Successful treatment of a novel generalized variant of canine discoid lupus erythematosus with oral hydroxychloroquine

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Abstract
Discoid lupus erythematosus (DLE) is a common canine autoimmune disease that usually manifests as a localized ulcerative and scarring nasal dermatitis. We report herein a generalized variant of canine DLE successfully treated with the antimalarial immunomodulator hydroxychloroquine (HCQ). A 9-year-old hairless Chinese crested dog was presented with annular and polycyclic hyperpigmented and scaly skin lesions with central erosions, hypopigmentation and/or scarring on the trunk, neck and lateral extremities. Associated systemic signs were not seen. The clinical diagnosis of generalized DLE was supported by the demonstration of lymphocyte-rich interface dermatitis with epidermal atrophy and dermo-epidermal deposition of immunoglobulins and activated complement. As for human DLE, treatment was initiated with HCQ at 5 mg/kg once daily along with 2 weeks of 0.1% tacrolimus ointment and restriction of sun exposure. Over the following year, complete remission was maintained with HCQ at 5 mg/kg orally once daily with the exception of three relapses; two occurred during treatment induction and the third arose when the frequency of HCQ administration was reduced to every other day. Disease flares were controlled with 0.1% tacrolimus ointment alternating with 0.1% prednicarbate cream once daily for 5–10 days. Altogether, adverse drug events were not seen with this regimen. In summary, clinically, histologically and immunologically, this dog’s disease mirrored the generalized discoid variant of chronic cutaneous lupus erythematosus of humans. The apparent benefit of HCQ, its safety and low cost warrant future investigations of its use for treatment of canine cutaneous lupus variants.

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Introduction
In humans, cutaneous lupus erythematosus (CLE) encompasses three main variants: acute, subacute and chronic CLE (CCLE). This division is based on the average duration of the disease, clinical features, histological changes and likelihood of development of concurrent systemic lupus erythematosus, which is highest in acute CLE and lowest for CCLE. The most common clinical subtype of CCLE is the so-called discoid lupus erythematosus (DLE), which can be either localized or generalized. Lesions of human DLE most often affect the scalp, face and ears, the sun-exposed areas of the body. The classical lesion of human DLE begins as a sharply demarcated purplish macule or papule that gradually evolves into a coin-shaped (discoid) plaque. With time, the lesional centre becomes atrophic and hypopigmented, and disfiguring scars frequently develop. Peripheral lesional scaling and hyperpigmentation are commonly seen.

First reported in 1979, canine DLE is believed to be a common autoimmune skin disease with a phenotype different from that of humans with the same dermatosis. In two large series, dogs with DLE were reported to be affected principally with a nasal-predominant erosive, ulcerated and crusted dermatitis associated with depigmentation and loss of architecture of the nasal planum. To the authors’ knowledge, a phenotype similar to that of human generalized DLE has not been reported in the canine species.

At present, the diagnosis of DLE in humans and dogs is based on clinical signs, exclusion of systemic signs suggestive of systemic lupus erythematosus (SLE) and demonstration of lymphocytic interface dermatitis on microscopic examination of lesional skin biopsy specimens. This diagnosis is further supported by the demonstration of autoantibodies and activated complement along the dermo-epidermal junction (the so-called ‘lupus band test’, LBT) by direct immunofluorescence (IF) of lesional and nonlesional skin sections.

The current standard of care for canine DLE involves sun avoidance and the use of oral and/or topical glucocorticoids, topical tacrolimus, niacinamide and tetracycline, or vitamin E. In cases refractory to these
interventions, cytotoxic drugs such as azathioprine are usually added. In general, the prognosis of canine DLE is considered good with appropriate therapy. As oral glucocorticoids are associated with common and often severe adverse effects that can lead owners to discontinue treating their dogs, there is a need for additional treatments that offer high efficacy with low risk and cost.

Besides photoprotection, topical glucocorticoids and calcineurin inhibitors, the treatment of choice for human DLE is the antimalarial drug hydroxychloroquine (HCQ). Antimalarials are known to decrease autoantigen, but not foreign antigen, presentation to immune cells, to downregulate the expression of proinflammatory cytokines and to be potent protectors against ultraviolet-light-induced cell damage and inflammation. As a result, they have immunomodulating properties rather than inducing systemic immunosuppression.

In this article, we report a novel generalized variant of DLE in a dog that exhibited skin lesions identical to those described in the human homologous disease. In this dog, the long-term remission of skin lesions was achieved with HCQ, while disease flares were controlled with short courses of topical anti-inflammatory drugs.

Case report

A 9-year old female spayed Chinese crested hairless dog was presented to the North Carolina State University Veterinary Teaching Hospital Dermatology service with a 9 month history of progressive skin lesions. Prior to this visit, the dog had been seen for evaluation of multiple circular erosions and scales on the neck and trunk. This dog had received various treatments that included a single steroid injection and two short courses of oral antibiotics, all of which resulted in an initial but transient improvement of skin lesions. The disease eventually progressed to involve hairless areas of the neck, the lateral trunk and all four legs. Interestingly, lesions affecting the face and sun-protected skin areas were not seen. The patient was not pruritic.

Upon presentation to the dermatologist, the dog was in good general health; apart from its skin problem, abnormalities were not noticed on physical examination. Annular to polycyclic, hyperpigmented, sometimes scaly and erythematous skin lesions with either central whorl macules, papules and plaques, crusts overlying deep erosions/ulcers or hypopigmented atrophic scars (Figure 1a–d) were detected on the trunk, neck and lateral extremities. The lesions ranged from 2 to 4 cm in diameter.

Three 8-mm-diameter punch biopsies were taken from the margins and the centre of polycyclic skin lesions. Samples were submitted for routine histopathology and direct IF testing. Histopathology revealed cell-rich, lymphocytic interface dermatitis (Figure 2) that was bordered by epidermal hyperpigmentation and progressed to deep erosions and shallow ulcers centrally in some lesions. Discrete zones of basal cell vacuolation, apoptosis and loss were associated with central areas of keratinocyte attenuation and elongation. Secondary epidermal atrophy was superimposed on a background of moderate epidermal hyperplasia, and mild spongiosis, compact to laminated orthokeratotic hyperkeratosis and mild neutrophilic crusting were also observed. Lymphocytic infiltration of the basal layer and satellitosis of apoptotic basal cells were accompanied by basal cell vacuolation and disorganization. A thick band- or filament of lymphocytes and fewer plasma cells, with intermixed individual melanophages, was present in the superficial to mid-dermis and intimately contacted the epidermis. These changes extended to involve subjacent hair follicle infundibulum. Sebaceous glands were present. Prominent thickening of the basement membrane zone was seen within areas of active inflammation and was especially visible after periodic acid Schiff staining. Histological evidence of chronic solar injury in poorly haired skin was minimal, but samples were from areas of chronically hyperpigmented skin that contained prominent inflammation and epidermal injury that might have obscured such actinic changes.

Direct IF revealed the deposition of IgG, IgM, IgA and C3 along the dermo-epidermal junction of lesional skin biopsies (Figure 3). Weaker deposition of reagents between epidermal layers was interpreted as reflecting the spongiosis in the haematoxylin and eosin stained sections. Indirect IF testing on canine salt-split buccal mucosa did not detect any circulating anti-basement membrane IgG autoantibodies at 1:10 serum dilution.

Taken together, the existence of these unique macroscopic skin lesions, along with a chronic clinical course, the presence of a cell-rich lymphocytic interface dermatitis on histology and the deposition of autoantibodies and complement along the basement membrane zone were suggestive of a CCLE variant similar to human generalized DLE, a dermatosis never before reported in veterinary medicine.

To rule out concurrent SLE, serum antinuclear antibody titre, a serum chemistry profile, complete blood count and urinalysis were obtained. The antinuclear antibody titre was considered negative at less than 1:20 serum dilution, and the complete blood count and urinalysis results were unremarkable. The only abnormalities noticed on the chemistry profile were elevated blood urea nitrogen and magnesium levels and activity of alanine aminotransferase. Based on these results and the lack of systemic signs, coexisting SLE was ruled out.

Treatment with oral HCQ (Gallop, St Paul, MN, USA) and topical tacrolimus 0.1% ointment (Protopic; Astellas Pharma, Deerfield, IL, USA) was initiated as recommended for humans with DLE. As HCQ is contraindicated in patients with pre-existing retinal disease, a complete ocular examination was performed by a veterinary ophthalmologist; retinal anomalies were not observed. The patient was subsequently started on 5 mg/kg HCQ orally once daily, along with twice-daily applications of tacrolimus ointment. To better evaluate the potential efficacy of tacrolimus, the owner was asked to treat only one side of the body with this ointment while leaving the other side untreated. Within 4 days of beginning this treatment, all tacrolimus-treated lesions were neither erythematous nor eroded, whereas untreated ones had enlarged in size, were more erythematous and had ulcerated. The owner was then advised to treat all skin lesions with tacrolimus ointment until a complete remission was achieved while maintaining the patient on

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generalized discoid lupus erythematosus. This figure provides a close up of the different lesions present on the thorax at treatment onset, as follows: (a) annular, micropapular and hyperpigmented lesion with focal hypopigmented scarring and erosions and healed normal central skin; (b) polycyclic and annular hyperpigmented lesions with focal depigmentation and scarring; (c) two annular hyperpigmented lesions, the leftmost one with more extensive central scarring; and (d) well-demarcated ulcer with peripheral scarring and focal crusting in a second smaller lesion.

daily HCQ. Over the following 2 weeks, most skin lesions had completely resolved, and the few remaining ones were no longer erythematous or eroded. Tacrolimus was discontinued, and the dog was returned for re-evaluation 3 weeks later. At that time, only rare hyperpigmented annular or polycyclic lesions with central scarring and depigmentation were present. The dog apparently tolerated HCQ very well, as adverse events were not noticed. At the same appointment, a 1-cm-long exophytic, hyperpigmented nodule over the right scapula - a lesion that had been there for several months - was removed under local anaesthesia. Histopathology was consistent with a benign melanocytoma, and further diagnostic or therapeutic steps were not undertaken. Interestingly, within 48 h of that visit, the patient’s DLE relapsed, with eroded, ulcerated and scaly lesions developing on the lateral hindlegs. Twice-daily applications of tacrolimus ointment were reinstituted, as during treatment induction, and all lesions resolved within 5 days of application; lesions then remained in remission for the following 2 months.

Three months later, while still being treated with HCQ at the same dosage, the dog developed ulcers on the lateral thorax and front legs shortly after having been boarded while the owner was on holiday. On this occasion, reinstituting twice-daily application of tacrolimus for 4 days resulted in only minor clinical improvement. As a result, the potent diester steroid 0.1% prednicarbide cream (Prasco Laboratories, Mason, OH, USA) was added. All lesions resolved within 5 days of using this combination. Both topical medications were stopped, and the patient was continued solely on oral HCQ. The dog's skin disease remained in complete remission over the following 7 months, until an attempt was made to taper the HCQ from once daily to once every other day. This resulted in an immediate relapse of skin lesions, suggesting that daily HCQ had been important in maint-
Figure 2. Canine generalized discoid lupus erythematosus. Biopsy of dorsal back skin. This figure illustrates cell-rich interface dermatitis and infundibular mural folliculitis with basal cell vacuolation as well as apoptosis (arrowhead) and lymphocytic satellitosis of basal keratinocytes (inset). A band-like (lichenoid) infiltrate of lymphocytes and plasma cells hugs the epidermis. Haematoxylin and eosin. Scale bar represents 100 μm.

Figure 3. Canine generalized discoid lupus erythematosus. Linear, irregular and thick deposits of immunoglobulins or activated C3 complement at the basement membrane zone (positive lupus band test; arrowheads) can be seen. Dermal plasma cells expressing intracellular IgG or IgM were present in the superficial dermis (a–c). Irregular low-intensity intercellular epidermal fluorescence probably reflected the spongiosis seen in histological sections. Direct immunofluorescence for detection of canine IgG (a), IgA (b), IgM (c) and C3 (d).

taining remission. Therefore, once-daily dosing with HCQ was restarted and combined with once-daily application of topical tacrolimus ointment and prednicarbacte cream. Complete remission was achieved within 5 days and topical treatment was subsequently discontinued. Twelve months after starting HCQ, mild retinal degeneration was noticed during an ophthalmology recheck examination. Even though these lesions were assessed as being most consistent with age-related changes, repeated ophthalmological examinations were scheduled every 6 months. At the time of writing, the patient’s DLE has been maintained in complete remission with HCQ once daily for more than 4 months (Figure 4).

Discussion

Clinically, histologically and immunologically, the skin lesions of this Chinese crested hairless dog mirrored those of the generalized classical variant of DLE in humans. Clinically, the annular to polycyclic hyperpigmented, scaly lesions with central erosions, skin atrophy and scarring are pathognomonic for human DLE. To the authors’ knowledge, there is only a single dog reported to have skin lesions similar but not identical to
those of the patient described herein. In this young adult female Spitz, skin lesions were solely described as dorsal truncal erythema and scaling, and a photograph suggested that these lesions were annular or polycyclic. In this Spitz dog, microscopic lesions were reported as a lymphoplasmacytic interface dermatitis with mural folliculitis with near complete absence of sebaceous glands. It is unclear whether or not this Spitz' disease represents a variant of CLE or a different entity.

An important factor that could explain the occurrence of CCLE in this Chinese crested dog is the absence of hair on the trunk and extremities, a characteristic of this breed. In humans, most CLE variants are photosensitive, and it is logical to expect that prolonged and repeated exposure to ultraviolet radiation of hairless body areas could have been a triggering factor for generalized DLE skin lesions in this dog.

In our patient, the clinical suspicion of generalized DLE was supported by compatible findings on histology (a cell-rich lymphocytic interface dermatitis with epidermal atrophy and basement membrane thickening), as well as the demonstration of three different immunoglobulin isotypes and activated complement along the basement membrane zone. The demonstration of immunoglobulins and complement by direct IF – a positive LBT – was initially thought to be pathognomonic for human CLE until such findings were also observed in other nonlupus-associated skin diseases. The diagnostic specificity of the LBT, however, increases markedly when two or more immunoreagents (immunoglobulins and/or complement) are seen deposited at the dermo-epidermal junction of lesional skin, or when the LBT is positive on samples from nonlesional sun-protected areas. In our dog, the LBT was positive on lesional skin for four immunoreagents (IgG, IgM, IgA and C3), thereby supporting the clinical and histological suspicion of DLE. Unfortunately, as this dog was nearly hairless, biopsies from nonlesional sun-protected areas were not available for direct IF testing. The diagnostic value of the LBT for canine DLE remains to be tested by comparing results obtained from sun-exposed lesional skin and sun-protected nonlesional skin samples of dogs with this disease, as well as those with other lesions resembling nonlupus dermatoses.

In this case, HCQ was selected because of its proven efficacy in human DLE, its relative safety at low dosages and its low cost. The main adverse effect limiting the use of HCQ in humans is the development of retinopathy. This adverse effect is rare and is mainly seen in high-risk patients, i.e. those using the medication for long periods (>5 years), those on higher dosages (>6.5 mg/kg/day), those with high body fat levels, nephro-, hepato- or retinopathies, and those over 60 years of age. In the dog described herein, HCQ was given orally at 5 mg/kg once daily. This dosage did not appear to
result in observable adverse drug events. It is unclear whether the mild retinal changes seen after 1 year of administration were due to HCQ administration and/or to the advanced age of the dog; the latter hypothesis was considered most likely by the ophthalmologist.

Interestingly, HCQ has only been reported once before in veterinary medicine for the treatment of exfoliative CLE in three German shorthaired pointers. In these dogs, HCQ administration, at 5–10 mg/kg once daily, led to the lack of perceived progression of their disease but not to lesion remission, and adverse effects were not noted. The lack of remission in these dogs with exfoliative CLE might be due to this lupus variant being associated with systemic signs and an overall poor prognosis. In contrast, the paucity of flares seen during HCQ therapy in our patient could be due to the restriction of lupus lesions to the skin. During the 1 year follow up of this patient, only three recurrences of CLE occurred. The first two flares happened when HCQ might not have been fully effective and/or when stressful events might have contributed to precipitate the flares. The last relapse occurred when HCQ was tapered from once daily to once every other day; this observation provides an indication of a likely dose-dependent efficacy. When HCQ was restarted with once-daily dosing, along with a 5 day course of topical medications, all lesions underwent remission, and they remained quiescent without the need for additional topical interventions for at least 4 months thereafter.

In this dog, at the time of treatment induction and after each flare, active lesions were treated with 0.1% tacrolimus ointment, a topical calcineurin inhibitor shown to be effective in humans with DLE. The efficacy of topical tacrolimus in the treatment of the localized nasal variant of the canine disease has been demonstrated. In the present case, the improvement of lesions on the side of the body treated topically with tacrolimus, but not on the untreated side, suggests that tacrolimus is also an effective treatment for generalized DLE.

In conclusion, the lesions seen in this dog resembled those of generalized DLE of humans, a unique dermatosis not reported previously in the canine species. The apparent benefit of HCQ to help induce and maintain remission, combined with its safety and low cost, warrant further exploration of HCQ for treatment of cutaneous lupus variants and other autoimmune skin diseases in the canine species.

References


Generalized discoid lupus erythematosus

observé. Le diagnostic clinique de DLE a été supporté par la mise en évidence d'une dermatite d'interface riche en lymphocytes avec atrophie épidermique et dépôt d'immunoglobulines et de complément activé à la jonction dermo-épidermique. Comme pour le DLE humain, le traitement a été initié avec l'HQC à 5mg/kg une fois par jour et deux semaines de tacrolimus 0.1% en pommade et l'éviction des expositions solaires. Au cours de l'année suivante, une rémission complète a été maintenue avec 5mg/kg d'HQC par os une fois par jour à l'exception de 3 rechutes : deux se sont produites au cours de l'induction et la troisième a eu lieu lorsque la fréquence d'administration d'HQC a été réduite à une fois tous les deux jours. Ces crises ont été contrôlées avec du tacrolimus en pommade à 0.1% en alternance avec une crème de prednicarbate à 0.1% une fois par jour pendant 5 à 10 jours. Aucun effet indésirable n'a été observé pour ces traitements. En résumé, d'un point de vue clinique, histologique et immunologique, la dermatose de ce chien correspond au variant de lupus discoïde généralisé de lupus érythémateux cutané chronique de l'homme. Les avantages manifestes de l'HQC, sa sûreté et son faible coût justifient de futures études sur son utilisation dans le traitement des variants du lupus cutané canin.

Resumen El lupus eritematoso discoide (DLE) es una enfermedad autoinmune canina bastante común que se manifiesta generalmente como una dermatitis nasal ulcerativa y dermatitis nasal fibrosante. Aquí publicamos una variante generalizada de DLE canino tratada con éxito con el fármaco antimalaria inmunomodulador hidroxicloroquina (HQC). Un perro de raza Chino de nueve años de edad se presentó con lesiones anulares, políciclicas, hipopigmentadas y descamantes con erosiones e hipopigmentación o cicatriz centrales en el tronco, el cuero y la parte lateral de las extremidades. No se observaron signos sistémicos asociados. El diagnóstico clínico de DLE generalizado fue apoyado por la demostración de dermatitis de interfase rico en linfocitos con atrofia de la epidermis y deposición en la unión dermo-epidermática de inmunoglobulinas y complemento activado. Al igual que en el DLE humano, se realizó tratamiento con HQC a dosis de 5 mg/kg una vez al día durante dos semanas junto con pomada al 0.1% de tacrolimus y restricción de la exposición al sol. Se mantuvo remisión completa durante el año siguiente con HQC a dosis de 5 mg/kg por vía oral una vez al día a excepción de tres recaídas: dos ocurrieron durante la inducción del tratamiento y la tercera se presentó cuando la frecuencia de la administración de HQC se redujo a días alternos. Las exacerbaciones de la enfermedad fueron controladas con pomada al 0.1% de tacrolimus altermando con crema al 0.1% de prednicarbato una vez al día durante cinco a diez días. En conjunto, no se observaron fenómenos adversos con este régimen de tratamiento. Resumiendo, a nivel clínico, histológico e inmunológico, la enfermedad de este perro reflejó la variante de DLE generalizada similar a la enfermedad en humanos. El aparente beneficio del tratamiento con HQC, su seguridad y el bajo coste estimulan futuras investigaciones de su posible uso para el tratamiento de variantes cutáneas caninas de lupus.

要約 円板状エリテマトーデス（DLE）は通常、限局した潰瘍性、癒状性の鼻の皮膚炎として生じる一般的な皮膚の免疫疾患である。我々はここに抗マラリア性免疫調整剤ヒドロキシンクロロキン（HCQ）を用いて治療に成功した全身性のDLEの症例を報告する。9歳男のチャイニーズクレステッドドッグが、体幹、顔部、四肢外側面に、硬癌あるいは多発性の色素沈着を示し、中央部にびらん、色素脱失を伴う潰瘍状の皮膚症状が認められたという主訴で来院した。関連する全身症状は認められなかった。全身性DLEの臨床診断は、表皮の萎縮を伴うリンパ球にとめられた病状や、真皮-表皮境界部の弾性グロプリンおよび活性型補体の沈着が支持された。ヒトのDLEの初期の治療はHCQを5mg/kgで1日1回投与し、それに0.1%タクロリムス軟膏を2週間外用し、同時に日光の暴露を制限した。その後1年間5mg/kgのHCQを1日1回投与し観察していたが、3回にわたり再発した：2度は治癒後中で、1度はHCQの投与を隔日に減少した時であった。再発したときには0.1%タクロリムス軟膏と0.1%プレドニゾールベットクリームを交代で1日1回、5-10日間外用した。全体的に、薬の副作用はこの投与法では認められなかった。要約すると、臨床的、組織学的、免疫学的にこのイヌの疾患はヒトの慢性皮膚エリテマトーデスの全身性円板状亜型と類似していた。HCQは明らかに利点、安全性と低価格があるため犬の皮膚病変および亜型の治療への今後の研究が期待される。

摘要 盤状紅斑狼疮（DLE）是常见的大体免疫病，通常表现为溃疡和瘢痕性局部鼻端皮炎。我们在此报道一例犬DLE自身发病的变异病例，使用有免疫调节性的抗疟药羟氯喹（HCQ）得到了有效治疗。一只9岁中国冠毛犬在躯干、颈部和肢端侧出现坏死和多环相连的色素过度沉着和皮损，并且病变中心糜烂、色素斑点和/或脱屑。未见相关的全身症状，此犬的组织病理发现淋巴细胞丰富的界面性皮炎，同时表皮萎缩以及球蛋白和激化的补体沉积真皮-表皮连接处，可以据此诊断为全身DLE。由于DLE的治疗，HCQ的起始治疗量为5mg/kg/日，联合使用0.1%他克莫司软膏2周，并且严格遮光。此犬在一年治疗中使用HCQ5mg/kg/日口服，症状完全缓解，期间复发三次，再次发生在治疗期间，第三次复发出现在HCQ给药剂量降至每日0.1mg/kg时，用0.1%他克莫司软膏替换每5日1次的0.1%泼尼松乳膏能够控制疾病。上述治疗方案完全没有出现药物副反应。总之，在临床症状、组织学和免疫学上，此犬的发病和人慢性皮肤红斑狼疮的全身状变异型相似。HCQ在此病很有效，而且安全，便宜，应当在犬皮肤疾病各种变异型的治疗上做更多调查。