INVESTIGATIVE REPORT

Experimental *Candida albicans* Lesions in Healthy Humans: Dependence on Skin pH

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The addition of suspensions of Candida albicans cells under occlusion to the left and right forearms, buffered at 2 different pH levels (6.0 and 4.5), resulted after 24 h in unilateral or bilateral lesions in 14 of 15 volunteers. The resulting skin-surface pH was 5.7 ± 0.3 and 5.1 ± 0.2, respectively. The lesions were more pronounced on the arm with the higher pH in all 14 subjects who reacted. In 11 cases, reactions occurred only on the arm with the higher pH. The pH-induced results are not due to inhibited growth of C. albicans. They may be due to a pH dependence of the yeast's virulence capacity and/or a modulation of the host's defence ability. The use of skin-occlusive products (e.g. dressings, diapers and panty liners) is known to raise skin pH and is associated with skin infections of C. albicans. An acidic buffer incorporated in such products could be a preventive measure for Candida-induced skin rash. *Key words: experimental infection; skin rash; skin-surface pH.*

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Several studies with experimentally induced *Candida albicans* infection in humans have shown that it is a predictable and reproducible test system. Maibach & Kligman observed about 1000 inoculations in approximately 150 subjects in their classic study (1). This test model was further refined (2). The model has been used in evaluating topical steroid–antibiotic combinations (3). An improved *in vivo* method for testing topical antimicrobial agents has also been described (4).

The original experimental model consists in principle of adding suspensions of $10^4 - 10^5$ cells of *C. albicans* to the skin and occluding it for 24 h (1). In 36–72 h small discrete pustules will emerge. A reaction occurs in 90–95% of subjects, proportional to the number of organisms added. The most important requirement for a successful reaction is occlusion. Unless the stratum corneum is water saturated, the organism cannot compete against the resident microflora.

The authors of the studies mentioned above consider the skin reactions to the experimentally induced *Candida* infection to be a biological contact dermatitis of the primary irritant type (1–3). However, the results of another study strongly suggest that contact sensitivity to *Candida* antigen plays an important role in causing the skin lesions (5). Delayed cutaneous hypersensitivity to *C. albicans* is so common in the adult population that testing with *C. albicans* antigen was proposed as a useful procedure for measuring the capacity of a person to manifest delayed-type hypersensitivity (6–8).

pH may be a factor of significance in the pathological status of *C. albicans in vivo*, but it does not exert its influence by affecting the growth of the fungus, *i.e.* the rate of increase of its biomass (9). Dimorphism, the ability of *C. albicans* to exist in a blastospore and a filamentous form, is reported to be influenced by pH (9, 10). The blastospore form is favoured by a slightly acidic pH and the filamentous form at neutral pH or above. Furthermore, it has been shown that microfilaments play an important role during pH-regulated morphological transition (11). Acid proteinases may play a pathogenic role in *C. albicans* infections *in vivo* and they could act over a wide range of pH conditions (12, 13). Genes have been described that regulate morphogenesis of *C. albicans* to adapt to environments of diverse pH (14, 15). A gene encoding a pH-regulated antigen that may play a role in candidal infection was described recently (16). Clinical observations of diabetic patients, in whom a higher skin-surface pH is possibly correlated to candidal intertrigo, have been reported (17), but to the authors' knowledge pH-related effects in experimental cutaneous *C. albicans* lesions have not been reported.

The aim of this study was to determine whether different environmental pH would affect experimentally induced *Candida* lesions on the skin.

MATERIAL AND METHODS

Subjects

Fifteen female, healthy Caucasians participated (mean age 44.5, range 30–63 years). The study was approved by the local research ethics committee. All participants signed a letter of consent.

Microorganisms

The *C. albicans* strain used was type H29, kindly provided by Professor Lars Edebo, Department of Microbiology, Sahlgrenska University Hospital, Göteborg, Sweden. Cells were grown on Sabouraud's glucose agar at 37 C for 24 h before use.

Experimental patches

The patches were made of common diaper materials and punched out as circular disks with a diameter of 70 mm. The outer side of polyethylene and the inner side (which will be placed towards the skin) of non-woven polypropylene were glued together with a 10 mm edge around the inner circular core of 50 mm diameter. The inner core consists of approximately 0.85 g cellulose pulp and approximately 0.15 g of superabsorbent polymer (IM 7100 and E127/97; Clariant, Germany). The superabsorbent polymer is a polyacrylic acid which was adjusted to either pH 4.5 or 6.0. It acts as a strong buffering system.

pH

Skin-surface pH was measured with a Courage+ Khazaka PH900, Mettler-Toledo flat electrode 304. Every morning and afternoon the instrument was calibrated at pH 4.0 and 7.0. The instrument is accurate to 0.1 units. A few drops of deionized water were added to the electrode before measurement and the average of 3 values was recorded.

Experimental design

The skin microflora was analysed and the skin-surface pH was measured at the start. A modification of the Williamson–Kligman scrub technique was used for assessment of the resident skin microflora and the added *C. albicans*. A stainless-steel ring 2.6 cm in internal diameter covering a 5.5 cm^2 area of the skin was used. One millilitre of sterile 0.075 M phosphate buffer (pH 7.9) containing 0.1% Triton X-100 was poured into the ring, the skin rubbed gently with a blunt sterile glass rod for 1 min and the fluid removed with a Pasteur pipette (18). Serial dilutions were performed in phosphate-buffered saline, and samples from the dilutions were plated out on Sabouraud's glucose agar media and incubated at 37 C for 48 h for analyses of yeast, and on blood agar at 37 C for 24 h for analyses of bacteria.

Candida albicans (10^4 cells/ml) was suspended in a physiological saline solution. Seven millilitres of this solution were added to each patch, 15 min before it was attached to the forearm. The 2 types of patch of different pH value were taped to the volar aspects of the forearms, randomly on either the left or the right arm. The patches were worn under strict occlusion for 24 h. The patches were then removed, and after approximately 2 min the skin pH was measured. The skin was examined visually and samples were taken to determine the number of colony-forming units (cfu) of *Candida*. After 48 h, the pH measurements and *Candida* sampling were repeated and the skin lesions were visually assessed (no reaction, faint reaction, moderate reaction or strong reaction). The reading was blinded and the type of patch was unknown to the assessor.

Statistics

The paired *t*-test was used to test for statistical significance between pH differences and differences in the number of *Candida* organisms. The differences in observed skin lesions were tested by the sign test.

RESULTS

Skin-surface pH

The skin-surface pH of the 15 volunteers was measured at the start and varied between 4.5 and 6.0. The individual variations between the left and right arms were small, with a mean value of 5.2. The pH was then measured when the patches were removed after 24 h occlusion and finally 24 h later. The mean values ± SD (*n* = 15) are presented in Fig. 1. After 24 h occlusion the pH was 5.1 ± 0.2 and 5.7 ± 0.3 at the 2 respective sites (*p* ω 0.0001).

Visual skin lesions

The visual assessment of skin reactions gave a very clear result. The "acidic site" (pH 4.5) had less severe reactions than the "ref-site" (pH 6.0) in all 14 subjects who reacted. In 11 cases, reactions occurred only on the arm of the ref-site (pH 6.0). The sign test showed a statistically significant difference at *p* ω 0.001 for difference in reactions at the 2 sites.
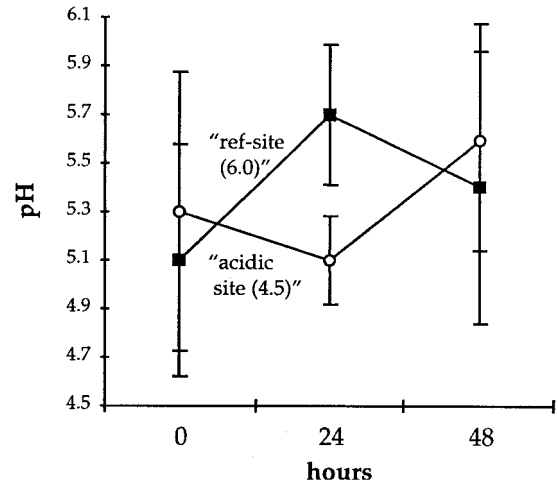

Fig. 1. Variations in skin pH at the start of the experiment, directly after patch removal following 24 h occlusion, and 24 h later. Values are means ± SD. Patches were buffered at 2 pH levels, the "acidic site" (pH 4.5) and the "ref-site" (pH 6.0).

Skin microflora and growth of Candida albicans

At the start of the trial, skin microflora was determined with the scrub-test. The overall conformity between the left and right arm was good. Coagulase-negative *Staphylococci* were found in all subjects, range 30–4500 cfu/cm^2. Only 2 individuals had *Diphteroid* sp., at densities of 440/220 and 9/15 cfu/cm^2, respectively. Two other persons had *Bacillus* sp. (220/160 and 73/9 cfu/cm^2 respectively) and none had *Staphylococcus aureus*.

No growth of *C. albicans* was found in any subject before the trial.

After 24 h, when the patches were removed, scrub-test samples were taken to assess the number of cfu/cm^2 of *C. albicans* on the skin. The acidic site (pH 4.5) had 180 ± 280 cfu/cm^2 (mean ± SD) and the ref-site (pH 6.0) had 150 ± 160 cfu/cm^2. No statistically significant difference (*p* = 0.64) was found between the 2 sites. Growth was found on both arms in 13 subjects. In 1 subject growth was found only on the acidic site (pH 4.5), and in another subject only on the ref-site (pH 6.0).

After 48 h, *i.e.* 24 h after the patches had been removed and the lesions had become visually evident, 9 subjects had no growth at all of *C. albicans*. Three subjects showed low numbers (1.8–24 cfu/cm^2) on the arm with the ref-site (pH 6.0) and the lesion pattern was stronger on this arm. One subject had an increased growth since the day before on both arms (250 and 780 cfu/cm², respectively), but only lesions on the arm with the ref-site (pH 6.0) (the arm with the lowest number of cfu). Two subjects were missing on this occasion, so no microbial data could be obtained.

DISCUSSION

The main purpose of the study was to investigate the effect of pH on the skin reactions induced by *C. albicans*, in a disposable diaper system. The visual assessment clearly verified pronounced reactions on the ref-site (pH 6.0). It was not considered necessary to include control patches, not inoculated with *C. albicans*, as the reaction pattern, typical for

the yeast with papules and pustules, was easily recognized, and there are years of experience with the diaper material under different conditions buffered at the 2 pH levels under investigation.

No correlation between the start pH of the skin and the severity of the reaction could be seen; however, the only person who did not react was the one with the lowest skin-surface pH at start.

Earlier studies have shown that the severity of the skin response to *C. albicans* is dependent on the initial number of organisms added (1, 2). In another study with experimental addition of *C. albicans* to people of different racial origin, black skin harboured a higher population of *C. albicans*, but reacted with fewer skin lesions than white skin (19). In the present study, the lower pH did not lead to a reduced number of organisms on the skin. Both sites showed a similar number of *C. albicans*, but a clear difference in reaction. Thus, the reduced reaction response is not due to an inhibited growth of the organisms.

The morphology of *C. albicans* was not investigated in this study. However, it is known from the literature that pH influences the dimorphism of *C. albicans*. An acidic pH favours the blastospore form and a higher pH the mycelial form (9, 10). It is known from clinical practice that the mycelial form is associated with pathogenicity of *C. albicans* and this has been verified in clinical studies (9, 20, 21).

Other mechanisms may be directly or indirectly dependent on pH. pH may act by influencing the properties of the yeast, making it more or less virulent, or it may act on the host's defence system. Support for the latter is given in studies describing how acidic skin-surface pH promotes barrier function and combats infection (22, 23). It is reported that the immunological defence against *C. albicans* is primarily cell mediated and that *C. albicans* appears capable of sensing the state of the immune system and responds accordingly (24).

It is also believed that contact sensitivity to *Candida* plays an active role in the response to cutaneous infection by *C. albicans* (5). It was beyond the scope of the present study to determine the nature of the response mechanism. However, some observations in connection with the study and in the preliminary pilot tests indicate an immunological response. After the skin lesions had healed, 1 subject was challenged with *C. albicans* again and showed a strong response reaction, although the challenge was for less than 1 h and non-occlusive. The skin lesions of 2 subjects in this study did not recover completely in 5 weeks, despite both antifungal and potent steroid therapy, but complete recovery was seen in a check-up approximately 4 weeks later.

The present study design originated from earlier mentioned studies (1–3), but it differed in some respects. The other studies were performed on male subjects, whereas this study involved females only. This difference could be of importance, since women in general have been more exposed than men to *Candida* through both gastrointestinal and vaginal colonization.

The *Candida* suspension was added to water-absorbent materials, whereas the other studies used only a plastic film to cover the suspension. The main absorbent constituent of the patches used here is a superabsorbent polymer, which is also the necessary component enabling the pH in the system (patch and skin) to be controlled. The 2 superabsorbent polymers have been assessed in a repeated insult test and found to be non-irritant to the skin (data on file, SCA Hygiene Products, September 1997). The water-absorption capacity of the superabsorbents varies depending on pH, with the acidic one having a lower capacity. The same amount of liquid (7 ml) was added to both the acidic patch (pH 4.5) and the reference patch (pH 6.0).

The association of *C. albicans*-induced skin rash with diapers has been described (25). It was found that *C. albicans* is commonly present both on the skin and in the faeces of subjects with clinically overt candidiasis of the napkin area. *Candida albicans* has previously been reported as a secondary invader to the already initiated dermatitis (26). However, since *C. albicans* easily induces cutaneous lesions when applied under occlusion to the skin, it is reasonable to believe that the yeast can occasionally be the primary source of diaper dermatitis (25).

A practical implication of the influence of pH on *Candida*-induced skin rash could be that for skin-occlusive products there is a new alternative to reduce the risk of skin rash. When it is not possible or suitable to eliminate the yeast with antimycotics, or to reduce the humidity sufficiently, an acidic buffer could be incorporated into the products as a preventive measure. Examples of products to be considered may include wound dressings, surgical tapes, diapers, sanitary towels and panty liners.

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