 10 μl of the indicated sensitizing agent was applied to the right ear pinnae. Data are presented as mean + SE ($n = 3$) of ear swelling units (defined in Materials and Methods) collected 48 h after challenge TDD vehicle, contents of the placebo transdermal delivery device, AL-TDD, contents of the AL transdermal delivery device, AL, arecoline, DNFB, dinitrofluorobenzene The AL and DNFB solutions were prepared in ethanol water : olive oil (4 4 : 1, by vol)

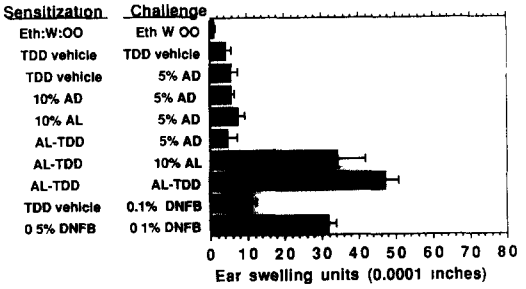

Fig. 5 Comparison of AL and AD as contact allergic dermatitis agents Mice were sensitized by applying 25 μl of the indicated agent to the abdomen on two consecutive days After 4 days, 10 μl of the indicated agent was topically applied to the right ear pinnae Data are presented as ear swelling units (mean + SE for $n = 3$) collected 48 h after challenge Eth . W · OO, ethanol water · olive oil (4 4 1, by vol.) vehicle, TDD vehicle, the contents of the placebo transdermal delivery device, AD, arecaidine; AL, arecoline, AL-TDD, contents of the AL transdermal delivery device, DNFB, dinitrofluorobenzene prepared in ethanol water olive oil (4 4 1, by vol)

the AL-TDD and their respective placebo vehicles (Fig. 5). Subsequent topical challenge of the previously AD-, AL- or AL-TDD sensitized mice with 5% AD in ethanol : water : olive oil vehicle did not elicit a significant ear swelling response above background, i.e., the control groups sensitized and challenged with ethanol : water : olive oil or the placebo TDD vehicle. In contrast, mice topically sensitized with AL-TDD elicited a significant ($p < 0.05$) ear swelling response (> 30 ear swelling units) with subsequent topical challenge to either 10% AL in the ethanol : water : olive oil vehicle or the contents of the AL-TDD. Again, the AL-induced ear swelling responses elicited were comparable to that produced by DNFB.

The inability of AD to elicit ACD was likely due to its more hydrophilic nature and thus, its poor penetration of the stratum corneum following topical application. To determine whether AD could elicit an ACD response in AL-sensitized mice if it were introduced into the skin, AD was dissolved into a physiologically compatible vehicle, PBS (pH 7.2) and immediately injected subcutaneously into the right ear pinnae of mice previously topically sensitized on the abdomen with the placebo TDD vehicle or 5% AL pre-

pared in the TDD vehicle (Fig. 6). AL and AD produced 1.5- and 3-fold greater ear swelling responses, respectively, following subcutaneous injection into TDD vehicle sensitized mice than similar concentrations applied topically (16 ± 1 and 6 ± 2, respectively, Figs 4 and 5). AL elicited an ACD response when injected subcutaneously into mice previously sensitized by topical application of 5% AL in the TDD vehicle as confirmed by the 2.5-fold increase in the ear swelling response above TDD vehicle sensitized animals (Fig. 6). In contrast, AD challenge appeared to produce an inflammatory response, but not an ACD response, as demonstrated by the similar ear swelling responses in the 5% AL in the TDD vehicle sensitized and nonsensitized placebo TDD vehicle control groups. Increasing the concentration of AD (4, 10 and 16%) subcutaneously injected into the skin, however, resulted in a linear increase in the mean inflammatory ear swelling response of 4.7, 19.2 and 31.0, respectively. The ear swelling response from the 16% AD treatment was not significantly different from that for subcutaneous challenge of 3% AL. Thus, while the inflammatory nature of AD was dose dependent, it was much less potent than AL. Further, the AD ear swelling response was not immunogenic, since subcutaneous challenge of 5% AD

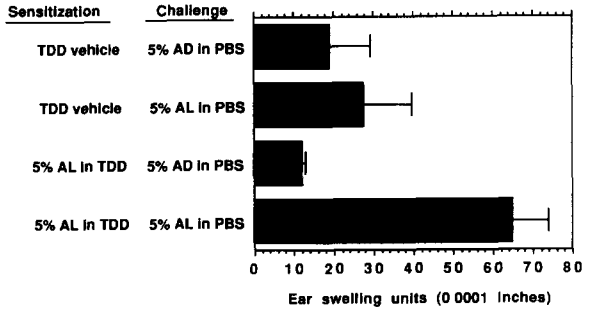

Fig. 6. Inflammatory actions of AL and AD following topical application in a variety of vehicles. Mice were sensitized by applying 25 μl of placebo TDD vehicle or 5% AL in TDD vehicle to the abdomen on two consecutive days. After 4 days, 10 μl of the indicated agent prepared in PBS was injected subcutaneously into the right ear pinnae Data are presented as the mean+SE of the ear swelling units ($n = 3$) measured 48 h after challenge TDD vehicle, contents of the placebo transdermal delivery device, AD, arecaidine; AL, arecoline; PBS, phosphate-buffered saline, pH 7.2

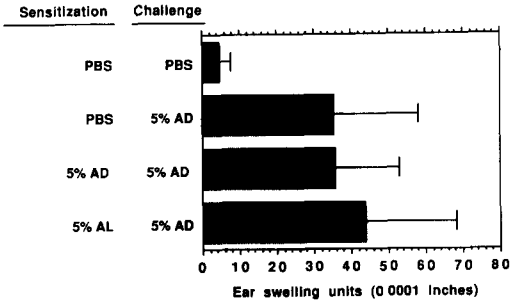

Fig 7. Inflammatory ear swelling response elicited by AD. Mice were sensitized by subcutaneous injection of 100 μl PBS, 5% AD in PBS or 5% AL in PBS into the abdominal skin on two consecutive days. After 4 days, 10 μl of PBS, 5% AD was injected subcutaneously into the right ear pinnae Data are presented as the mean+SE of ear swelling units ($n = 3$) measured 48 h after challenge PBS, phosphate-buffered saline, pH 7 2, AD, arecaidine; AL, arecoline AL and AD solutions were freshly prepared in PBS, pH 7 2

into the ear pinnae of mice previously topically sensitized to PBS, 5% AD or 5% AL in PBS into the abdomen was equivalent. The AD ear swelling response following subcutaneous challenge of 5% AD into the above topically sensitized mice was 9-fold greater than that of the PBS control mice (Figs 6 and 7). This 9-fold difference was clearly outside the experimental variability and likely represents nonspecific inflammation. It is interesting, nonetheless, that a 2-fold greater ear swelling response to subcutaneous 16% AD challenge was observed in mice sensitized to 0.01% AL (72 ± 8, mean $\pm$ SE, $n = 3$) compared with PBS-sensitized controls (31 ± 7, $n = 3$). These data suggest that at certain doses, AL induction of ACD may exacerbate the AD-induced inflammatory ear swelling response.

Adoptive transfer of AL- and AD-sensitized lymphoid cells to naive mice

To further confirm the inflammatory and immunological activities of AL and AD, adoptive transfer experiments were performed (Fig. 8). Mice were sensitized on two consecutive days by subcutaneous injection of either 3% AL or 3% AD in PBS into the abdominal skin. After 5 days, lymph node cells from these animals were transferred into naive syngeneic recipients. All animals were challenged by subcutaneous injection of AL

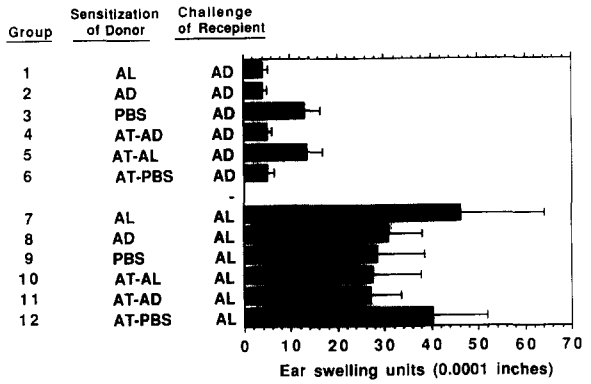

Fig 8 Adoptive transfer of delayed-type hypersensitivity induced by AL and AD Syngeneic donors were sensitized on two consecutive days by subcutaneous injection of PBS, 3% AL in PBS, 3% AD in PBS into abdominal skin. After 4 days, lymph node cells from donor mice, designated as AT-PBS, AT-AL and AT-AD, respectively, were transferred by intravenous injection into naive recipients. Tranfers were made at the ratio of one donor to one recipient Recipient mice were then challenged by subcutaneous injection of 10 μl of 3% AL or 3% AD into the right ear pinnae. Control animals (groups 1–3 and 7–9) were sensitized as per donor mice and then challenged after 4 days as per recipients. Data are presented as the mean + SE ($n = 3$) of ear swelling units collected 48 h after challenge. AL, arecoline; AD, arecaidine; PBS, phosphate-buffered saline, pH 7 2, AT-AL, adoptive transfer of AL sensitized mouse lymph nodes; AT-AD, adoptive transfer of AD sensitized mouse lymph nodes

or AD into the right ear pinnae. Ear swelling measurements were performed 48 h after the challenge.

The minimal extent of ear swelling elicited by AD challenge was not significantly different between the AD-treated animals (groups 2 and 4) and controls (groups 3 and 6), confirming that AD was not an immunological agent.

Confirmation of the antigen-specific ACD response by AL was inconclusive in these experiments, due to the high, but equivalent ear swelling responses elicited by subcutaneous challenge of 3% AL, regardless of the source of donor cells (groups 10–12). Indeed, AL is a potent inflammatory agent upon subcutaneous injection, as demonstrated by the high extent of swelling observed in the non-sensitized control animals (groups 9 and 12). In contrast to the hydrolytic product, AD, AL appears to have immunological properties, as demonstrated by the greater extent of ear swelling in animals sensitized and challenged with AL (groups 7 and 10), compared with animals sensitized and challenged with AD (groups 2 and 4). Although suggestive, this experiment was not conclusive due to the strong irritant effect produced by subcutaneous injection of AL. Direct comparison of this type was further hindered by the inability of AD to elicit a response when applied topically.

Discussion

The development of a transdermal delivery device (TDD) to systemically deliver arecoline for the management of cognitive memory loss associated with Alzheimer's disease and affective neurological disorders was halted due to severe skin inflammatory reactions produced by the TDD in guinea pigs and humans (Kurihara-Bergstrom et al., 1991). Given that the half-life of AL in blood is very short and that AL is rapidly hydrolyzed to a pharmacologically inactive metabolite, AD, in the skin (Boyland and Nery, 1969), we postulated that the adverse skin reaction to the transdermal delivery device could be the result of an inflammatory or allergic contact dermatitis (ACD) response elicited by either the parent compound or hydrolytic product. Alternatively, the observed skin reaction could be caused by the vehicle within the transdermal device. A series of experiments were systematically designed in the present study to elucidate the chemical species responsible for this inflammatory reaction and determine whether the inflammatory reaction was ACD or irritant dermatitis.

Elucidation of the chemical species eliciting the observed inflammatory reaction required (1) pure parent and metabolite, (2) an appropriate vehicle to deliver the individual chemical species and (3) an animal model system with which to measure the inflammatory and immunological responses. Isolation of adequate quantities of AL and AD was accomplished with chemical extraction. Stability and solubility of AL in aqueous and alcohol vehicles identified an ethanol mixture in olive oil as an appropriate vehicle for topical delivery of AL. Pronounced hydrolysis of AL to

AD occurred in aqueous vehicles, likely reflecting solvolysis, the degradation of an active drug through reaction with the vehicle, which acts as a nucleophile and attacks electrophilic ester centers of the drug molecule (Leffler and Grunwald, 1963; Hammett, 1970). Solvolysis occurred to the greatest extent in solutions that approximated the pK_a of arecoline (6.84), consistent with that of the water and PBS vehicles. In contrast to the aqueous vehicles, hydrolysis of AL in the alcohol vehicle was negligible. Neither mouse skin nor human skin increased the hydrolysis of AL to AD in any of the tested vehicles. These studies demonstrate that the hydrolytic activity of AL is very sensitive to the vehicle environment and could play an important role in the skin response observed in previous and present immunological studies.

Topical sensitization and challenge of mice with PBS or the ethanol:olive oil:water vehicle demonstrated negligible ear swelling responses. Subcutaneous injection of PBS also produced minimal ear swelling responses in this animal model.

Topical AD challenge in all of the above vehicles produced a minimal ear swelling response to control and previously AL- or AD-sensitized mice. This lack of response was likely due to the poor penetration of AD into the skin. AD, being more polar than AL, would be less able to penetrate the epidermis and thus would remain primarily on the skin surface. Subcutaneous injection delivered AD directly into the skin and resulted in a 5-fold increase in the ear swelling response compared with topical administration of AD. Further, increasing the concentration of AD subcutaneously injected linearly increased the ear swelling response. These data support the hypothesis of the inflammatory nature of AD. The lack of significant ear swelling following subcutaneous challenge with AD to mice previously sensitized with AL or AD in the adoptive transfer experiments, however, confirmed that AD was not an immunogen. That a 2-fold greater ear swelling response to subcutaneous 16% AD challenge was observed in mice previously sensitized to very low (0.01%) concentrations of AL compared with PBS-sensitized controls suggested that at particular doses, AL induction of ACD may exacerbate the AD-induced inflammatory ear swelling response.

AL produced both a nonspecific inflammatory response, as demonstrated by the similarity in the ear swelling response to subcutaneous AL challenge of mice previously sensitized to AL, AD or PBS alone, and an antigen-specific ACD reaction, when AL was applied topically to AL-sensitized mice. AL therefore appeared to play a critical role in exacerbating the skin response to AD by inducing a local, nonimmune-mediated inflammatory response. Local inflammation would increase the number and/or activity of the inflammatory and lymphoid cells recruited into the inflamed skin site and likely enhance the irritant reaction elicited by AD. Similarly, during the elicitation phase of an ACD response to AL, the presence of AD could heighten the severity of the reaction through the recruitment of inflammatory cells. This hypothesis is consistent with a recent report where it was shown that irritants enhance the ACD response elicited in humans by known allergens (McLelland et al., 1991).

In summary, these data demonstrate that an otherwise nominal hydrolytic reaction of a topical drug may, in the appropriate environment, be exacerbated to an allergic contact dermatitis response. The present data support the hypothesis that AL penetration into the skin induces a local nonspecific inflammatory response and is hydrolyzed to its pharmacologically inactive product, AD. Both the parent compound and the hydrolytic product may be irritating and induce inflammation, the latter in a concentration-dependent manner. The resulting local inflammation is likely characterized by the recruitment of increased numbers of lymphocytes into the skin and their recognition of AL as an immunogen. Further elucidation of this complex physicochemical and immunological interaction between AL and AD is needed. Nonetheless, this topical drug serves as an example of how an active agent and its pharmacologically inactive hydrolytic product may synergize in an immunologically active fashion to result in dramatic, unforeseen and unacceptable side effects of transdermal and topical drug delivery.

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