

Protection of cattle from infection with vaccinia virus by bovine interferon α C

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The efficacy of bovine interferon α C as an antiviral compound was evaluated in calves infected with vaccinia virus. Calves treated with the interferon did not develop characteristic vaccinia lesions, demonstrating a clear protective effect of treatment. The only side effect observed was hyperthermia. After intramuscular injection, interferon appeared quickly in the blood and was cleared within 24 hours. The *in vivo* antiviral activity of bovine interferon α C was confirmed by its ability to induce 2'5'A synthetase, an enzyme implicated in the antiviral state, in lymphocytes.

VIRAL diseases cause important economic losses in cattle and a broad spectrum antiviral compound would therefore be of great value. In 1982, the authors obtained the first evidence for the efficacy of interferon as an antiviral agent in cattle (Werenne and others 1983). At that time, the only interferon available in large quantities was genetically engineered human interferon α 2. Bovine interferon has now been expressed in bacteria (Capon and others 1985, Velan and others 1985) and this interferon exerts antiviral activity *in vitro* (Gillespie and others 1985, Fulton and others 1986, Shafferman and others 1987) and can be tested *in vivo*. There are some advantages in using homologous interferon; bovine interferon is probably more effective than non-homologous interferon for the treatment of viral diseases in cattle and the use of human interferon in cattle may lead to antibody production, thus limiting its repeated use (Roney and others 1985).

Experimental infection of calves with vaccinia virus is a reliable system with which to test the potential value of interferon (Werenne and others 1983, 1985) because the infection is localised at the site of injection and can be monitored during its development. The aims of the present study were to determine whether interferon α C was effective against experimental vaccinia virus infection in calves, to follow its pharmacokinetics and to monitor its ability to induce the antiviral enzyme 2'5'A synthetase in lymphocytes.

Materials and methods

Six calves about six months old were used for the experiment; two were infected with vaccinia virus, two were infected and treated with interferon, one was uninfected and treated with interferon and one served as an untreated control. On day 0, the calves were inoculated intradermally on a shaved area on the flank (12 sites on each animal) using a bifurcated needle, with vaccinia virus (strain Elstree, IHE, Brussels, 3×10^8 plaque forming units/ml) or with phosphate

buffered saline. A similar series of mock inoculations of phosphate buffered saline was performed in each animal as an internal control.

Bovine interferon α C (10^8 U/ml, purity 98 per cent, specific activity 2×10^8 U/mg protein) at a dose of 10^6 U/kg bodyweight was injected intramuscularly daily for seven consecutive days, starting on day -1. Untreated animals received intramuscular injections of equivalent volumes of phosphate buffered saline.

Skin lesions were photographed daily and scored on an arbitrary scale. The size of the vaccinia lesions was the main objective criterion used for scoring. Other factors, however, are directly related to the pathogenic effect of the virus and they were taken into account by evaluating the clinical stage of the infection from the visual aspect of the lesions (from papula to crust). The score derived from measurements of the lesions was modulated in this way so that the regression of the severity of the signs could be followed after their maximal intensity had been reached, although at this stage the size of the lesions, including an inflammatory zone, had not begun to decrease. Because the evaluations were to some extent subjective, two independent observers scored the lesions separately, using their own criteria, and allocating a score of 6 to the most advanced stage of the lesions. These scores could easily be normalised from one experiment to the other by using photographs taken during the previous studies. The two independent scores for each lesion were then averaged and a total score for each animal was obtained summing the mean scores of each individual lesion, thus taking into account the number of positive infection sites.

Plasma samples for the determination of circulating antiviral activity were collected on day -1 immediately after and then one, two, five, eight, 12, 16, 20 and 24 hours after the injection of interferon, on days 0 to 4 just before the injection of interferon, on day 5 just before and two and five hours after the injection of interferon and on days 6, 7, 8, 11 and 14 just before the injection of interferon. Lymphocytes were prepared from each blood sample except when complete pharmacokinetic studies were being performed.

Body temperature was measured just before each blood sample was taken.

Circulating antiviral activity was determined by the technique of Stewart (1979). 2'5'A synthetase was assayed by the procedure of Vanden Broecke and others (1985).

Results

Fig 1 shows the development of the total lesion score during the course of the experiment. In two untreated calves the vaccinia lesions appeared at each site of inoculation within 48 to 72 hours and reached a maximum after six to eight days. In both treated calves a clear protective effect of interferon was observed. Most inoculation sites did not develop any lesions and only a few very small crusts appeared after five to eight days and these healed within two to three days. There were no lesions either in the two uninoculated controls or at the sites of mock inoculation in the infected animals.

Interferon treatment was well tolerated although a significant hyperthermia was observed in the treated animals, with a maximum (about $+1^\circ$ C) five to eight hours after the injection of interferon.

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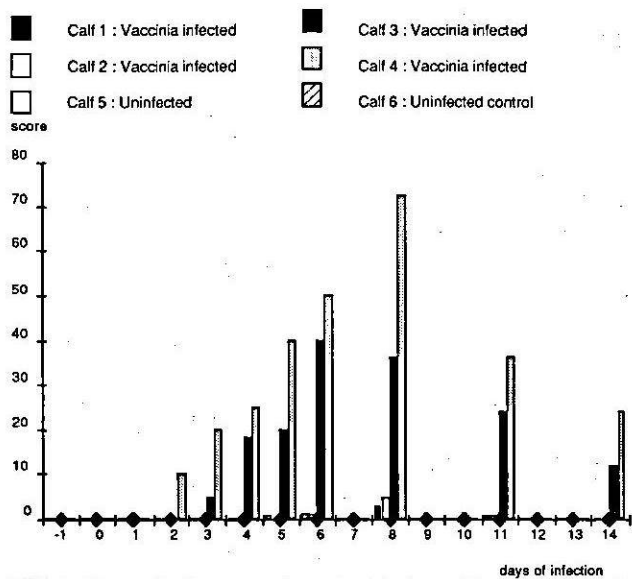


FIG 1: Normalised scores of vaccinia lesions. Normalisation takes into account both the score of the individual infection sites and the total number of such sites

No circulating antiviral activity was detected before the beginning of treatment. Interferon appeared quickly in the circulation, reached a peak two to five hours after injection (100 to 120 U/ml) and was completely cleared within 24 hours. No accumulation of interferon in blood was observed after repeated treatment. Bovine interferon α C induced 2'5'A synthetase in the lymphocytes of treated animals (Fig 2). The enzyme level increased gradually three to five days after the beginning of treatment and remained high for at least 48 hours after the last injection of interferon. Untreated controls never showed any detectable circulating antiviral activity and there was no increase in their basal level of 2'5'A synthetase.

Discussion

Bovine interferon α C, like human interferon α 2, (Werenne and others 1985) was well tolerated and clearly active against experimental vaccinia virus infection in calves. As shown in Fig 1, the interferon treated animals developed only a few short-lived lesions at two or three inoculation sites.

As observed for human interferon, bovine interferon appeared rapidly in the circulation and was completely cleared within 24 hours, justifying a daily treatment schedule. A good correlation was observed between the maximal level of circulating antiviral activity and the peak of body temperature; both occurred about five hours after the intramuscular injection of interferon.

The ability to induce 2'5'A synthetase in lymphocytes is considered to be a criterion for the biological activity of interferon in vivo (Vanden Broecke and others 1985). The enzyme appeared within two to three days after the beginning of interferon treatment. In contrast vaccinia infection had no detectable effect on the basal level of the enzyme in the two untreated calves; this correlates well with the observation that no endogenous interferon was induced in these animals.

The present data provide clear evidence that bovine interferon α C is biologically active in cattle in vivo, whereas no effect of interferon on vaccinia virus is detectable in vitro (Schellekens and others 1979). Thus in vivo trials are essential for the assessment of the potential of bovine interferon as an antiviral compound.

Although Babiuk and others (1985) reported that bovine interferon had no significant effect on the excretion of bovine rhinotracheitis virus by experimentally infected cattle, the evidence presented here suggests that bovine interferon α C is a promising compound for the protection of cattle against some viral infections.

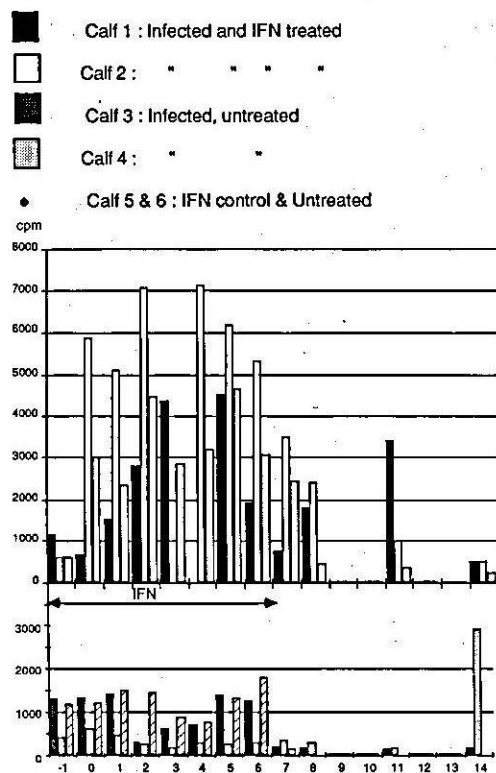


FIG 2: 2'5'A synthetase activity in lymphocytes. Blood samples were taken at the indicated days (at day -1 to 6 the samples were taken just before interferon treatment). Lymphocytes were isolated from buffy coat by Ficoll-Hypaque density gradient and cell extract were prepared for 2'5'A synthetase assay, which was measured by monitoring the polymerisation of tritiated adenosine triphosphate into ppp(2'Ap)nA oligomeres (14, 15). At the end of the experiment (after day 8) calf 6 showed an increased 2'5'A synthetase activity due to an accidental infection unrelated to the present study. Those values are therefore not included in the figure. Top: interferon treated calves. Bottom: control animals

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