Research Article

Therapeutic Paint of Cidofovir/Sucralfate Gel Combination Topically Administered by Spraying for Treatment of orf virus Infections

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Abstract. The aim of the research was to study a new cidofovir/sucralfate drug product to be used as a spray for treating the mucosal and/or skin lesions. The product, i.e., a water suspension of sucralfate (15% w/w) and cidofovir (1% w/w), combines the potent antiviral activity of the acyclic nucleoside phosphonate cidofovir ((S)-1-[3-hydroxy-2-(phosphonomethoxy)propyl]cytosine) and the wound healing properties of sucralfate gel (sucrose octasulphate basic aluminum salt). The product was characterized *in vitro* with respect to compatibility between drug and carrier, spray particle size, spray deposition, drying kinetics, and drug content and release. An interaction between the two active substances was found. The interaction between sucralfate and cidofovir was counteracted by introducing sodium dihydrogen phosphate (16% w/w) in the preparation. The spray formulation containing cidofovir/sucralfate gel painted the skin and dried quickly to a scab, remaining firmly adhered to the lesions. The therapeutic paint was tested *in vivo* on lambs infected with orf virus by treating the animals with different cidofovir/sucralfate formulations (0.5% or 1% cidofovir+sucralfate 15% + NaH₂PO₄ 16% w/w) and with sucralfate gel suspension alone as control. The treatment with formulations containing cidofovir and phosphate salt for four consecutive days resulted in a rapid resolution of the lesions, with scabs containing significantly lower amounts of viable virus when compared with untreated lesions and lesions treated with sucralfate suspension alone.

KEY WORDS: cidofovir; orf virus; skin infections; sucralfate; therapeutic paint; topical.

INTRODUCTION

Cidofovir ((S)-1-[3-hydroxy-2-(phosphonomethoxy)propyl]cytosine) is a drug used for the topical therapy of skin infections caused by DNA viruses (1-3). The activity of cidofovir against orf virus has already been demonstrated *in vitro* (4), *ex vivo* (5) in previous studies and in clinical treatments of human orf infections, being used, in that instance, as 1% w/v preparation in Beeler base cream (6). Orf virus is the prototype of the Parapoxvirus genus and it is the causal agent of a worldwide contagious skin infection of sheep and goats known as contagious ecthyma, contagious pustular dermatitis, or scabby mouth. The virus infects via broken, scarified, or otherwise damaged skin and replicates in epidermal cells following direct contact with infected animals

or with contaminated fomites. Three to four days post-infection the lesions become evident and progress from the stages of erythema to pustule and resolve with the formation of scabs. Scabs contain millions of virus particles which, when they dry up and drop off the animal, contaminate the environment. The disease has a considerable economic impact on the agricultural sector, particularly in the third world where it is regarded as one of the top 20 most important viral diseases affecting the welfare of farmed sheep and goats (7). Transmission of orf virus to humans may occur after contact with infected or recently vaccinated animals and/or fomites in conjunction with skin trauma (8). No drug products are currently marketed for the treatment of this infection and vaccination represents the only option to limit the severity of the disease (9,10).

Recently, the efficacy of semisolid formulations, namely ointment and cream, for the topical treatment of orf virus experimentally infected lambs has been demonstrated (11). Despite their efficacy on localized lesions, semisolid preparations are inconvenient when a large number of animals needs to be treated, as is the case when entire sheep flocks are affected. Thus, an improved method for delivering the drug suitable for use and easy to administer on the farm is required. Such a method has to cover the skin or mucosa and to stay in contact with the lesions, resisting the mechanical stresses due to animal activity. Paradoxically, this

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formulation must be thick enough for adhesion to the tissue but fluid enough to be applied easily. Thus, a formulation meeting those requirements and that could be applied by spray rapidly drying after application, was considered to be the ideal solution.

Sucralfate, the basic aluminum salt of sucrose octasulphate, is a safe and effective drug administered by the oral route in the treatment of gastric and duodenal ulcers (12). The efficacy of a new sucralfate gel formulation on wound healing has also been demonstrated (13,14). This sucralfate gel dispersed in water appears as a white paint exhibiting a thixotropic behavior of suspension i.e., thick at rest and fluid after agitation. The gel form of sucralfate exhibits a very strong adhesiveness to mucosa and skin. It has been used as the base for beauty mask formulation (15), but has the potential to be an effective base for the formulation of a therapeutic paint (16,17).

Therefore, the aim of present work was to study a new cidofovir/sucralfate drug product for use as a spray for treating the mucosal and/or skin lesions. Due to the bioadhesiveness of sucralfate gel, it was thought that its combination with cidofovir would be ideal for maintaining contact between the antiviral drug and the skin and/or mucosa even during normal animal activity. The product, named therapeutic paint, was characterized *in vitro* with respect to compatibility between drug and carrier, spray particle size, spray deposition, drying kinetics, and drug content and release. The therapeutic paint was then tested *in vivo* on lambs experimentally infected with orf virus in order to evaluate the behavior and efficacy of cidofovir administered in this manner.

MATERIALS AND METHODS

Materials

Cidofovir was kindly donated by Gilead (Lot. 1966-C-1, Foster City, USA). Sucralfate humid gel raw material (21% w/w sucralfate content) was a gift of Lisapharma S.p.A., Erba, Italy (Lot. 01090312, Eutichals, Lodi, Italy). Sodium dihydrogen phosphate was purchased from Fluka (Buchs, Switzerland). Water, methanol, and other solvents were of analytical grade.

Methods

Formulation Preparation

Spray formulations containing sucralfate 15% w/w, cidofovir 0.5% or 1% w/w, and sodium dihydrogen phosphate 6%, 11%, or 16% w/w were prepared by diluting sucralfate humid gel raw material with aqueous solutions containing the antiviral drug and the salt. The white mixture was stirred for 1 h using an Ultraturrax homogenizer (11,000 rpm; T25B, Ika-Werke, Staufen, Germany).

For determining the dissolved cidofovir content, suspension was centrifuged (5,500×g, 5 min) and cidofovir concentration in the supernatant was measured by high-performance liquid chromatography (HPLC) (Agilent 1100 apparatus, Agilent, Waldbronn, Germany) using the following conditions: column Alltech RP C8 Mixed Mode 4.6×250 mm (Alltech, Milan, Italy); mobile phase, 50 mM sodium dihydrogen phosphate pH 4.4/acetonitrile (85:15); flow rate 1 ml/min; and

detector wavelength 278 nm. The method was linear from 0.99 to 99 µg/ml, with a precision of 0.7% RSD and a limit of quantitation of 0.16 µg/ml.

As reference formulation, a semisolid preparation containing cidofovir 1% w/w in Beeler base cream was used because it had been employed in a previous assessment of the anti-viral drug (2,11).

Formulation Characterization

A Jasco FT-300E apparatus (Tokyo, Japan), supported by Jasco FT software, was used for Fourier transform infrared (FTIR) spectroscopy. Spectra were collected in the 4,000–650 cm⁻¹ wavenumber range. In the case of the sucralfate gel suspensions, the samples were completely dried in an oven at 50°C and mixed with KBr in ratio 1:10. The mixture was then compressed with a hydraulic press to obtain a thin disc.

Rheology measurements on sucralfate gel suspensions were performed at 25°C with a Rheostress 600 Haake (Henko, Spinea, Italy) equipped with a C35 cone-plate measurement system (35 mm Ø, 1° angle).

The particle size distribution of sucralfate gel raw material and formulations was evaluated using laser diffraction-based particle size analyzer (Mastersizer X, Malvern Instruments Ltd., Malvern, UK). Measurements were performed by direct dilution under mechanical stirring of 0.5 ml of the suspensions in approximately 100 ml of water (freshly distilled, degassed, and filtered on 0.45 µm cellulose acetate filters) using the Small Volume Dispersion Unit (Hydro SM, Malvern Instruments Ltd., Malvern, UK).

Cidofovir release from formulations was studied *in vitro* using Franz type diffusion cells equipped with a membrane of regenerated cellulose cut from a dialysis tubing membrane as barrier (MW cut-off 12,000–14,000 Da, tubing width 32–34 mm, Dexstar Visking, Medicell International Ltd, London, UK). The tubing membrane was first boiled in distilled water for 30 min in order to remove air from the pores and then cut into square-shaped pieces of about 5.8 cm² in area that were inserted between donor and receptor compartments (0.58 cm² permeation area). The receptor compartment was filled with degassed distilled water and the assembled system was allowed to equilibrate at 37°C for half an hour. Then 0.5 g of each test formulation (corresponding to 5 mg of cidofovir) was introduced into the donor compartment, which was sealed with a screw cap to prevent evaporation. The receptor compartment was magnetically stirred to avoid any boundary layer effects. All experiments were carried out over a 3-h period of time. At predetermined time-points, samples were withdrawn from the receptor solution analyzed by HPLC for cidofovir content.

For spraying the formulation onto the skin during the *in vivo* trial, conventional commercially available trigger sprays (Yuyao Paojie Plastics Co Ltd, China; www.bj-sprayer.com) mounted on screw-capped amber glass bottles (25 mm neck diameter, 100 ml capacity) were used. An air peak flow rate during actuation of 0.1–0.2 l/min (Mass Flow meter, TSI, Shoreview, USA) and a nozzle of 0.5 mm characterized the sprayer.

After priming, the amount of suspension sprayed was measured by weighing (scale precision 0.1 mg) the device before and after ten consecutive actuations. Using a circular

piece of filter paper (23 cm² area) placed in front of the sprayer nozzle at a distance of 15 cm, the number of actuations and the amount of formulation required to coat the filter surface area was determined by weighing.

Spray droplet size was measured using a laser diffraction particle size analyzer (Mastersizer X, Malvern Instruments Ltd., Malvern, UK). The measurements were performed by placing the sprayer perpendicularly to the laser beam, at 15 cm from the beam and the lens, respectively. Aspiration (50 l/min) was placed directly at a distance of 30 cm in front of the sprayer, in order to maximize the amount of droplets crossing the laser beam.

The drying of spray formulation deposited was evaluated by spraying approximately 2 g of formulation on a Petri dish (8.5 cm diameter) and introducing it in an oven at 32±1°C. The weight loss was registered every 5 min.

In Vivo Experiments

The fully virulent IT 987/04 wild-type orf virus strain was used to infect orf virus naive lambs. The animals were raised in a containment facility at the Faculty of Veterinary Medicine, University of Bologna, Italy.

The animal studies were approved by the ethics and scientific committee of the University of Bologna and were conducted in accordance with Regulation 86/609/EEC, "Protection of animals used for experimental and scientific purposes".

The lambs were fed with ultra high temperature sterilized milk until 30 days of age, but from 14 days old until the end of the study, solid food and clean water were freely available at all times. Nine lambs were housed in three groups of three animals. The first group was spray painted with the suspension of sucralfate gel 15% w/w, the second group with a suspension of sucralfate gel 15% w/w containing cidofovir 0.5% w/w and NaH₂PO₄ 16% w/w and the third group with a suspension of sucralfate gel 15% w/w containing cidofovir 1.0% w/w and NaH₂PO₄ 16% w/w. All animals were infected by scarification (18) on the inner side of both hind thighs. The scarification sites on the right leg were subjected to treatment, while the virus scarification sites on the left legs were left untreated, so each animal served as its own control.

In vivo experiments were performed blind, with the identity of the treatments not revealed until the end of the trial. Starting from day 4 post-infection, each animal was treated once daily for four consecutive days covering the lesion completely with the spray formulation; lesions were not cleaned before each new treatment. The spraying was performed manually by positioning the tip of the sprayer at a 15 cm distance from the lesion and actuating the lever three times in order to paint the area affected. The preparation was sprayed on the lesions and the deposited material was left to dry for 1–2 min, before allowing the animal to move freely. The infected areas were examined daily for 28 days. The progression of the lesions was evaluated daily, by measuring the thickness of the skin at the site of lesions. Briefly, the thickness of the animal skin was measured by clipping it with a manual caliper and exerting a standard pressure across the line of scarification.

The scab material was collected from each animal and stored at -80°C. The effect of treatment on viral replication

was determined by monitoring the recovery of viable virus from the treated and untreated animals by *in vitro* virus growth and assessing the total amount of viral DNA in the samples using real-time Taqman[®] PCR (19).

For *in vitro* virus growth, 25 mg of pooled scab material taken from treated or untreated lesions were ground in 1 ml PBS containing 10% penicillin, streptomycin, and amphotericin B solution (Gibco, UK). The scab suspension was centrifuged at 2,000×g for 5 min and the clarified supernatant filtered through a 0.45 µm membrane before being used to inoculate primary lamb keratinocytes (PLKs). The cells were viewed microscopically after infection and the presence or absence of viral cytopathic effect was recorded. The viral titre was calculated by end point dilution assay. Virus titration was performed on monolayers of PLKs grown in 96-well plates. Viral suspensions were diluted from 10⁻¹ to 10⁻⁸ and 50 µl of each dilution was used to inoculate a separate series of five wells on the plate. Cultures were incubated at 37°C, 5% CO₂ and the cytopathic effect evaluated after 4 days. The final titre, expressed as 50% tissue culture infectious dose (TCID₅₀/ml), was calculated using the Reed and Muench method for the determination of the 50% endpoint.

The extraction of viral DNA from the supernatants derived from centrifuged pooled scab was performed using the easy DNA kit (Invitrogen, UK) following the manufacturer's instructions. The quantification of viral DNA was obtained by real-time polymerase chain reaction (PCR) performed using the Rotor-Gene 3000 system (Corbett Research, Australia). The absolute quantification of DNA is achieved by plotting tenfold dilutions of a standard plasmid containing a fragment of the orf virus B2L gene against the corresponding threshold cycle value.

Statistical Analysis

The data obtained from the daily trial scores were statistically analyzed by one-way ANOVA with Bonferroni's post-test, while the real-time PCR data were analyzed using ANOVA, Tukey's multiple comparison test. The statistical analyses were carried out by GraphPad Prism version 4.00 (GraphPad Software, San Diego CA, USA). Results were considered significant when *p* was <0.05.

RESULTS AND DISCUSSION

In Vitro Experiments

The topical formulations consisting of sucralfate 15% w/w suspensions in which cidofovir was dissolved, appeared as a white paint that spread on the skin surface and dried to leave a protective coating. The objective was to have a suspension liquid enough to cover the lesions of infected animals by spraying, but with a short drying time and with sufficient adhesiveness to maintain close contact between the lesions and the antiviral drug for prolonged time. The mean volume diameter (*d_{v, 0.5}*) of sucralfate gel particles in suspensions was in the range between 6.4 and 7.7 µm.

Chemical assay of the suspensions containing sucralfate and cidofovir revealed that while the sucralfate content was as expected, no cidofovir was detected in the supernatant.

Fig. 1. FT-IR spectra of cidofovir (A), sucralfate gel (B), and of the mixture 1:1 of the two active principles (C)

The possibility of an interaction between cidofovir and sucralfate gel particles was thus investigated.

FTIR spectra of sucralfate, cidofovir, and of sucralfate gel/cidofovir mixtures were recorded. The FTIR spectrum of cidofovir (Fig. 1 (A)) shows two characteristic absorption peaks at 1,132 and 1,014 cm^{-1} , corresponding to the stretching vibration of P=O and P-O bonds, respectively, and a forked

Fig. 2. Diffusion of cidofovir through a regenerated cellulose membrane from cidofovir 1% aqueous solution (filled triangle), Beeler base 1% cidofovir (filled square), sucralfate 15%+cidofovir 1% (filled diamond), and sucralfate 15%+cidofovir 1%+NaH₂PO₄ 16% (filled circle) formulations (mean value $\pm$ SD, $n=3$)

Fig. 3. Shear stress versus shear rate for sucralfate gel 21% (filled circle), sucralfate gel 15% (filled square), sucralfate gel 15%+NaH₂PO₄ 16% (filled diamond) (mean value $\pm$ SD, $n=6$)

peak at 1,378 and 1,359 cm^{-1} corresponding to the bending of OH group. In the sucralfate gel spectrum (Fig. 1 (B)), besides the broad absorption band at around 3,400 cm^{-1} related to the OH groups, two strong peaks at 1,639 and 1,253 cm^{-1} , corresponding to the bending vibration of C=O and to the S=O stretching vibration, were also evident. A typical peak corresponding to aluminum oxide (Al-O) was observed in the 1,049–1,008 cm^{-1} range. The mixture of two drugs showed for sucralfate a shift of the fork of OH bending to 1,398 and 1,367 cm^{-1} and for cidofovir a shift of the absorption peak attributed to the P=O bond from 1,132 to 1,120 cm^{-1} (Fig. 1 (C)). The shift of these signals suggested the adsorption of cidofovir

Fig. 4. Loss of weight of a layer of spray deposited sucralfate 15%+cidofovir 1%+NaH₂PO₄ 16% formulation at 32°C and consistency of deposited material (mean value $\pm$ SD, $n=3$)

Fig. 5. Appearance of lesions after 4 days of treatment. *Left column a*=15% sucralfate suspension; *b*=sucralfate 15%+cidofovir 0.5%+NaH₂PO₄ 16% formulation; *c*=sucralfate 15%+cidofovir 1%+NaH₂PO₄ 16% formulation. *Right column* untreated controls

on sucralfate gel particles in suspension. It is likely that the interaction involved the phosphate group of cidofovir and the aluminum ion of sucralfate and that the interaction was strong enough to sequester the cidofovir molecule from the solution.

Cidofovir diffusion through a membrane of regenerated cellulose from sucralfate gel/cidofovir formulations was studied with the aim to verify the effect of sucralfate/cidofovir interaction on drug availability (Fig. 2). Cidofovir dissolved in water permeated freely through the cellulose membrane. On the contrary, the formulation containing cidofovir and sucralfate gel did not provide quantifiable amount of antiviral drug

in the receptor compartment of diffusion cell, demonstrating that the interaction proposed above limited the cidofovir availability. Thus, it appeared that the sucralfate particles were able to adsorb the cidofovir as it has been reported for other biologically active molecules (20).

We then attempted to prevent this adsorption and to displace the cidofovir by adding as competing ion an increasing amount of phosphate to sucralfate gel suspensions. At 6% of sodium dihydrogen phosphate, the lowest amount used, a significant ($p<0.001$) concentration of free cidofovir was detected, i.e., 0.91 ± 0.1 mg/ml, in comparison with the formula-

Fig. 6. Skin thickness of lesions in treated groups of animals: sucralfate suspension 15% (filled diamond), sucralfate 15%+cidofovir 0.5%+NaH₂PO₄ 16% formulation (filled square); sucralfate 15%+cidofovir 1%+NaH₂PO₄ 16% formulation (filled circle). Untreated lesions are shown as control (unfilled circle)

tion not containing phosphate ions (0.08 ± 0.02 mg/ml). By increasing the content of sodium dihydrogen phosphate to 11%, the concentration of cidofovir in the supernatant raised to 8.0 ± 0.5 mg/ml. The further increase of the content of sodium dihydrogen phosphate to 16% resulted in pretty complete displacement of cidofovir. The displacement of cidofovir by phosphate was demonstrated also via the permeation experiments through the cellulose membrane. Figure 2 shows that, after the addition of the competing phosphate ion, cidofovir significantly diffused in the receptor compartment of the Franz cell. The *in vitro* permeation studies also revealed that the transport rate of cidofovir was greater in the sucralfate/phosphate/cidofovir formulation than in the semisolid formulation (Beeler base).

Thus, the development of topical formulation containing sucralfate and cidofovir required the addition of phosphate ions to counteract the interaction between cidofovir and sucralfate gel particles. During preliminary *in vivo* studies, we found that the interaction between the two drugs completely inhibited the antiviral efficacy of formulation, even if some benefit to lesion resolution appeared due to the wound healing properties of sucralfate (data not shown). The concentration of cidofovir in the

supernatant of the sucralfate/cidofovir suspension containing 16% sodium dihydrogen phosphate was determined to be 10.4 ± 0.2 mg/ml. This concentration is significantly higher than both the reported *in vitro* 50% inhibitory concentration of 2.2 ± 1.8 μ g/ml and the 50% cytotoxic concentration of ≥ 38.6 μ g/ml on primary lamb keratinocytes (21).

The flow characteristics of this drug formulation will be critical to its intended use as a spray. Sucralfate gel exhibits rheological properties similar to colloidal clays. This typical thixotropic behavior of highly structured systems has been described previously (16). Sucralfate gel is characterized at rest by a viscosity that decreases rapidly by increasing the shear rate because of the destruction of the three-dimensional structure formed at rest. Sucralfate humid gel raw material (21% w/w sucralfate content) showed a viscous yield value of 60–70 Pa. The diluted sucralfate suspension used for the cidofovir formulations (15% w/w sucralfate) had a significant reduction of this value (to 5–10 Pa) that allowed for spraying of the product. The addition of phosphate salt to the 15% sucralfate suspension did not affect significantly the viscosity of the formulations (Fig. 3).

For a successful application of cidofovir/sucralfate formulations during the *in vivo* experiments, the sprayer device should effectively deposit a homogeneous layer of product on skin lesions. Thus, the spray capacity of covering an area roughly corresponding to the skin lesions was evaluated *in vitro*. Experiments were performed using the formulation containing 16% of phosphate used for *in vivo* studies. The amount of formulation sprayed at each actuation was close to 1 g (1.01 ± 0.07 g, $n=15$). Taken into account the actual area infected with the virus in the model animals, a target surface area close to 23 cm² was considered and the number of actuations necessary to cover it with a homogeneous layer of product was determined. Three actuations of the trigger sprayer covered the target completely, depositing on its surface 0.86 ± 0.18 g of the paint.

The size distribution of droplets emitted from the trigger sprayer was quite broad due to the low flow rate produced manually; the droplet size distribution was around 300 μ m ($d_{v,0.1}=133$ μ m; $d_{v,0.5}=295$ μ m; and $d_{v,0.9}=1,139$ μ m).

In vitro evaluation of product deposition and subsequent drying found a steady diminution in the water content of the layer of sucralfate gel deposited (Fig. 4). In particular, in the first 10–15 min, a weight loss around 15% in weight led to a thick paste typical of concentrated sucralfate suspensions. The evaporation of 40% of water content produced a residue having the appearance of an almost dry solid product, firmly adherent to the deposition substrate. This type of behavior was considered beneficial because the formulation would not drip away completely from the animal skin after administration and would remain in contact with the lesions.

Table I. Logarithm of Total Viral DNA Amount (Copies DNA/ μ l) and of 50% Tissue Culture Infectious Dose of Vital Viruses (TCID₅₀/ml) Found in Scabs

Formulations	Controls untreated	Sucralfate 15%	Cidofovir 0.5% + Sucralfate 15% + NaH ₂ PO ₄ 16%	Cidofovir 1% + Sucralfate 15% + NaH ₂ PO ₄ 16%
Copies DNA	5.50 ± 0.29	5.10 ± 0.67	4.07 ± 0.30	3.88 ± 0.72
TCID ₅₀	5.31 ± 0.38	4.83 ± 0.70	3.02 ± 0.19	<1

In Vivo Experiments

All infected lambs started to develop the typical orf virus lesions 3–4 days after virus scarification. The lesions are characterized by an intense erythema and edema along the lines of scarification and the appearance of vesicles and pustules. No significant differences were seen between animals at this stage indicating that all the lesions were developing normally and were not being influenced by individual, animal-related, factors. Scagliarini and coworkers (11) previously demonstrated that the treatment regimen was critical to the final outcome of therapy with the best results obtained from treating the animals for four consecutive days starting as soon as the lesions were evident to the shepherd (3–4 days post-infection). This approach is also applied with other antiviral drugs such as acyclovir used for the topical treatments of herpes virus infections. It is well recognized that the best results are generally obtained by treating as soon as the clinical signs begin to appear. In this study we show that the formulations containing cidofovir/sucralfate led to a quicker resolution of the lesions with less production of scabs (Fig. 5). This latter point is considered particularly beneficial since the scabs are thought to represent the principal means by which the environment becomes contaminated by the virus.

Blood samples were taken prior to the scarification and thereafter weekly, in order to monitor markers of renal function (22). The blood levels of urea and creatinine remained within normal limits throughout the experiment, suggesting lack of renal toxicity arising from systemic absorption. At the same time, a possible systemic effect of cidofovir was also unlikely, because drug applied topically on one thigh did not affect the development of the untreated lesion on the contralateral thigh.

After 4 days of treatment the animals treated with cidofovir/sucralfate formulations showed the formation of a white scab formed by dried sucralfate gel. By day 8 post-infection, after 4 days of topical treatment, a significant difference ($p < 0.001$) in skin thickness (Fig. 6) was found between lesions treated with the sucralfate alone and lesions treated with formulations combining cidofovir, sucralfate, and sodium dihydrogen phosphate. However, no significant difference was found in lesion skin thickness between the animals treated with different cidofovir concentrations (0.5% *w/w* cidofovir; 1% *w/w* cidofovir). The treated animals developed white dried scabs that were easily removed between days 10 and 17 post infection and the treated lesions were completely resolved by day 13 to 15 post infection. The amount of the viral DNA in the scabs, measured by quantitative PCR, was significantly higher ($p < 0.05$) in the animals treated with sucralfate alone in comparison with the animals treated with formulations of sucralfate containing the antiviral drug (Table I).

The virus recovered from the pooled scabs was also cultured to detect the presence of viable virions. The presence of viable virus, as measured by CPE, was observed in all the samples, but again the viral titre was significantly higher ($p < 0.05$) in scabs recovered from animals treated with sucralfate alone when compared to the groups treated with the two different concentrations of cidofovir combined with sucralfate.

These results show that the sucralfate/cidofovir preparations exerted an antiviral effect that was evidenced by the significantly lower quantity of viable virus in the scabs of the animals treated with 1% cidofovir/sucralfate. Taken together,

these results were similar to those obtained in a previous study with a cidofovir 1% cream preparation in terms of clinical course and presence of viable virus in the scabs of the animals (11).

In summary, the *in vivo* studies demonstrated both the efficacy of the therapeutic paint spray formulation and the ease with which it could be administered even when performed with a very simple device such as a trigger sprayer.

CONCLUSIONS

Sucralfate gel has been demonstrated as a suitable carrier in topical formulations designed to keep cidofovir in contact with cutaneous lesions. The capability of the sucralfate gel to adsorb the drug was counteracted by introducing sodium dihydrogen phosphate in the preparation. The innovative spray formulation containing cidofovir/sucralfate gel paints the skin and dries quickly to a scab, remaining firmly adhered to the lesions. The treatment of model orf virus infections in sheep led to a rapid healing of the lesions. Thus, this new sprayable therapeutic paint represents an effective tool for orf virus treatment in addition to the semisolid formulations previously proposed.

The spray application of sucralfate-based paints permits an efficient coating of infected area independent of its location and gives a rapid and easy means for veterinary practitioners to treat large numbers of animals.

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