In-vitro antiviral efficacy of ribavirin and interferon-alpha against canine distemper virus

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Abstract

Canine distemper is a highly contagious disease with high incidence and lethality in the canine population. The objective of this study was to evaluate the efficacy of antiviral action with ribavirin (RBV), interferon-alpha (IFNα), and combinations of RBV and IFNα against canine distemper virus (CDV). Vero cells inoculated with CDV were treated with RBV, IFNα, and combinations of these drugs. The efficacy to inhibit viral replication was evaluated by adding the compounds at different times to determine which step of the viral replicative process was affected. Both drugs were effective against CDV *in vitro*. The IFNα was the most active compound, with an average IC_{50} (50% inhibitory concentration) value lower than the IC_{50} of the RBV. Ribavirin (RBV) was more selective than IFNα, however, and neither drug showed extracellular antiviral activity. The combination of RBV and IFNα exhibited antiviral activity for the intra- and extracellular stages of the replicative cycle of CDV, although the intracellular viral inhibition was higher. Both RBV and IFNα showed high antiviral efficacy against CDV, and furthermore, RBV + IFNα combinations have shown greater interference range in viral infectivity. These compounds could potentially be used to treat clinical disease associated with CDV infection.

Résumé

La maladie de Carré est une maladie très contagieuse avec une forte incidence et un degré de mortalité élevé parmi la population canine. L'objectif de cette étude était l'évaluation de l'efficacité de l'action antivirale de ribavirine (RBV), interféron-α (IFNα) et des combinaisons de RBV et IFNα contre le virus de la maladie de Carré (CDV, canine distemper virus). Des cellules Vero inoculées avec CDV ont été traitées avec RBV, IFNα et des combinaisons des deux. L'efficacité d'inhiber la réplication virale a été évaluée en ajoutant les composants à des moments différents afin de déterminer l'étape où le processus de la réplication virale est touché. Les deux médicaments se sont avérés efficaces contre le virus CDV in vitro. L'interféron-α était le composant le plus actif en démontrant une valeur moyenne de IC_{50} (concentration inhibitoire à 50 %) plus basse que celle du IC_{50} pour RBV. Par contre RBV était plus séléctif que IFNα et aucun des deux ne démontraient une activité antivirale extracellulaire. La combinaison de RBV et IFNα montraient une activité antivirale pour les phases intra- et extracellulaire du cycle de réplication du virus, avec une inhibition virale plus forte du côté intracellulaire. RBV et IFNα ont démontré une forte efficacité antivirale contre le virus de la maladie de Carré, de plus avec une plus grande portée d'interférence dans l'infectiosité virale pour les combinaisons de RBV + IFNα. Ainsi ces composants pourraient potentiellement être utilisés comme traitement de la maladie clinique associée à l'infection par le virus CDV.

(Traduit par les auteurs)

Introduction

Canine distemper is caused by an enveloped viral pathogen that is highly contagious, with a single-stranded, negative-sense ribonucleic acid (RNA) genome. The etiologic agent belongs to the genus *Morbillivirus*, family *Paramyxoviridae*, and often causes fatal disease in dogs and various members of the order *Carnivora*. Canine distemper virus (CDV) causes severe immunosuppression and multisystemic commitment usually associated with viral dissemination to the central nervous system, resulting in progressive and multifocal demyelinating leukoencephalopathy (1,2). Although there is extensive vaccination, the absence of specific antiviral drugs for the treatment of canine distemper enables the persistence of CDV as one of the most important pathogens of domestic dogs (3).

The nucleoside analog 1-(β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (ribavirin, RBV) shows inhibitory effects for a wide variety of deoxyribonucleic acid (DNA) and RNA viruses (4). The RBV molecule is the only commercially available compound with known antiviral action against various members of the *Paramyxoviridae* family (5–7). The mechanism of intracellular viral inactivation described for RBV comprises its initial conversion into ribavirin-5′-monophosphate (RMP) by adenosine kinase; RMP is a potent inhibitor of inosine-5′-monophosphate dehydrogenase (IMPDH), promoting decreased levels of intracellular guanosine

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triphosphate (GTP). The resulting imbalance of nucleotides causes the replacement of GTP for alternative nucleotides, thereby increasing the frequency of viral mutation and the production of defective genomes (8).

The products of cellular metabolic RBV may also reduce viral protein translation and RNA replication, and may interfere with the effectiveness of the addition of the cap in RNA (9). Despite its low selectivity and divergent results, RBV has been used in various experimental protocols for the treatment of subacute sclerosing panencephalitis due to measles in humans. Canine distemper and measles *morbilliviruses* (MeV) are genetically related and cause similar clinical signs in the host (7,10).

Interferons (IFNs) are proinflammatory cytokines that are synthesized by the innate immune system of the host response to viral infections. Interferons enable a state of viral resistance and play an important role in modulating the adaptive immune system (11). An important pathway of the antiviral response consists of the production of type I interferons, especially interferon-alpha/beta (IFNα/β) (11,12). Interferon-alpha (IFNα) has strong antiviral, antiproliferative, and immunomodulatory properties. The action of IFNα promotes the production of CD8+ T-lymphocytes, which were identified in rhesus monkeys as a major resource for the inhibition of MeV replication (13).

Some important *morbilliviruses*, such as rinderpest virus (RPV), peste-des-petits-ruminants virus (PPRV), MeV, and CDV, inhibit interferon signaling pathways and block the action of IFN I (α/β) and IFN II (γ). *Morbillivirus* proteins V, C, and N suppress transcriptional responses induced by IFN I and II and the establishment of the antiviral state (14,15). Infections caused by *morbilliviruses* also lead to reduced production of IFNα/β, probably due to translational suppression of IFNα signaling (16).

This study aimed to investigate the antiviral activity of ribavirin and interferon-α *in vitro* against CDV. The compounds were evaluated both separately and combined in order to determine the inhibitory activity of the individual compounds and any possible synergistic effects of the combination of these drugs. Due to the absence of previous research about the effects of the combination of ribavirin and IFNα on replication of CDV, the present study investigated the resulting impact from this association against CDV *in vitro*. Antiviral research is essential in the search for therapeutic strategies to effectively control canine distemper.

Materials and methods

Compounds

Two antiviral drugs, RBV (Ribavirin; 250-mg capsule, Blausiegel Laboratory, Cotia, SP, Brazil) and IFNα (Human recombinant interferon-α; Blauferon A, α-interferon 2a, 3 MIU/mL, Blausiegel Laboratory) were considered in this study. Ribavirin was initially diluted in DMSO (dimethyl sulfoxide; Sigma-Aldrich, St. Louis, Missouri, USA) at a stock concentration of 100 mM (24.42 mg/mL) and stored at 4°C. The recombinant human IFNα was diluted in ultra-pure water at a concentration of 100 000 IU/mL (3.7 μg/mL). The concentrations used in the tests were diluted in MEM (Minimal essential medium; Cultilab, Campinas, SP, Brazil) at the time of use.

Cells and viruses

Vero cells were maintained in MEM supplemented with 10% FBS (Fetal bovine serum; Cultilab), 1.6 mg/L of penicillin (Penicillin G potassium salt; Sigma-Aldrich), and 0.4 mg/L of streptomycin (Streptomycin sulfate salt; Sigma-Aldrich). The sample of CDV (Rockborn strain, 11th passage; Federal University of Minas Gerais, Belo Horizonte, MG, Brazil) was titered through the determination of $TCID_{50}$ (50% infective dose in tissue culture) (17).

Cytotoxicity

The cytotoxicity of the separate and combined compounds was determined by microscopic evaluation of cell viability and morphology, and confirmed and measured by a colorimetric method based on the reduction of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma-Aldrich, Deisenhofen, Germany] by mitochondrial enzymes (18). Vero cells were distributed into 96-well microplates (96-well cell culture, cluster tissue culture treated plates; TPP, Trasadingen, Switzerland) and after 4 h of incubation at 37°C and 5% carbon dioxide (CO_2) for cell adherence, the test compound diluted in MEM at several different concentrations was added. After the dilution in MEM, the microplates were incubated at 37°C and 5% CO_2 for 72 h and the effects on cell morphology were observed microscopically. The cells were then washed twice with phosphate-buffered saline (PBS) solution pH 7.2 with 5% FBS and incubated with MTT (1 mg/mL) for 4 h. The salt formed was solubilized by adding DMSO (100 μL/well) and agitating for 15 min at 37°C in a shaker. The microplates were incubated at 37°C and 5% CO_2 for 1 h. Optical densities (OD_{550}) were determined by an absorbance microplate reader (ELX800 Absorbance Microplate Reader; BioTek, Winooski, Vermont, USA).

The evaluation of the compounds included analysis of 6 wells for each concentration, including the untreated control cells. The percentage of viable treated cells was calculated relative to the untreated control (% control cell = $OD_{exp}/OD_{cell\ control} \times 100$). The CC_{50} (50% cytotoxic concentration) was defined as the compound concentration related to a 50% reduction of the absorbance of the control cell.

Time-of-addition study

This assay aimed to investigate the interference of compounds at different stages of the CDV replicative cycle. The study was conducted according to the method described in a previous study (19) with some modifications. Ninety-six-well microplates with Vero cell monolayers (1×10^5 cells/well) were incubated with RBV and IFNα for 1 h (-1 h: effect on pre-treatment), washed twice with PBS solution, and inoculated with dilutions from 10 to 10^5 $TCID_{50}$/mL of CDV. Other cell monolayers in microplates were inoculated with the viral suspensions before incubation and treated with the compounds at the same time, then incubated for 1 h at 4°C (0 h: effect on adsorption). Some inoculated and untreated microplates were incubated at 37°C with compounds diluted in MEM for 1 h (1 h: effect on penetration). Two h after viral inoculation, other microplates with infected cells were treated with compounds diluted in MEM (2 h: intracellular effect). The antiviral activity was measured at each stage by reducing the viral titer of the treatments compared to the control viral titer after 72 h of incubation (17). Assays were done in

Table I. Cytotoxicity, anti-CDV activity, and selectivity indexes of the compounds tested

Compounds	Ciytotoxicity CC_{50}[a] (μg/mL)	Antiviral activity IC_{50}[a] (μg/mL)	Selectivity index (SI)[b]
RBV	353 ± 48	8.32 ± 0.004	42.4
IFNα	0.36 ± 0.06	0.024 ± 0.001	15
DMSO (%)	2.11 ± 0.11	—	—

[a] CC_{50} (50% inhibitory concentration) and IC_{50} (50% cytotoxic concentration) values represent mean ± standard error of the mean values. Experiments were done in triplicate.
[b] Selectivity index (CC_{50}/IC_{50}).
CDV — canine distemper disorder; RBV — ribavirin; IFNα — interferon-alpha; DMSO — dimethyl sulfoxide.

triplicate and DMSO was evaluated under the same conditions to determine the possible interference of this solvent.

The 50% inhibitory concentration (IC_{50}) corresponds to the concentration of the compound that leads to a 50% reduction in viral activity. The selectivity index (SI) of the compounds was obtained by calculating the ratio between the values of CC_{50} and IC_{50} for each stage of viral replication. The antiviral activity of the compounds was determined, considering the minimum reduction of 1 $\log_{10}$ (90%) of the virus productivity (20).

Analysis of combinatorial effects between the drugs

Associations between RBV and IFNα were evaluated in the time-of-addition assay (TofA), as previously described. To determine the combination index (CI), both potency (*Dm* value) and shape of the dose-effect curve (*m*-value) of each drug were obtained from the median-effect equation using CompuSyn software (21). The combinatorial effects of 2 drugs can be classified as synergistic ($CI \leq 0.5$), non-antagonistic ($0.5 > CI \leq 4$), or antagonistic ($CI > 4$) (22).

Statistical analyses

Statistical analyses were carried out using the program Sisvar (Statistical Analysis and Design of Experiments program, Sisvar 5.1 Build 72, Federal University of Lavras, Lavras, MG, Brazil). The cytotoxicity and time-of-addition assays were analyzed by 1-way analysis of variance (ANOVA) and 2-way ANOVA, respectively. A significant viral inhibition was determined for the concentrations of the compounds that showed a minimum reduction of 90% (1 $\log_{10}$) of viral yield and confirmed by statistical analysis. Values of CC_{50} and IC_{50} were calculated from a linear regression equation. The Tukey test was used to compare means and *P*-values < 0.05 were considered significant.

Results

Analysis of cytotoxic effect

The cytotoxicity of the compounds was determined by microscopic examination of cell morphology and measurement of cell viability by the MTT colorimetric method. The intensity and variety of cellular morphological changes (loss of cell monolayer, granulation, vacuolization in the cytoplasm, stretching and narrowing of cell extensions, and darkening of the cell borders) were dose-dependent.

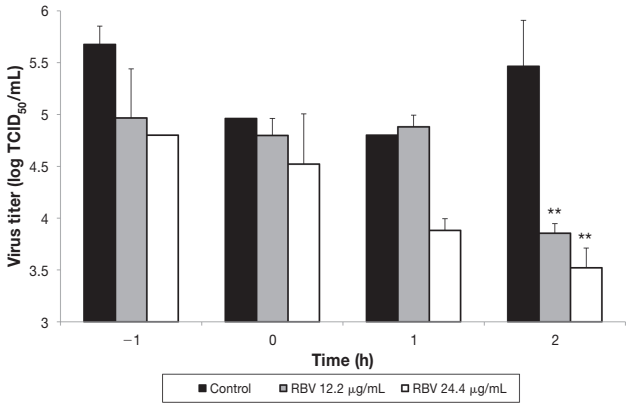

Figure 1. Inhibitory effects of adding ribavirin (RBV) at different stages of the replication cycle of canine distemper virus (CDV). The concentrations of RBV (12.2 and 24.4 μg/mL) were added at the times corresponding to the stages of pre-treatment (-1 h), adsorption (0 h), penetration (1 h), and intracellular (2 h).
**** Concentrations statistically different compared to control ($P < 0.01$) and inhibitory activity ≥ 1 $\log_{10}$.**

The CC_{50} of DMSO was higher than the concentration of DMSO used for dissolving ribavirin, which ensured the absence of cellular toxicity in the volumes of DMSO used and its use as an inert compound. The CC_{50} values of the evaluated drugs are shown in Table I. Interferon-alpha (IFNα) was approximately 980 times more toxic for Vero cells than RBV, showing a CC_{50} value of 0.36 ± 0.06 μg/mL compared to a CC_{50} of 353 ± 48 μg/mL for RBV. Combinations of RBV + IFNα at combination 1 (C1) (12.2 + 0.03 μg/mL) and combination 2 (C2) (12.2 + 0.06 μg/mL) sorted for antiviral studies showed over 90% cell viability in the cytotoxicity assay compared to untreated cells.

Analysis of antiviral activity

The antiviral activity of the isolated compounds and the combined compounds was determined by the time-of-addition assay. The addition of compounds relating to time-to-viral-infection allowed us to investigate the inhibition according to the stage of the viral replicative cycle. The IC_{50} values and SI (CC_{50}/IC_{50}) of the compounds are shown in Table I.

As the TofA assay for DMSO showed no significant antiviral activity in any of the stages of the replicative cycle of CDV, its use as a solvent does not interfere with the analysis of the antiviral action of the compounds.

The efficiency of antiviral activity of RBV on the canine distemper virus (CDV) was demonstrated at the time 2 h (intracellular; IC_{50} 8.32 ± 0.004 μg/mL and SI 42.4) of viral replication, with no significant differences between the concentrations C1 (12.2 μg/mL) and C2 (24.4 μg/mL), which presented, respectively, 98% and 99% inhibition of viral yield (Figure 1).

As shown in Figure 2, IFNα interfered in the CDV infectivity at the time 2 h (IC_{50} 0.024 ± 0.001 μg/mL and SI 15). Only C2 (0.06 μg/mL) showed an inhibitory effect, with 91.2% reduction in viral yield. Interferon-alpha (IFNα) was the most active compound, with an average value of IC_{50} lower than the IC_{50} of RBV. Ribavirin (RBV)

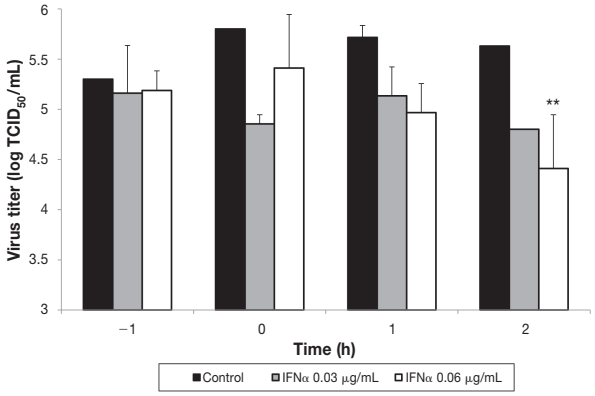

Figure 2. Inhibitory effects of adding interferon-alpha (IFNα) at different stages of the replication cycle of canine distemper virus (CDV). The concentrations of IFNα (0.03 and 0.06 μg/mL) were added at the times corresponding to the stages of pre-treatment (-1 h), adsorption (0 h), penetration (1 h), and intracellular (2 h).
**** Concentrations statistically different compared to control ($P < 0.01$) and inhibitory activity ≥ 1 $\log_{10}$.**

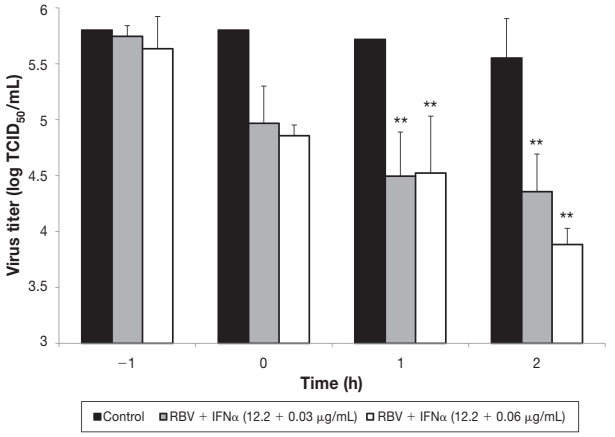

Figure 3. Inhibitory effects of adding the combination ribavirin + interferon-alpha (RBV + IFNα) at different stages of the replication cycle of canine distemper virus (CDV). The concentrations of the RBV + IFNα (12.2 + 0.03 μg/mL and 12.2 + 0.06 μg/mL) were added at the times corresponding to the stages of pre-treatment (-1 h), adsorption (0 h), penetration (1 h), and intracellular (2 h).
**** Concentrations statistically different compared to control ($P < 0.01$) and inhibitory activity ≥ 1 $\log_{10}$.**

was more selective than IFNα, however, and neither drug showed extracellular antiviral activity.

The RBV + IFNα combination demonstrated inhibitory effects at the times 1 h (penetration) and 2 h, with no significant difference between the concentrations C1 (12.2 + 0.03 μg/mL) and C2 (12.2 + 0.06 μg/mL) (Figure 3). The association of the drugs resulted in an inhibition of 92% and 91% (C1 and C2, respectively) of cytopathic effect at the time 1 h and 93% and 98% at the time 2 h. The efficacy of antiviral action was higher at the intracellular stage than at the penetration stage.

The isolated compounds and their combinations are compared in Table II. There was no difference between the inhibitory effects of isolated compounds and their combination at the intracellular stage, but only the combination of drugs showed a significant extracellular viral inhibition.

The combinatorial effects of the compounds are shown in Table III. The calculation of the combination index (CI) resulted in values that indicate a non-antagonistic association between RBV and IFNα against the stage of intracellular replication of CDV (Table III). Although the combination of the compounds also showed antiviral action for the penetration stage, calculating the CI and the associated interpretations was unfeasible due to the absence of significant antiviral activity (≥ 1 $\log_{10}$) of the isolated drugs at that stage.

Discussion

Infection with the canine distemper virus exhibits high rates of lethality in dogs, which persists as there is still no specific and efficient treatment. Although regular vaccination can provide satisfactory control of canine distemper, occasional outbreaks are still reported in several regions of the world. This occurs even in groups of vaccinated dogs, which is probably due to the transmission of genetically distinct viral strains (2,23). In the present work, the effectiveness of *in-vitro* antiviral activity of ribavirin and interferon-α against canine distemper virus was evaluated for both the isolated and combined compounds.

As canine distemper and measles are closely related viruses that cause comparable diseases, this relationship allows ownership of molecules with therapeutic potential against MeV for canine distemper treatment and vice versa (24,25). Ribavirin is an example of such extrapolation and has demonstrated great antiviral efficiency against MeV (26). The antiviral assay with RBV demonstrated the effectiveness of the drug in intracellular viral inhibition, which indicates a possible interference of the molecule in the replicative process of CDV. Previous studies also reported the activity of RBV against CDV *in vitro* in the post-infective stage (3,27,28). According to a study that evaluated the anti-CDV activity of RBV *in vitro* (3) by comparing the IC_{50} of RBV for CDV (6.5 to 12.5 μg/mL) with the IC_{50} of RBV for MeV (8.6 to 66.7 μg/mL), the IC_{50} of RBV (8.316 to 8.324 μg/mL) observed in this study indicates that the compound has shown anti-CDV efficacy similar to that described here.

The evaluation of the antiviral activity of IFNα against CDV was justified by its direct relationship with the cellular antiviral response and also by the mechanism of IFNα depletion observed in infectious processes for *Morbillivirus*. The time-of-addition assay of IFNα demonstrated antiviral efficacy for the intracellular stage in CDV replication. Interferon direct interference pathways in the intracellular viral cycle comprise the induction of nucleases production that attack the viral genome and the inhibition of viral protein synthesis due to phosphorylation changes in the translation in initiation factors (29).

The interferon treatment in Vero cells induces the 2′,5′-oligoadenylate synthetase that is responsible for enzymatic activity (RNase L activation) correlated with the cellular antiviral state against nonsegmented negative-sense RNA viruses (30,31). *Morbilliviruses* (RPV, PPRV, MeV, and CDV) blocked the induction of the cellular antiviral state upon stimulation by small amounts of IFNα (10 or 100 IU/mL) (15). When considering the stimulation by higher amounts of IFNα (1000 IU/mL), however, the *morbilliviruses* tested showed variable

Table II. Relationship between viral titers corresponding to the positive controls and treatments with RBV (12.2 μg/mL), IFNα (C1 0.03 μg/mL and C2 0.06 μg/mL), and RBV + IFNα (C1 12.2 μg/mL + 0.03 μg/mL and C2 12.2 μg/mL + 0.06 μg/mL) for CDV stages of replication

	Log viral titer C+/Log viral titer [C]							
	-1 h		0 h		1 h		2 h	
Treatments	C1	C2	C1	C2	C1	C2	C1	C2
RBV[1]	1.14[a]	1.14[a]	1.03[b]	1.03[a]	0.98[b]	0.98[b]	**1.42[a]**	**1.42[a]**
IFNα	1.06[a]	1.02[a]	1.19[a]	1.08[a]	1.11[b]	1.15[b]	1.17[b]	**1.29[a]**
RBV + IFNα	1.01[a]	1.03[a]	1.17[a]	1.19[a]	**1.28[a]**	**1.27[a]**	**1.27[a,b]**	**1.42[a]**
F value	3.57 NS	5.55 NS	8.46	4.34 NS	10.0*	6.27*	9.0*	1.30 NS
CV (%)	6.21	4.65	4.60	6.34	7.21	8.95	5.62	8.48

Values in bold indicate a significant reduction in virus titer compared to the virus control ($P < 0.05$) and inhibitory activity ≥ 1 $\log_{10}$.

Averages represented by the same lower-case letter (column) and upper-case letter (row) did not differ by Tukey test.

C+ — untreated infected cells (positive control); [C] — treated infected cells; -1 h — pre-treatment; 0 h — adsorption; 1 h — penetration; 2 h — intracellular; RBV — ribavirin; IFNα — interferon-alpha; NS — not significant.

* Antiviral action is statistically significant by Tukey's test, $P < 0.05$.

[1] C1 = C2 = 12.2 μg/mL, single RBV concentration used in association with IFNα.

CV — coefficient of variance.

Table III. Combination effects and indexes of ribavirin (RBV) and interferon-alpha (IFNα) on intracellular stage of canine distemper virus (CDV), as obtained using CompuSyn software

			Drug combination (μg/mL)	
Drug	*Dm*[a] (μg/mL)	*m*[b]	Combination 1	Combination 2
RBV	0.28658	1.05287	12.2	12.2
IFNα	0.00386	0.85426	0.03	0.06
Effect			0.9356	0.9808
CI[c]			3.69045	1.17081

[a] Median-effect dose. Experiments were done in triplicate. Values represent the mean value.

[b] Slope, or kinetic order, of the dose-effect curve.

[c] Combination index. Synergy (CI $\leq$ 0.5), non-antagonism (0.5 $<$ CI $\leq$ 4), or antagonism (CI $>$ 4).

efficacy for interfering in the induction of the cellular antiviral state, showing little effect for most viruses. Accordingly, the concentrations of 0.03 μg/mL (9000 IU/mL) of IFNα and 0.06 μg/mL (18 000 IU/mL) considered in this study showed values higher than the potential suppression of the cellular antiviral state of CDV, alhough only the higher concentration resulted in significant inhibition of CDV. Interferon-alpha (IFNα) and RBV did not diverge on the antiviral efficacy against CDV.

The association of drugs with different mechanisms or modes of action may provide therapeutic synergism of several diseases simultaneously or enable more efficient performance over the same disease (32). Combining IFNα and RBV has been adopted as the first choice in treating patients with chronic hepatitis C and high viral load, due to the potentiation observed with the effects of the drugs involved (33). Having evaluated the association of these 2 compounds in antiviral assays against CDV, we can see that in addition to the individual drugs inhibiting the viral intracellular stage, the antiviral activity of the combined drugs was also effective in the extracellular phase of the replicative cycle.

Analysis of the combinatorial effects of RBV and IFNα in the post-infectious stage of CDV demonstrated the existence of a non-antagonistic relationship between the compounds, so that the effectiveness of antiviral activity of the combined drugs did not differ from the inhibitory potential demonstrated by the isolated drugs in the intracellular stage. The absence of synergy could be explained by viral inhibition through a common route to both compounds. The probable common route among these drugs that results in a non-antagonistic relationship against CDV, which is described in this study, is unknown, however, just as the precise mechanisms related to synergistic effects of IFNα and RBV against hepatitis C virus are unknown (34).

Another important result of the antiviral test of RBV + IFNα demonstrates viral inhibition in the CDV replicative cycle in the initial stage. This unexpected combinatorial effect provides greater antiviral efficacy for the combination RBV + IFNα, since it expands the spectrum of inhibitory interference in the CDV replicative cycle and enables the viral infection to be more dynamically controlled. As these isolated drugs have not yet been reported to have extracellular antiviral activity against CDV, the possible mechanisms related to the combination of RBV + IFNα and the results observed in the stage of viral penetration will require further studies in order to be elucidated. The existence of synergism between the compounds for extracellular viral inhibition cannot be ascertained due to the absence of significant antiviral activity of the isolated drugs at the penetration stage.

In conclusion, the compounds RBV, IFNα, and RBV + IFNα that were evaluated in this study showed antiviral efficacy against CDV. The compounds exhibited strong inhibitory potential for the intracellular stage of viral replication and the combinatorial effects

resulted in viral inhibition by intra- and extracellular pathways not yet elucidated. The combination of RBV and IFNα featured a greater therapeutic efficiency to broaden the spectrum of activity in the CDV replicative cycle. The evaluation of clinical applicability of drugs and combinations considered in this study is promising based on the antiviral efficacy of these compounds.

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