

study reviewed 609 patients with recurrent urticaria who received laboratory investigations of CRP and protein electrophoresis, and found that none fulfilled criteria for SchS⁵. We speculated that genetic factors and unrecognized cases might contribute to the low incidence in Korea. Physicians should suspect SchS in cases of recurrent urticaria with atypical clinical features.

CONFLICT OF INTEREST

The authors have nothing to disclose.

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Differential Effects of Digoxin on Imiquimod-Induced Psoriasis-Like Skin Inflammation on the Ear and Back

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Dear Editor:

Psoriasis is a chronic inflammatory skin disease in which interleukin (IL)-17-producing T helper (Th) 17 cells play a crucial role¹. Topical application of the toll-like receptor 7 agonist imiquimod (IMQ) on the ear and/or back skin of mice is a widely used IL-17-dependent model of psoriasis-like skin inflammation². The transcription factor reti-

noid acid-related orphan receptor γ t (ROR γ t) is required for IL-17 production by Th17 cells³. IMQ-induced skin inflammation is reduced both in ROR γ t-deficient mice and upon pharmacological inhibition of ROR γ t activity with novel small molecule selective ROR γ t inhibitors, indicating that ROR γ t is crucial for IMQ-induced psoriasis-like skin inflammation in mice and suggesting a potential role

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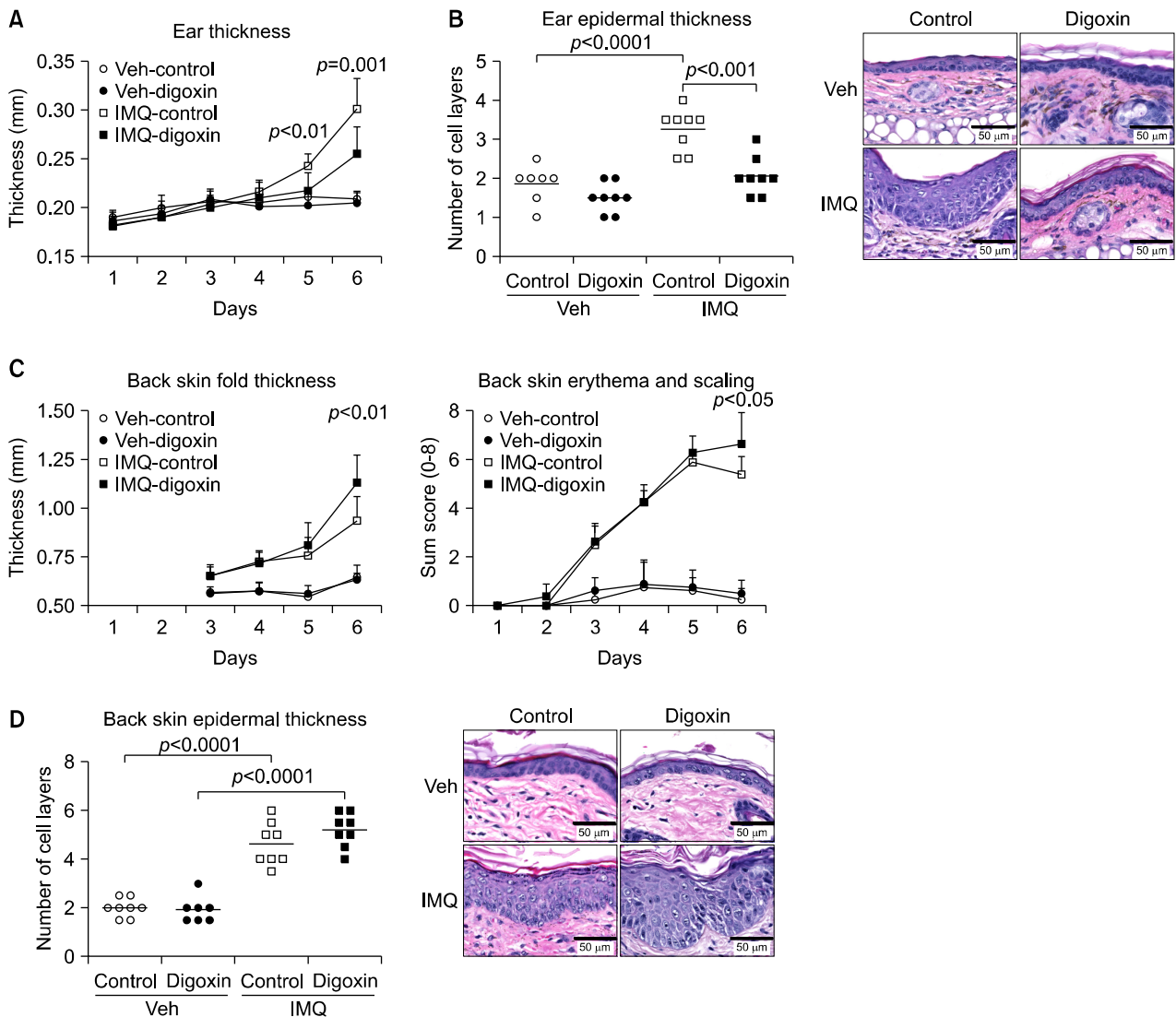


Fig. 1. Differential effects of digoxin on imiquimod (IMQ)-induced psoriasis-like skin inflammation on the ear and back. IMQ-induced skin thickening was assessed in the ear (A, B) and in the back skin (C, D). Thicknesses of the ear (A) and the back skin fold (C; left panel) were measured daily with a micrometer, from day 1 or 3 (A and C, respectively) until day 6. The back skin was visually scored for erythema and scaling each on a scale from 0~4 and the sum score (0-8) is presented (C; right panel). Values are presented as mean \pm standard deviation. Mean numbers of epidermal cell layers in the ear (B) and back skin (D) were assessed in H&E stained sections. Representative photos are also displayed (scale bar: 50 μ m). Statistics were performed using two-way ANOVA with Tukey's correction for multiple comparisons. Due to technical difficulties, data from one mouse are missing from the vehicle cream (Veh)-control group (B) and from the Veh-digoxin group (D), respectively.

for ROR γ t inhibition for treatment of psoriasis^{4,6}. The cardiac glycoside digoxin, which has been used for centuries to treat congestive heart failure and atrial fibrillation, binds to ROR γ t which leads to selective suppression of Th17 cell differentiation and IL-17 production *in vitro*, with therapeutic effects of digoxin demonstrated in mouse models of IL-17-dependent disease, e.g., experimental autoimmune encephalomyelitis and atherosclerosis, respectively^{7,8}. To our knowledge, the effects of digoxin in models of psoriasis have not been reported and

the aim of the current study was to investigate whether digoxin affects development of IMQ-induced psoriasis-like ear and back skin lesions in mice.

A detailed description of study materials and methods can be found in Supplementary File 1. In brief, at 8 weeks of age, female C57Bl/6J mice received topical Aldara (5% IMQ) or vehicle cream daily for 5 days (45 mg on the shaved back [1 \times 2 cm area] and 5 mg on the right ear). All mice were treated with either intraperitoneal (i.p.) digoxin (20 μ g/mouse on day 1, 10 μ g/mouse on day 3, and 20

Table 1. Effects of digoxin on plasma concentrations of IL-17A and SAA and on spleen weight and BW

	Total (n)		Control	Digoxin
IL-17A (pg/ml)	32	Veh	5.36 ± 2.06	6.05 ± 2.28
		IMQ	19.05 ± 5.22**	31.60 ± 13.29****, †
SAA (μg/ml)	28	Veh	38.8 ± 44.5	13.6 ± 5.6
		IMQ	171.2 ± 111.1*	221.6 ± 98.8***
Spleen weight (mg/g BW)	30	Veh	3.21 ± 0.74	3.14 ± 0.60
		IMQ	8.06 ± 1.00****	7.71 ± 0.62****
BW (% of weight on day 1)	31	Veh	100.13 ± 2.00	101.73 ± 1.95
		IMQ	102.34 ± 3.31	101.90 ± 4.56

Presented data were collected at study termination in mice receiving topical IMQ or Veh applied to one ear and the back skin. Values are presented as number only or mean ± standard deviation. Statistics were performed with two-way ANOVA using Tukey's multiple comparisons. In analyses where total n was not 32 (n=8 in each group), this was due to technical difficulties. The minimal detection limit for the IL-17A assay was 5 pg/ml, but although the measured IL-17A levels were below 5 pg/ml in 6 out of 16 mice receiving vehicle cream, these data were included in the two-way ANOVA. IL: interleukin, SAA: serum amyloid A, BW: body weight, IMQ: imiquimod, Veh: vehicle. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ Veh-control vs. IMQ-control or Veh-digoxin vs. IMQ-digoxin; † $p < 0.01$ IMQ-control vs. IMQ-digoxin.

μg/mouse on days 4 and 5) or saline (control group). The digoxin dose was somewhat lower than dosages (20~40 μg/d) previously used in other Th17-dependent disease models ameliorated by digoxin^{7,8}, but we found that the mice were in marked acute distress after the first dose, wherefore treatment was avoided on day 2. The mice (n=32; 8 mice/study group) were sacrificed on the 6th day, 24 hours after the last cream application and i.p. treatment. All experiments were performed according to the principles stated in the Danish law on animal experiments and were approved by the Animal Experiment's Inspectorate, Ministry of Justice, Denmark (no. 2012-15-2934-00119). The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the European Parliament (EU directive 2010/63/EU). The ethical policy of the University of Copenhagen complies with that of the National Institutes of Health (A5846-01).

In mice receiving topical application of vehicle cream, there were no discernible skin lesions and digoxin had no significant effects on measured variables (Fig. 1, Table 1). Digoxin attenuated the IMQ-induced ear thickening at days 5 and 6 (day 6: 0.26 vs. 0.30 mm, $p = 0.001$; Fig. 1A). Histological evaluation showed an inhibitory effect of digoxin on IMQ-induced epidermal hyperplasia in the ear (2.1 vs. 3.3 epidermal cell layers, $p < 0.001$; Fig. 1B). However, digoxin treatment increased the IMQ-induced back skin thickening at day 6 (1.13 vs. 0.94 mm, $p < 0.01$; Fig. 1C), and increased erythema and scaling on the back skin (sum score 6.6 vs. 5.4, $p < 0.05$; Fig. 1C). Also, there was no statistically significant effect of digoxin on IMQ-induced epidermal hyperplasia in the back skin (Fig. 1D). IMQ application had systemic inflammatory effects with induction of splenomegaly, as well as increased plasma

levels of IL-17A and the acute-phase protein, serum amyloid A (SAA) (Table 1). Surprisingly, in mice receiving topical IMQ, digoxin administration led to increased plasma concentrations of IL-17A (31.60 ± 13.29 vs. 19.05 ± 5.22 pg/ml, $p < 0.01$) whereas plasma levels of SAA, IMQ-induced splenomegaly, and body weight at study termination were not affected by digoxin (Table 1).

Our data showed that digoxin exerted differential effects on IMQ-induced psoriasis-like skin lesions at different anatomical locations, i.e., with reduction of skin lesion formation on the ear and exacerbation of back skin lesions, respectively. Interestingly, a similar differential effect on ear and back IMQ lesions in mice has been observed after topical treatment with sphingosine-1-phosphate (a cell growth and immune modulator)⁹. This may relate to differences of target tissues (thinner skin, less hair follicles, minimal subcutaneous adipose tissue, and diverse lymph drainage in ear vs. back skin), the IMQ application procedure (shaving vs. no shaving before application) and IMQ dose (5 vs. 45 mg Aldara), respectively. Also, digoxin bioavailability may differ at the two sites and future studies should include local assessment of IL-17A and ROR γ t expression, Th17 cell accumulation, and kinetics of radio-labelled digoxin, respectively, in addition to experiments with other ROR γ t inhibitors^{4,6}. We unexpectedly found increased plasma IL-17A levels which is in contrast to reduced levels reported in hyperlipidemic apoE-deficient mice treated with digoxin for 12 weeks, but the relationship between target tissue and systemic levels of IL-17A in experimental models of disease remains poorly defined⁸. Importantly, off-target effects of digoxin may play a role since in addition to ROR γ t inhibition the agent inhibits the sodium potassium adenosine triphosphatase (Na⁺/K⁺

ATPase) present in all cells and mainly in the heart. Indeed, systemic treatment with novel potent selective ROR γ t inhibitors appear to suppress IMQ-induced skin inflammation on both the ears and back of mice, albeit that there are no reports on simultaneous effects of these ROR γ t inhibitors on both anatomical locations in the same animals^{4,6}. In addition, it was recently reported that development of psoriasiform lesions after topical IMQ application in mice was dependent on unintended oral IMQ ingestion and it is possible that some of the systemic effects observed in our study were elicited by systemic IMQ uptake rather than skin-driven inflammation¹⁰.

The mechanisms responsible for differential effects of digoxin on IMQ-induced psoriasis-like skin inflammation on the ear and back in mice clearly require more study, but our results underline the complexity of the IMQ model and support caution in extrapolation of results of targeted interventions in this model to human psoriasis.

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SUPPLEMENTARY MATERIALS

Supplementary data can be found via <http://anndermatol.org/src/sm/ad-30-485-s001.pdf>.

CONFLICTS OF INTEREST

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