Effect of Phosphonoformic Acid in the Development of Bovine Embryos in Vitro

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CLINICAL RELEVANCE

This research evaluated the ability of phosphonoformic acid to inhibit bovine herpesvirus 1 (BHV-1) in cumulus cells commonly used in coculture with bovine in vitro–produced embryos. At 200 and 400 µg/ml, phosphonoformic acid inhibited 4 logs of BHV-1. Subsequently, phosphonoformic acid (200 and 400 µg/ml) added to both in vitro fertilization and culture medium resulted in a decrease in the proportion of developed blastocysts, and the number of cells per blastocyst was lower in the treated embryos. Therefore, while phosphonoformic acid can effectively inhibit replication of BHV-1 in coculture cells, it also inhibits development of in vitro–produced bovine embryos.

INTRODUCTION

Bovine herpesvirus-1 (BHV-1) is a ubiquitous viral pathogen of cattle. Infections with this alphaherpesvirus can result in reproductive and respiratory disease.[1,2] It is the etiologic agent of infectious pustular vulvovaginitis, infectious balanoposthitis, and infectious bovine rhinotracheitis.[3] Transmission of disease caused by BHV-1 occurs through close contact with nasal, oral, or genital secretions. The genital form of the disease is characterized by small pustules on the vestibular, preputial, and penile mucosa.[3–5] Seminal plasma from affected bulls contains BHV-1, but the virus does not enter the spermatozoa.[4,6] Infectious bovine rhinotracheitis is characterized by fever, anorexia, depression, dyspnea, nasal discharge, and hyperemic nasal mucosa.[3,7] BHV-1 is the most frequently diagnosed cause of viral abortion in North American cattle.[8] Aborting cattle may or may not show other clinical signs. If seen, the manifested clinical signs are generally respiratory. Abortions are rarely seen with the genital form of the disease.[2,8] BHV-1 infection can also lead to prolonged infertility due to intrauterine infection, endometritis, and severe necrotizing oophoritis.[2,5]

Routine application of standard handling

procedures for in vivo–produced embryos of cattle has greatly reduced risks of infectious disease transmission via embryo transfer. However, the risks differ between in vivo– and in vitro–produced embryos.[9] In vitro–produced embryos are at special risk for contamination with BHV-1, which can occur via exposed cumulus–oocyte complexes and spermatozoa or from contaminated products of animal origin that are used in media for washing, fertilization, and culture.[2,10,11] Many of these biologic products come from abattoirs where little of the history of the donor animal is known. In vitro–produced embryos require more manipulation and culture than in vivo–produced embryos, thus increasing the risk of viral entry and replication.[11] In addition, the zona pellucida of in vitro–produced embryos allows viruses to attach more readily than that of in vivo–produced embryos,[2] and standard embryo-washing procedures are less efficient for removing virus from in vitro–produced embryos. Bielanski and Dubuc[10] experimentally exposed heifers to BHV-1 and then collected their oocytes and used them for in vitro production of embryos. Embryos, oviductal cells, and uterine fluid were all positive for BHV-1, demonstrating that apparently normal transferable in vitro–produced embryos can have the virus associated with them. Such embryos provide the potential to spread BHV-1 to the recipient. Thus, the addition of an appropriate antiviral agent to the in vitro–production (IVP) system might minimize viral replication and allow the embryo to develop normally.

A number of antiviral agents shown to be effective against human herpesviruses have been evaluated against BHV-1. Phosphonoformic acid and phosphonoacetate inhibit viral replication by specifically inhibiting viral DNA polymerase through attachment to the pyrophosphate-binding site.[12–16] Use of phosphonoacetate is limited because it is topically irritating and toxic to laboratory animals and it accumulates in bones.[14] Phosphonoformic acid does not irritate the skin or genital mucous membranes and has only minor effects on normal cellular metabolism.

An antiviral agent that is effective against BHV-1 and nontoxic to embryos would be beneficial in embryo production. The objectives of this study were to evaluate the anti–BHV-1 activity and cytotoxic effects of phosphonoformic acid in primary bovine cell cultures and the impact on developmental efficiency of bovine embryos when media were supplemented with phosphonoformic acid.

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MATERIALS AND METHODS

Cell Lines

Madin–Darby bovine kidney (MDBK) cells, purchased from the American Type Culture Collection (Manassas, VA), were propagated and cultured in 1× minimum essential medium (MEM).[17]

Monolayers of cumulus cells were established from cumulus–oocyte complexes (oocytes surrounded by multiple layers of dense cumulus cells) that were aspirated from ovaries at an abattoir in Omaha, Nebraska, placed in matu-

ration medium in a portable incubator set at 38.5°C, and shipped by courier to Auburn University. The oocytes were matured in 25 mM HEPES-buffered tissue culture medium 199 with Earle's salts (Gibco, Invitrogen, Carlsbad, CA)[17] while they were in transit (20 to 24 hours). On arrival of the 300 oocytes, the expanded cumulus cells were removed by washing in HEPES-TALP[17] and vigorous pipetting. The media containing the cumulus cells was centrifuged and the pellet resuspended in α MEM. Cells were cultured in a four-well plate (2 cm^2) with 1 ml α MEM. On day 2, the cells were transferred to a 25-cm^2 flask. The cells were transferred seven to eight times before they were cryopreserved at –80°C until needed.

Antiviral Compounds

Phosphonoformic acid trisodium salt hexahydrate (also known as *foscarnet* and *sodium phosphonoformate tribasic hexahydrate*) was obtained from Sigma-Aldrich (St. Louis). The drug was supplied as a dry powder and dissolved in 1× MEM. The solution was filter sterilized (0.2 µm) before use.

Virus

BHV-1 (Colorado strain) was obtained from American Type Culture Collection (catalog number VR-864, lot number 1222901). It was propagated in MDBK cells.

Virus Isolation

Virus isolation procedures were used to determine the effect of the antiviral agent on the presence of BHV-1. One milliliter of diluted antiviral agent was added to each well of a 24-well plate (2 cm^2 monolayer), or 100 µl was added to each well of a 96-well plate (0.32 cm^2) previously seeded with MDBK cells or cumulus cells. The plate was incubated for 15 minutes at 38.5°C before BHV-1 was added. BHV-1 was diluted to provide a multiplicity of infection (MOI) of 0.005 to 0.5. The plate was incubated for 5 days at 38.5°C in an atmosphere of 5% carbon dioxide and air. The cells were examined daily for evidence of cytopathic effect. At day 2, media from cultures showing cytopathic effect were frozen for later viral quantification using plaque assay.

Plaque Assay

Plaque assays were used to quantify the amount of virus remaining after culture with an antiviral agent. Day 2 samples of media were assayed as previously described.[18]

Cell Cytotoxicity Assay

The effects of phosphonoformic acid (400 and 200 µg/ml) on MDBK cells were quantified using Cell Counting Kit-8 (Dojindo, Gaithersburg, MD). A water-soluble tetrazolium salt is reduced by dehydrogenases in cells to give a yellow-colored product (formazan). The amount of formazan dye produced by the dehydrogenase activity in the cells is directly proportional to the number of living cells.

Embryo Production

Embryo production was performed as previously described.[19] Briefly, following arrival of oocytes, cumulus-intact matured oocytes were washed in TL HEPES (Cambrex, Walkersville, MD). Matured oocytes were fertilized in drops containing Charles Rosenkrans medium (CR2) (10 ml) and bovine serum albumin (BSA) (Sigma-Aldrich) 6 mg/ml (0.06 g).[20] This medium was also used for in vitro culture drops up to day 3.5. Presumptive zygotes were cultured from day 3.5 to day 7.5 in in vitro culture (IVC) drops containing CR2 and 10% fetal bovine serum (FBS) (HyClone, Logan, UT).[19] The equine serum, BSA, and FBS used in this research were determined to be free of BHV-1 and anti–BHV-1 antibodies by virus isolation and virus neutralization.

Experimental Design

The first experiment evaluated noncytotoxic viral inhibition by phosphonoformic acid of BHV-1 in MDBK cells. The second experiment assessed the efficacy of phosphonoformic acid against four concentrations of BHV-1 in MDBK cells. The third experiment evaluated noncytotoxic viral inhibition by phosphonoformic acid of BHV-1 in primary cell cultures of bovine cumulus cells. The final experiment evaluated the development rate of bovine embryos fertilized and cultured in the presence of phosphonoformic acid.

Experiment 1

Four replicates were performed. Twofold dilutions of phosphonoformic acid trisodium salt hexahydrate (400, 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.56, and 0.78 µg/ml) were tested. Virus isolation was performed using 2×10^5 to 1×10^6 plaque-forming units (PFU) BHV-1/ml for an MOI between 0.05 to 0.5. Controls included (1) MDBK cells with phosphonoformic acid but no virus (to evaluate toxicity of agent to cells), (2) MDBK cells with BHV-1 (positive control for viral cytopathic effect), and (3) MDBK cells but no test antiviral or virus (negative control). The percent of virus inhibited for each test concentration was determined by comparison to equivalent samples from temporal control cultures in which no compound was added before or after inoculation.

Experiment 2

One trial was performed with five replicates. The purpose of this experiment was to determine whether the selected concentrations of phosphonoformic acid would completely inhibit BHV-1 in MDBK cells. The following dilutions of phosphonoformic acid were tested: 400, 200, 100, 50, and 25 µg/ml. Subsequently, the following dilutions of virus were used: 6×10^5 PFU/ml, 6×10^4 PFU/ml, 6×10^3 PFU/ml, and 6×10^2 PFU/ml. This allowed for an MOI of 0.2, 0.02, 0.001, and 0.001, respectively. Controls included (1) monolayer plus BHV-1 but no antiviral and (2) monolayer of MDBK cells and no chemical or virus.

Experiment 3

Four replicates were performed to evaluate noncytotoxic viral inhibition by phosphonoformic acid of BHV-1 in primary cell cultures (cumulus cells). The purpose of this experiment was to identify the minimal inhibitory concentrations for BHV-1 and maximal nontoxic concentrations of phosphonoformic acid in primary cell cultures. This was necessary to determine the therapeutic index (margin of safety between cytotoxic dose and effective antiviral dose).

The following dilutions of phosphonoformic acid were tested: 400, 200, 100, 50, and 25 µg/ml. Subsequently, BHV-1 was added to the cells in a single well at an MOI of 0.005 to 0.5. The inhibition of BHV-1 in incubating cells was quantified via plaque assay for virus in media (day 2). Controls included (1) monolayer of cumulus cells with phosphonoformic acid but no virus (to evaluate toxicity of agent to cells), (2) monolayer plus BHV-1 but no antiviral, and (3) monolayer of cumulus cells and no chemical or virus. The percent viral inhibition for each treatment was determined.

Experiment 4

Four replicates were performed. Three hundred oocytes were distributed as follows: 100 oocytes were used as an untreated control, and 200 oocytes were used in the treatment, with the specified antiviral concentration placed in both in vitro fertilization (IVF) and IVC media. Thus, the IVF and IVC media were supplemented with either 200 or 400 µg/ml of phosphonoformic acid. The use of 300 oocytes

$$\text{Percentage of virus inhibited} = \frac{\text{(Quantity of virus in the control sample lacking the compound} - \text{Quantity of virus in the compound sample)}}{\text{Quantity of virus in the control sample lacking the compound}} \times 100$$

with an anticipated minimal blastocyst development rate of 25% for the control oocytes allowed a difference of 5% in the treated oocytes to be significant at $P = .05$ using Pearson's chi-square statistic.

After embryonic development was assessed on day 7.5, embryonic quality and viability were evaluated. Embryonic quality was assessed by grading the embryo as described in the Manual of the International Embryo Transfer Society.[21] Embryonic viability also was assessed by counting the nucleated cells of developed blastocysts.[22]

Statistical Analysis

The cytotoxicity assay was analyzed using Student's *t*-test (JMP Software, SAS Institute, Cary, NC).

For experiments 1 and 3, the percent of virus inhibited for each test antiviral agent at each concentration was determined by comparison to equivalent samples from temporal control cultures in which no compound was added before or after inoculation (see box, above).

In experiments 1 and 3, the 99% inhibitory concentration (IC_{99}) was calculated using the JMP Software.

For experiment 2, the Reed–Muench method[23] was used to determine the phosphonoformic acid concentration at which virus infected only 50% of the cell cultures.

In experiment 4, the ratio of embryos that developed to blastocysts in each treatment group was compared with those in the control group using Pearson's chi-square test. The number of nucleated cells from the embryos and the grades of the embryos in each treatment group were compared with those in the control group using an analysis of variance paired *t*-test with JMP Software.

RESULTS

Cytotoxicity Assay

Phosphonoformic acid (400 and 200 µg/ml) was not cytotoxic to MDBK cells ($P < .05$). Phosphonoformic acid at a concentration of 200 µg/ml tended to enhance cell growth (105% of control), whereas a concentration of 400 µg/ml tended to reduce cell growth (95% of control); however, these differences were not significant ($P = .5$).

Experiment 1

Phosphonoformic acid at a concentration of 200 µg/ml inhibited greater than 4 logs of virus on MDBK cells (Figure 1). The IC_{99} was 46 µg/ml for phosphonoformic acid. Phosphonoformic acid produced no visible cytotoxicity in the MDBK cells at the concentrations tested. Cell morphology of the treated groups did not appear to differ from cells grown in control medium, and cell detachment was not observed.

Experiment 2

Phosphonoformic acid (400 µg/ml, 1333 µM) was able to completely inhibit viral replication of all four concentrations tested (6×10^5 PFU/ml, 6×10^4 PFU/ml, 6×10^3 PFU/ml, and 6×10^2 PFU/ml). At 200 µg/ml (666.6 µM) of phosphonoformic acid, complete inhibition of BHV-1 was seen at 6×10^4 PFU/ml, 6×10^3 PFU/ml, and 6×10^2 PFU/ml. At 6×10^5 PFU/ml, 6×10^4 PFU/ml, 6×10^3 PFU/ml, and 6×10^2 PFU/ml, the IC_{50} was 235 µg/ml, 143 µg/ml, 143 µg/ml, and 35.7 µg/ml phosphonoformic acid, respectively.

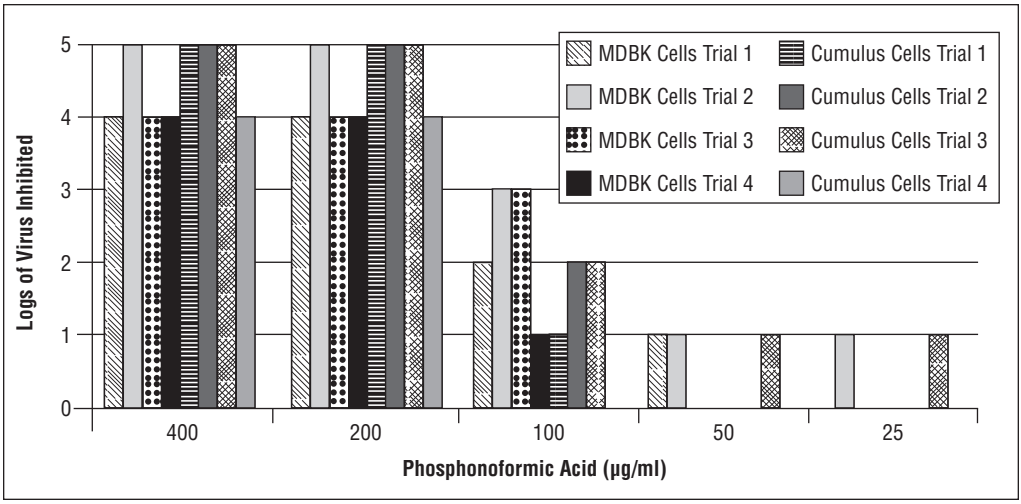

Figure 1. *Inhibition of BHV-1 by phosphonoformic acid at concentrations used in experiment 1 (Madin–Darby bovine kidney cells [MDBK cells]) and experiment 3 (cumulus cells). The logs of virus inhibited for each trial are shown.*

Experiment 3

Phosphonoformic acid was effective at inhibiting greater than 4 logs of virus at 200 µg/ml (Figure 1). The IC_{99} was 91 µg/ml for phosphonoformic acid. Phosphonoformic acid was not visibly cytotoxic to the cumulus cells at the concentrations tested. Cell morphology of the treated groups did not appear to differ from cells grown in control medium, and cell detachment was not observed.

Experiment 4

Embryos were able to develop in the presence of phosphonoformic acid (Table 1). However, the blastocyst development rates of the treated groups were significantly reduced from that of the control group. In addition, the nucleated cell counts of embryos from the treated groups were lower than those of the control group. The appearance of viable embryos (embryo grade) was not significantly different (P = .7) between the control group and the 200 µg/ml phosphonoformic acid group.

DISCUSSION

BHV-1 is a major economic concern in cattle-producing countries.[24] Because the virus has been shown to associate with in vitro–produced embryos,[10,25] it is possible that transfer of these embryos could result in transmission of disease to recipient cattle. Thus, use of an effective antiviral agent in the IVP system could provide a useful deterrent to transmission of disease to recipients. With this as a motivation, the specific aims of this project were to determine the viral IC_{99} of phosphonoformic acid against BHV-1 and to determine whether bovine in vitro–produced embryos would develop normally in the presence of effective antiviral concentrations of phosphonoformic acid.

There are several circumstances by which a contaminant can enter the IVP system. Virus can be present in the cumulus oophorus cells, follicular fluid, and materials used in fertilization and culture media.[11,26] Virus can also be associated with the sperm. Vanroose et al[11] have shown that presence of BHV-1 in all three

TABLE 1. Embryonic Development and Viability of Control and Treated (200 or 400 µg/ml Phosphonoformic Acid) Embryos from Experiment 4

Stage of Embryonic Development and Assessment of Viability		*Control Group*	*Phosphonoformic Acid*	
			200 µg/ml	*400 µg/ml*
Day 3.5 development (>4 cell embryos/ oocytes fertilized)	Trial 1	58/91 (64%)	68/89 (76%)	38/89 (43%)[a]
	Trial 2	80/103 (78%)	44/100 (44%)[a]	27/107 (25%)[a]
	Trial 3	46/104 (44%)	58/99 (59%)	22/99 (22%)[a]
	Trial 4	39/76 (51%)	31/79 (39%)	17/89 (19%)[a]
	Cumulative	223/374 (60%)	201/367 (55%)	104/384 (27%)[a]
Day 7.5 development (blastocysts/oocytes fertilized)	Trial 1	28/91 (31%)	28/89 (31%)	6/89 (7%)[a]
	Trial 2	43/103 (42%)	15/100 (15%)[a]	3/107 (3%)[a]
	Trial 3	19/104 (18%)	20/99 (20%)	2/99 (2%)[a]
	Trial 4	18/76 (24%)	11/79 (14%)	1/89 (1%)[a]
	Cumulative	108/374 (29%)	74/367 (20%)[a]	12/384 (3%)[a]
Nucleated cell no. (mean of day 7.5 blastocysts)		84 ± 42	57 ± 27[b]	27 ± 26[b]
Embryo grade (mean of day 7.5 blastocysts)		1.1 ± 0.3	1.1 ± 0.3	1.3 ± 0.5[b]

[a]Ratio differs significantly from control ($P \leq .01$).
[b]Means differ significantly from control ($P \leq .01$).

phases of the IVP system (in vitro maturation [IVM], IVF, and IVC) as well as in each phase separately will decrease blastocyst production. In a worst-case scenario, all three phases could contain virus. Since IVM was initiated at and oocytes were shipped from another location, it was not feasible to add antivirals to IVM. However, we did elect to test the antiviral agent in both IVF and IVC simultaneously.

Phosphonoformic acid, also known as foscarnet and trisodium phosphonoformate, has been used to effectively treat human cytomegalovirus infection and acyclovir-resistant herpes simplex virus infection. Phosphonoformic acid is a nonnucleoside antiviral and a pyrophosphate analogue.[12,15,27–29] It functions by selectively inhibiting virus-specific DNA polymerase at the pyrophosphate-binding site. By preventing cleavage of pyrophosphate from deoxynucleotide triphosphate, viral replication is inhibited.[15,29,30] Phosphorylation is not required as with nucleoside analogues; therefore, patients that are refractory to acyclovir, valacyclovir, penciclovir, and ganciclovir can be treated with phosphonoformic acid.[15] Resistance to phosphonoformic acid involves alteration in binding to viral DNA polymerase.[28,29] The safety of phosphonoformic acid in human pregnancy has not been thoroughly evaluated.[28] However, a single case report of treatment in a patient at 32 weeks' gestation did not indicate any adverse effects in the infant.[27]

Schwers et al[14] demonstrated that phosphonoformic acid was effective in reducing the number of plaques produced by BHV-1 (Los Angeles strain) on Georgia bovine kidney cells

as well as decreasing the titer of virus.[14] They showed that 500 and 1,000 μM of phosphonoformic acid significantly reduced the number of plaques produced by BHV-1 (3 logs). They also demonstrated a significant reduction in plaque size with 10 μM of phosphonoformic acid, and this reduction increased as the concentration of the drug was increased to 1,000 μM. In addition, they demonstrated that the drug was safe for uninfected control cells that were grown in MEM with 10% FBS. Similarly, Helgstrand et al[12] showed that 500 μM phosphonoformic acid reduced BHV-1 plaque formation on calf kidney cells by 99.9%. The concentrations that we used are similar to those used by Sarisky et al[31] to demonstrate that phosphonoformic acid was effective against strains of herpes simplex virus serotype 1 resistant to penciclovir and acyclovir. After our initial testing in MDBK cells, cumulus oophorus cells were used in additional screening assays because they represent primary cultures of somatic cells that are commonly used in cocultures with bovine and human in vitro–produced embryos. Consistent with the above findings, we demonstrated that phosphonoformic acid was effective at reducing viral concentration by greater than 4 or 5 logs both on MDBK cells and bovine cumulus cells while exhibiting no signs of cytotoxicity. In addition, virus could be inhibited completely if a lower concentration of virus (6×10^4 PFU/ml) or a higher concentration of phosphonoformic acid (400 μg/ml) was used. Inhibition of viral replication and nontoxicity in cumulus cells was encouraging; however, when phosphonoformic acid (400 and 200 μg/ml) was placed in the IVF and IVC media of developing bovine embryos, blastocyst development was reduced compared with the control group. In addition, the number of cells in the treated embryos was lower than in the control embryos. The cell number is an indication of developmental potential and has been shown to be a better indicator of embryo quality than visual morphologic assessment.[32] As the cell number decreases, developmental potential of the embryo decreases. Trial 2 of the 200 μg/ml treatment group was the only trial that demonstrated decreased day 3.5 and day 7.5 development. When the results of this trial were omitted from the cumulative results, embryonic development was not significantly different from the control ($P > .5$); however, the nucleated cell count was still significantly lower than in the control embryos ($P < .0001$). Thus, if subsequent trials were performed, phosphonoformic acid might be shown to allow embryos to develop at a rate comparable to that of the controls, but the quality of the embryos will still be diminished from that of the controls.

Phosphonoformic acid is nontoxic to bovine cumulus cells and inhibits replication of BHV-1 in these cells, but it is detrimental to embryonic development.

As stated above, the cumulus cells showed no signs of morphologic change induced by phosphonoformic acid during the screening assays. Also, during IVF and IVC, no change was seen in the cumulus cell monolayers of the treated groups compared with those of the control groups. Thus, the negative effect on embryonic development was more likely the result of a direct effect on the embryonic cells as opposed to an indirect effect via the cocul-

tured cells. In addition, hyperactivation of spermatozoa was not affected by the presence of phosphonoformic acid and day 3.5 development was not significantly different between the control group and the group treated with 200 µg/ml phosphonoformic acid. Thus, when used at the lower concentration, the drug had no effect on fertilization or cleavage rates that was apparent at these stages of observation.

Blastocysts were able to develop; however, they developed at a reduced rate, and their developmental potential was lower as determined by the nucleated cell count. Thus, the fertilization and development of bovine embryos were more sensitive indications of the toxic effects of the antiviral agent than were division and growth of either MDBK or cumulus cells. Use of the antiviral during both IVF and IVC might have enhanced the negative impact on embryonic development. A reasonable approach in future research might be to use the antiviral only during IVC because the opportunity for viral replication during IVF is limited by the short time (6 hours) in that phase of embryo production.

Another possibility for the difference in toxicity of the antiviral agent in the embryos compared with the MDBK and cumulus cells lies in the concentrations of serum used in the different media. The antiviral agent was reconstituted in 1× MEM with 10% equine serum. The MDBK cells were cultured in 1× MEM with 10% equine serum, and the cumulus cells were cultured in α MEM with 15% FBS. The embryos were fertilized and cultured first in CR2 with BSA (days 0 to 3.5) followed by CR2 with 10% FBS in the second phase of culture (days 3.5 to 7.5). Serum has been shown to have detoxifying effects. Others have shown that the concentration of FBS present in the medium can affect the results of cytotoxicity studies of various agents.[33,34] Tognon et al[33] showed that cytotoxicity of an anticancer drug decreased (75% cytotoxicity versus 40%) as FBS concentration increased from 2.5% to 10%, respectively. In comparison, when the drug was diluted with human serum albumin (HSA), cytotoxicity was 100%. Use of the drug dissolved in HSA and diluted in medium with FBS yielded a lower cytotoxicity but not as low as when the drug was dissolved in FBS. It is believed that FBS provided protection to the cells by preventing complete uptake of the drug by the cells. Tognon and colleagues[33] showed that the intracellular concentration of the drug was lower in the presence of 10% FBS compared with HSA. Similarly, Bohets et al[34] showed that FBS concentration affected the cytotoxicity of nephrotoxic agents. One agent (mercuric chloride) was less cytotoxic with 5% to 10% FBS than with 0% to 1% FBS in the medium. This could be a result of FBS binding to mercury. The cytotoxicity of the other agent (paracetamol [acetaminophen]) was not affected by the concentrations of FBS. Thus, to ideally determine the cytotoxicity of an agent, the lowest concentration of FBS required to maintain the cells should be used. The potential cytoprotective effect of FBS on phosphonoformic acid is unknown. Based on the above studies, further research using various concentrations of FBS in the presence of phosphonoformic acid should be conducted to determine if a specific concentration of FBS might mitigate the reduction of embryonic development without reducing the antiviral effects of phosphonoformic acid. Of course, consideration would have to be given to the possible detrimental effects on embryonic development of manipulating the concentration of serum in various segments of embryo production.

CONCLUSION

Completion of this research confirmed that phosphonoformic acid is nontoxic to bovine cumulus cells and can be used to inhibit repli-

cation of BHV-1 in these cells. However, as used in this study, the compound was detrimental to embryonic development. Future research should evaluate whether strategic use of FBS might mitigate the negative effects of phosphonoformic acid without compromising its antiviral effect or negatively impacting embryo development. Also, additional studies should be initiated to evaluate embryonic development when the antiviral is only used in IVC as opposed to its simultaneous use in both IVF and IVC.

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