In vitro cytotoxicity testing of a nanocrystalline silver dressing (Acticoat) on cultured keratinocytes

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ABSTRACT

Acticoat is a polyethylene mesh coated with nanocrystalline silver. It has been used widely as a dressing for chronic wounds, acute partial-thickness burn wounds and donor sites. In this study, the *in vitro* cytotoxicity of Acticoat on cultured keratinocytes is tested. Human keratinocytes are cultivated on a pliable hyaluronate-derived membrane (Laserskin) using dermal fibroblasts as the feeder layer. When the cultured Laserskin (CLS) is sub-confluent it is covered by Acticoat, which is exposed to water (Group 1), phosphate-buffered saline (Group 2) or culture medium (Group 3). The control group is not exposed to the Acticoat. After 30 minutes incubation at 37°C, the inhibitory effect of the nanocrystalline silver on keratinocyte growth is measured by an MTT assay. Compared with the control, the relative viability of the CLS dropped to 0%, 0% and 9.3%, respectively. Thus, Acticoat is cytotoxic to cultured keratinocytes and should not be applied as a topical dressing on cultured skin grafts.

KEY WORDS: Cytotoxicity. Keratinocytes. Silver.

Introduction

Skin is the body's protective layer against a hostile external environment. Damage to this natural barrier as a result of thermal injury results in bacterial colonisation and an imbalance of homeostasis leading to significant morbidity and mortality.

Early excision of the burned tissues and skin grafting of the exposed areas has proved to be an effective means of reducing morbidity and shortens hospitalisation.[1] The cultured epidermal autograft (CEA) provides an alternative epidermal covering for major burns victims whose donor sites are limited. The CEA and other human skin substitutes comprise merely a few layers of keratinocytes and are fragile and vulnerable to bacterial infection.

The routine use of systemic antibiotics is usually not effective because of the blockage to the microcirculation by coagulation that follows thermal injuries. The delivery of systemic antibiotics to the wound is further reduced by inflammatory oedema and tissue ischaemia. Therefore, topical agents that can attain a high therapeutic level are usually used to control bacterial colonisation in the treatment of burns.

Silver and its compounds have a long history of use as astringents and antiseptics in human healthcare.[2,3] The US Food and Drug Administration approved silver ion solutions as antimicrobial agents in 1920s. Its wide-spectrum activity against Gram-positive and Gram-negative organisms, the lack of acquired antimicrobial resistance and the low incidence of local or systemic sensitivity are several advantages to the use of silver as a topical agent for skin grafts and donor sites.[4–6]

In addition to argyria, silver nitrate solution is toxic to cells on the wound surface because nitrate is a potent oxidising agent. Of the silver compounds available, silver sulphadiazine (SSD) is the most commonly used topical agent for burn wounds. However, it is cytotoxic to cultured skin grafts.

Acticoat is a recently introduced topical dressing material that delivers silver to the surface of the wound.[7,8] It comprises a nylon/polyester core between two layers of high-density polyethylene mesh, and incorporates nanocrystalline silver by means of a patented physical deposition technique. Ionic silver and silver radicals are released in a high concentration when Acticoat is exposed to water. The nanocrystalline silver in Acticoat provides strong antimicrobial activity within 30 min of application to the wound surface. Acticoat is proven to be an effective dressing for chronic wounds, acute partial-thickness burn wounds and donor sites.[9] However, the biocompatibility of Acticoat with CEA or other human skin substitute has not been investigated.

In this study, the *in vitro* cytotoxicity of the nanocrystalline silver of Acticoat on cultured keratinocytes is evaluated by MTT (3-[4,5-dimethylthiazol-2yl]-2,5-diphenyl-tetrazolium bromide) assay.

Materials and methods

Cultured Laserskin

Laserskin (Fidia Advanced Biopolymers, Abano Terme, Italy) is a benzyl-esterified hyaluronate derivative. It acts as a scaffold on which keratinocytes can proliferate.

With full informed consents from the patients involved, keratinocyte and dermal fibroblast cultures were established from foreskin tissue removed during circumcision. After overnight trypsination at 4°C, the epidermis was separated

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from the dermis. The isolated epidermal cells were cultivated on mitomycin C-treated 3T3 cells.[10] Keratinocytes were maintained in Dulbecq's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), epidermal growth factor (10 ng/mL), cholera toxin (10 ng/mL) and insulin (5 µg/mL). The 3T3 cells are a mouse fibroblast cell line which is transformed by two different viruses.[11]

Secondary keratinocyte culture was seeded on pieces of Laserskin (2.5 cm x 3.3 cm) using human dermal fibroblasts as a feeder layer.[12] The dermal fibroblasts were established from the dermis of the foreskin and cultivated with DMEM supplemented with 10% FBS.

Acticoat cytotoxicity testing

When the keratinocytes on Laserskin achieved sub-confluence, the cultured Laserskins (CLS) were divided into four groups, and three of the four were covered with Acticoat (Smith & Nephew, Canada). The Acticoat was moistened with water (Group 1), phosphate-buffered saline (PBS; Group 2) or culture medium (Group 3). The control group was not exposed to Acticoat.

After incubation for 30 min at 37°C in 5% CO_2, the Acticoat was removed. The viability of CLS was measured by an MTT assay, as described previously.[13] The CLSs were placed individually in a six-well cluster plate containing 1.3 mL MTT (2 mg/mL) solution in each well. After 4 h incubation at 37°C, the MTT was aspirated. Dimethyl sulphoxide (DMSO; 1.3 mL) was added and the plate was mixed on an orbital shaker for 30 min. Absorbance (A) of the resulting supernatant was measured at 650 nm. Plain Laserskin similarly treated with MTT and DMSO was used as a reference blank. Tests were run in triplicate.

Results

The relative cellular viability of the CLS was the ratio of its A (Groups 1, 2 and 3) to that of the control group. Acticoat exerted a severe cytotoxic effect on keratinocytes. Those in Groups 1 and 2 (delivered by water and PBS, respectively) were killed by the nanocrystalline silver. In group 3, only 9.3% of the keratinocytes survived when the nanocrystalline silver was delivered by culture medium.

Discussion

Thermally coagulated burn eschar provides an optimal environment for bacterial colonisation. The clinical efficacy of systemic prophylaxis is limited by the avascularisation of the eschar. In the 1960s, Fox[14,15] introduced SSD as a topical burns therapy. It was synthesised from silver nitrate and SSD, and complexed to propylene glycol, stearyl alcohol and isopropyl myristate. The bacteriostatic and bactericidal activities of SSD are attributed to its inhibition of DNA replication.[16]

When SSD reacts with cellular DNA it dissociates and the silver ions combine with the DNA; thus, DNA replication is hindered. Cell growth will resume at a normal rate when the ratio of silver to DNA falls below a critical level. The take-up of silver ions into bacteria also affects the oxidation of glucose, glycerol, fumarate, succinate and lactate in the respiratory process. The silver reacts with the respiratory chain at the cytochromes and in the NADH-succinate dehydrogenase region.[17] Silver sulphadiazine shows a wide spectrum of activity against *Pseudomonas aeruginosa* and other Gram-negative organisms. Bacterial resistance during therapy has not been reported.

Considerable efforts have been made to improve the take rate of CEA. Bacterial colonisation in the wound is one of the major causes of failure of CEA to engraft. Unlike native skin grafts, the incomplete epidermal barrier and lack of vascular and immune components make all models of cultured skin graft more susceptible to wound infection. Moreover, the organelles of cultured skin grafts are more primitive than those of natural skin or split-thickness skin grafts.[18]

These biological deficiencies of cultured skin grafts make them more susceptible to the cytotoxicty of topical agents. Thus, an ideal topical agent or dressing for burn wounds should maintain the cultured keratinocytes in a viable state and free from infection. Commonly used topical agents such as modified Daiken's solution, chlorhexidine, providone-iodine solution, SSD and silver nitrate solution were found to be cytotoxic.[19–21] After 15-min exposure to 0.05% silver nitrate solution and 0.03% SSD in microsuspensions, cultured keratinocytes demonstrated mean survivals of 7.6% and 47%, respectively, when determined by flow cytometry.[21] The therapeutic levels of these two silver compounds are 0.5% and 1%, which are much higher than the concentrations tested in the *in vitro* cytotoxicity experiments.

Acticoat consists of nanocrystalline silver coated on polyethylene mesh by a patented process. Owing to the greater surface area on the mesh, Acticoat can provide a faster release of silver. Compared with CEA, the cultivation of keratinocytes on Laserskin offers a definite advantage for cytotoxicity testing of dressing material. As the CLS is pliable, dispase treatment to harvest the graft is not required. The damaging effect of dispase treatment on CEA may be variable and difficult to control.

The MTT assay is used widely for the measurement of *in vitro* growth of cancer cell lines after exposure to anticancer drugs.[22] Mitochrondrial succinate dehydrogenase in viable cells reduces the MTT, whereas there is no reduction of MTT by the dead cells.

In vitro study showed that Acticoat can attain its full antimicrobial activity within 30 min. Therefore, the cultured Laserskin was exposed to Acticoat for 30 min in the experiments reported here. Although Acticoat generated a local concentration of silver of 0.005% to 0.01%,[7] which were much lower than toxic levels (0.03% to 0.05%) reported elsewhere,[21] a severe inhibitory effect on keratinocyte growth on Laserskin was observed.

No keratinocytes survived in Groups 1 and 2. Thus, the nanocrystalline nature of Acticoat produced a significant detrimental effect on bacteria as well as normal cells. In Group 3, some cells (9.3%) survived when the nanocrystaline silver was delivered by culture medium. The viability of CLS in this group was greater than that seen in Groups 1 and 2, which was expected because the bactericidal activity of silver drops in the presence of the chloride, phosphate and protein anions that produce an insoluble salt with silver.[16]

In conclusion, Acticoat is cytotoxic to cultured keratinocytes and should not be applied clinically as a topical dressing on cultured skin grafts.

Reference

1 Deitchy EA. A policy of early excision and grafting in elderly burn patients shortens the hospital stay and improves survival. *Burns* 1985; **12**: 109–14.

2 Klasen HJ. Historical review of the use of silver in the treatment of burns. I Early uses. *Burns* 2000; **26**: 117–30.

3 Klasen HJ. Historical review of the use of silver in the treatment of burns. II Renewed interest for silver. *Burns* 2000; **26**: 131–8.

4 Fuller FW, Parrish M, Nance FC. A review of dosimetry of 1% silver sulfadizine cream in burn wound treatment. *J Burn Care Rehabil* 1994; **15**: 213–23.

5 Stern HS. Silver sulphadiazine and the healing of partial thickness burns: a prospective clinical trial. *Br J Plast Surg* 1989; **42**: 581–5.

6 Wassermann D. Schlotterer M, Lebreton F, Levy J, Guelfi MC. Use of topically applied silver sulphadizine plus cerium nitrate in major burns. *Burns* 1989; **15**: 257–60.

7 Tredget EE, Shankosky HA, Groeneveld A, Burrell R. A matched-pair, randomized study evaluating the efficiacy and safety of Acticoat silver-coated dressing for the treatment of burn wounds. *J Burn Care Rehabil* 1998; **19**: 531–7.

8 Yin HQ, Langford R, Burrell RE. Comparative evaluation of the antimicrobial activity of ACTICOAT antimicrobial barrier dressing. *J Burn Care Rehabil* 1999; **20**: 195–200.

9 Yin HQ, Langford R, Tredget EE, Burrell RE. Effect of Acticoat antimicrobial barrier dressing on wound healing and graft take. *J Burn Care Rehabil* 1999; **20**: S231.

10 Slacker KL, Williams ML, Goldyne M. Mitomycin C-treated 3T3 fibroblasts used as feeder layers for human keratinocyte culture retain the capacity to generate eicosanoids. *J Invest Dermatol* 1987; **89**: 536–9.

11 Todato GJ, Habel K, Green H. Antigenic and cultural of cells doubly transformed by polyoma virus and SV40. *Virology* 1965; **27**(2):179–85.

12 Lam PK, Chan ESY, To EWH, Lau CH, Yen SC, King WWK Development and evaluation of a new composite laser skin graft. *J Trauma* 1999; **47**: 918–22.

13 King WWK, Lam PK, Li AKC. Anti-proliferative activity of interferon-a on human squamous carcinoma of tongue cell lines. *Cancer Lett* 1994; 85: 55–8.

14 Stanford W, Rappole BW, Fox CL. Clinical experience with silver sulphadiazine. *J Trauma* 1969; **9**: 377–88.

15 Fox CL. Silver sulphadiazine: a new topical therapy for pseudomonas in burns. *Arch Surg* 1968; **96**: 184–8.

16 Russell AD, Hugo WB. Antimicrobial activity and action of silver. *Prog Med Chem* 1994; **31**: 351–70.

17 Williams RL, Doherty PJ, Vince DG *et al*. The biocompatibility of silver. *Critical Review Biocompatibility* 1989; **5**: 221–43.

18 Monafo W, Freedman B. Topical therapy for burns. *Surg Clin North Am* 1987; **67**: 133–6.

19 Boyce ST, Warden GL, Holder IA. Cytoxicity testing of topical antimicrobial agents on human keratinocytes and fibroblasts for cultured skin grafts. *J Burn Care Rehabil* 1995; **16**: 97–103.

20 Cooper ML, Boyce ST, Hansbrough JF, Foreman TJ, Frank DH. Cytotoxicity to cultured human keratinocytes of topical antimicrobial agents. *J Surg Res* 1990; **48**: 190–5.

21 Smoot EC, Kucan JO, Roth A, Mody N, Debs N. *In vitro* toxicity testing for antibacterials against hyuman keratinocytes. *Plast Reconstr Surg* 1991; **87**: 917–24.

22 Mosmann T. Rapid colorimetric assay for cellular growth and survival: application of proliferation and cytotoxicity assays. *J Immunol Methods* 1983; **65**: 55–63.