

EFFECT OF DEXAMETHASONE ON THE MEAN PLAQUE SIZE OF BOVINE HERPESVIRUS 1

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Résumé

EFFET DE LA DEXAMÉTHASONE SUR LA TAILLE MOYENNE DES PLAGES PRODUITES PAR LE BOVINE HERPESVIRUS 1. — L'effet de la dexaméthasone sur le virus de la rhinotrachéite infectieuse bovine (Bovine herpesvirus 1; BHV 1) a été étudié en mesurant la taille moyenne des plages produites en culture de cellules par huit souches de ce virus, soumises à deux concentrations de phosphate de dexaméthasone (1 mM et 0,1 mM). La dexaméthasone a produit une diminution significative de la taille moyenne des plages, pour toutes les souches virales étudiées. Des différences ont été observées dans le comportement des souches de BHV 1 en fonction de la concentration en dexaméthasone.

Infectious bovine rhinotracheitis virus (Bovine herpesvirus 1; BHV 1) is known to persist in a latent stage in cattle previously infected (Sheffy and Davies, 1972). Reactivation of latent BHV 1 can occur by means of several stimuli: viral superinfection (Mensik *et al.*, 1976), *Dictyocaulus viviparus* infestation (Msolla *et al.*, 1983), oral administration of 3-methylindole (Espinasse *et al.*, 1983) and by injection of glucocorticoids, especially dexamethasone (Pastoret *et al.*, 1979a).

Intermittent reactivations, leading to viral reexcursions, are observed in cattle, without external stimuli (Snowdon, 1965): this can be explained by the effect of increased level of endogenous glucocorticoids, for example in stressing conditions (Kent and Ewbank, 1983).

The mechanism of BHV 1 reactivation is a problem still unsolved. There are two main hypotheses: the first claims that BHV 1 is reactivated by depression of the immune system, induced by glucocorticoid treatment, for example (Roth and Kaerberle, 1982). The second hypothesis explains the viral reactivation by a direct effect of glucocor-

ticoids on the latently infected cell (Costa *et al.*, 1974; Pastoret, 1979).

In vitro experiments are needed to determine the exact mechanism of reactivation. Unfortunately, an *in vitro* model of BHV 1 latency is not available in cell culture. In this paper, the effect of dexamethasone was therefore investigated on acutely BHV 1-infected cells. The mean plaque size produced by BHV 1 in monolayer was compared between infected cell cultures treated with dexamethasone phosphate or not. This work extends the preliminary results obtained by Pastoret *et al.* (1979b), by the fact that we studied a set of eight plaque-purified BHV 1 strains and dexamethasone phosphate. The use of this soluble salt of dexamethasone ensures an equal distribution of the compound in the whole cell culture medium.

Materials and Methods

Cell culture and viruses

Georgia Bovine Kidney (GBK) cells were cultured in

Table 1. — Effect of dexamethasone on the mean plaque size of eight BHV 1 strains; transformed data (square roots).

Minimum Essential Medium (MEM) as previously described (Thiry *et al.*, 1981).

The BHV 1 strains used were: IBR/Cu3 isolated in Belgium from a case of metritis; IBR/Cu6 and IBR/Cu7 are Belgian respiratory isolates; IPV/3760 and IPV/2144 are Belgian genital isolates, kindly provided by Dr. G. Wellemans, Brussels, Belgium; IBR/LA is the Los Angeles strain; two vaccine strains were also used: thermosensitive *ts* RLB 106 strain (Zygraich *et al.*, 1974) and IBR-IPV vaccine strain from Bayer. Each BHV 1 strain was plaque-purified three times and passaged once or twice in cell culture before the experiment.

Production of plaques

Confluent cell monolayers, grown in Petri dishes (30 mm diameter) were inoculated with 0.2 ml of tenfold dilutions of each virus strain and incubated for 1 hour at 37 °C. Plaques were obtained under MEM supplemented with 5% of anti-BHV 1 bovine immunoserum (neutralizing titre: 1/64). For each virus strain, dexamethasone phosphate was added to the medium at 1 mM and 0.1 mM final concentrations. Control infected cells were also covered with medium devoid of dexamethasone phosphate. Preliminary experiments have shown that the concentrations used were not cytotoxic for confluent GBK cells. Dexamethasone-treated monolayers infected with *ts* RLB 106 strain were incubated at 35 °C and 37 °C. After four days, infected cells were fixed and stained with hydro-alcoholic solution of crystal violet. The experiment was carried out in two parts, the first involving IBR/Cu3, IBR/Cu6, IBR/Cu7 and IBR/LA strains; the second, IBR-IPV vaccine strain, IPV/3760 and IPV/2144 strains; *ts* RLB 106 strain at 35 and 37 °C.

Measurement of the mean plaque size

Cell cultures inoculated with the optimal viral dilution causing a sufficient amount of isolated plaques were chosen and the plaque areas were measured with a Leitz ASM image analysing system by the direct transmission of the image of plaques through the optical microscope to the Leitz ASM. For each virus strain and for each dexamethasone concentration, the area of 50 plaques was measured, except in two cases: control IPV/3760 strain (26 data) and *ts* RLB 106 strain at 37 °C for 1 mM dexamethasone (18 data). Each plaque area was measured three times and the arithmetic means were taken as data.

The normality of the plaque size distributions was tested for skewness and kurtosis. One-way analysis of variance and Duncan's test were used as statistical tests.

Results

To ensure a normal distribution in all cases, the square roots of the data were taken before variance analysis and Duncan's test. Addition of

	dexamethasone phosphate (mM)		
	0	0.1	1
IBR/Cu3			
N	50	50	50
mean	1.44	1.15	1.05
SD	0.34	0.33	0.31
Duncan	A ^a	B	B
IBR/Cu6			
N	50	50	50
mean	0.53	0.38	0.24
SD	0.09	0.06	0.05
Duncan	A	B	C
IBR/Cu7			
N	50	50	50
mean	1.47	1.07	0.95
SD	0.56	0.39	0.43
Duncan	A	B	B
IBR/LA			
N	50	50	50
mean	1.47	1.15	1.02
SD	0.39	0.33	0.32
Duncan	A	B	B
IBR-IPV vaccine strain			
N	50	50	50
mean	2.02	1.44	1.21
SD	0.26	0.41	0.34
Duncan	A	B	C
IPV/3760			
N	26	50	50
mean	1.79	1.29	1.16
SD	0.28	0.33	0.30
Duncan	A	B	B
IPV/2144			
N	50	50	50
mean	1.94	1.54	1.20
SD	0.46	0.38	0.29
Duncan	A	B	C
<i>ts</i> RLB 106 37° C			
N	50	50	18
mean	0.65	0.57	0.25
SD	0.14	0.27	0.05
Duncan	A	A	B
<i>ts</i> RLB 106 35° C			
N	50	50	50
mean	0.83	0.85	0.65
SD	0.19	0.19	0.14
Duncan	A	A	B

a: means with the same letter are not significantly different.

dexamethasone phosphate to the overlay medium influenced significantly the plaque size of each virus strain ($P < 0.001$). Duncan's test allowed the distinction to be made between two or three groups for each virus strain (table 1). In one case (ts RLB 106), the mean size of plaques obtained under 0.1 mM dexamethasone phosphate was not significantly different from control plaques; in other cases, the three plaque size populations were clearly distinguished ($P < 0.05$). Finally, some strains expressed no difference between the plaque size populations obtained under the two dexamethasone phosphate concentrations, which nevertheless significantly differed from control ($P < 0.05$) (table 1; fig. 1 and 2).

Discussion

The enhancing effect of dexamethasone on virus multiplication is well documented for retroviruses as mouse mammary tumour virus (Parks *et al.*, 1974). In this case, the direct effect of glucocorticoid on the proviral genome is demonstrated (Scheidereit *et al.*, 1983). The multiplication of other viruses, e.a. polyomavirus (Morhenn *et al.*, 1973), is also stimulated by corticosteroids.

The reports of the effects of glucocorticoids on

herpesviruses are controversial: the plaque size of herpes simplex virus 2 (HSV 2) is decreased or increased, depending on the virus strain or the cell culture used (Costa *et al.*, 1974). The multiplication of BHV 1 is enhanced with corticosterone (Hall and Minocha, 1977), but the plaque size is reduced with dexamethasone (Pastoret *et al.*, 1979b).

Our results confirm the decrease of the mean plaque size, whatever the strain used (fig. 1 and 2), in a different cell line from that used by Pastoret *et al.* (1979b). It remains to be investigated whether the cell culture system may influence the results as reported for HSV 2 by Costa *et al.* (1974).

If our overall results indicate an inhibiting effect of dexamethasone on the multiplication of BHV 1, some differences between strains can be shown: either no difference in the mean plaque size whatever the concentration of dexamethasone used (four out of eight strains), or no difference between control plaques, and plaques obtained under 0.1 mM dexamethasone (1/8), or significant differences between control and each concentration (three out of eight strains) (table 1).

The results obtained from our experiments and those reported above cannot solve the problem of

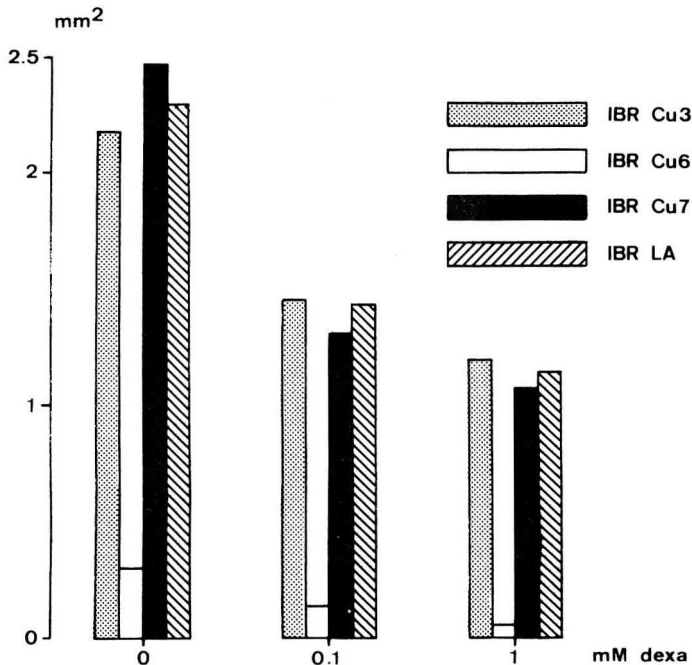


Fig. 1. — Effect of dexamethasone on the mean plaque size (expressed in mm^2) of BHV 1 strains; first part of the experiment. dexta: dexamethasone phosphate.

glucocorticoid action on BHV 1 reactivation. If a stimulating effect on virus multiplication had been described in all the reports, there would be no doubt about the direct action of glucocorticoid on latently infected cells. In addition, as pointed out by Pastoret *et al.* (1979b), the absence of stimulation does not exclude a possible cellular action of dexamethasone, because glucocorticoids act at a transcriptional level: transcription could start without enhancing the replication process.

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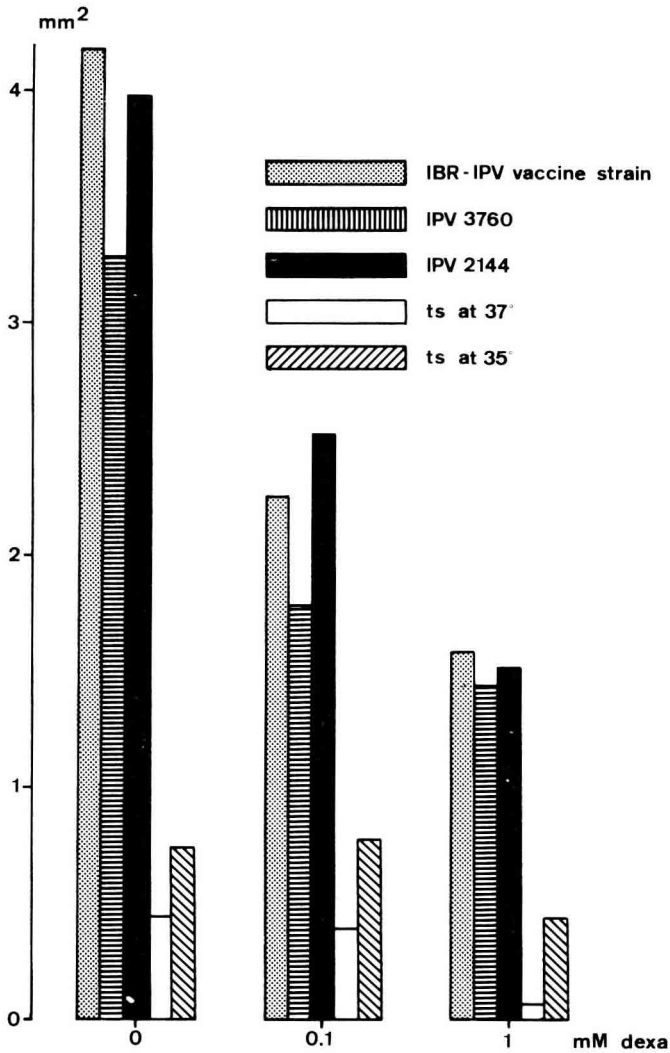


Fig. 2. — Effect of dexamethasone on the mean plaque size (expressed in mm²) of BHV 1 strains; second part of the experiment. dexa: dexamethasone phosphate.

Summary

The effect of dexamethasone on infectious bovine rhinotracheitis virus (Bovine herpesvirus 1; BHV 1) was studied by measuring the mean plaque size produced by eight virus strains under two concentrations of dexamethasone phosphate (0.1 and 1 mM). Dexamethasone induced a significant reduction of the mean plaque size, whatever the strain used. Some differences were noted between the BHV 1 strains studied, depending on the dexamethasone concentration.

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