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EXPERIMENTAL ROTAVIRUS DIARRHOEA IN COLOSTRUM-DEPRIVED NEWBORN CALVES: ASSAY OF TREATMENT BY ADMINISTRATION OF BACTERIALLY PRODUCED HUMAN INTERFERON (Hu-IFN α 2)

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Résumé

DIARRHÉE EXPÉRIMENTALE À ROTAVIRUS CHEZ LE VEAU NOUVEAU-NÉ: ESSAI DE TRAITEMENT PAR L'ADMINISTRATION D'INTERFÉRON HUMAIN α 2 PRODUIT EN BACTÉRIES. — Sept veaux nouveau-nés privés de colostrum ont été inoculés oralement, dans les 24 heures suivant la naissance, avec du rotavirus bovin. Trois d'entre eux ont reçu des injections intramusculaires d'interféron humain α 2 produit en bactéries (10^6 UI/kg par jour). Les quatre animaux témoins ont souffert de diarrhée pendant 48 heures au moins, tandis qu'un seul des trois veaux traités a présenté un bref épisode de diarrhée. L'interféron humain α 2 produit en bactéries semble donc à même de contrôler efficacement la diarrhée à rotavirus chez le jeune veau, bien qu'il n'empêche ni l'excrétion de particules infectieuses ni l'apparition d'anticorps spécifiques. Par ailleurs, le traitement a été parfaitement toléré par les trois animaux. L'efficacité de l'interféron humain α 2 dans le traitement de cette importante infection virale du bétail semble dès lors démontrée.

Bacterially produced human interferon Hu-IFN α 2 (Nagata *et al.*, 1980) was shown to be non toxic and clearly active *in vivo* against experimental vaccinia virus infection in young calves (Goossens *et al.*, 1982; Wérenne *et al.*, 1983, 1985). A daily treatment schedule at a dose of 10^6 IU/kg body weight provided complete protection in some animals. Although this experimental system was particularly convenient to quantify the protection conferred by interferon treatment, it remained to determine if human interferon might be useful for the treatment of some important viral diseases of the calf.

Bovine rotavirus infection is one of the major causes of diarrhoea in newborn calves (Mebus *et al.*, 1971; Mc Nulty, 1978; Flewett and Woode, 1978; Scherrer and Cohen, 1978) and is respon-

sible for important economical losses for breeders. Bovine rotavirus was shown to be highly sensitive to the antiviral effect of conventionally obtained human α interferon (Dagenais *et al.*, 1981) as well as of bacterially produced Hu-IFN α 2 (Goossens *et al.*, 1984) *in vitro*, in cell cultures of bovine or simian origin, although it appears more resistant to the effect of bovine interferon (La Bonnardière *et al.*, 1980). Moreover, the endogenous interferon response of calves experimentally infected with bovine rotavirus seems to play an important role in the control of rotavirus pathogenicity (Schwers *et al.*, 1983).

Indeed, in calves inoculated with high doses of bovine rotavirus, circulating interferon appeared very early and no diarrhoea was observed, while in calves inoculated with lower virus doses, interfe-

ron production was delayed and preceded by a severe episode of diarrhoea (Vanden Broecke *et al.*, 1984).

Therefore, experimental bovine rotavirus infection of newborn calves was chosen as a model to test the *in vivo* antiviral efficiency of bacterially produced Hu-IFN $\alpha 2$ (Nagata *et al.*, 1980) against an ubiquitous viral disease of calves.

Materials and Methods

Virus and cell cultures

Rotavirus strain 81/36F, adapted in cell culture but still strongly pathogenic for newborn calves (Castrucci *et al.*, 1983a and b), was kindly supplied by Professor G. Castrucci, from the University of Perugia, Italy. It was used after nine passages on rhesus monkey kidney cells MA 104 and one passage on Georgia bovine kidney cells GBK. Virus titration was performed by plaque assay on MA 104 cells, following the technique of Matsuno *et al.* (1977).

Interferon

Bacterially produced human $\alpha 2$ interferon (Hu-IFN $\alpha 2$) (Nagata *et al.*, 1980) was kindly provided by Drs. C. Weissmann and M. Fountoulakis, from the University of Zürich. Its specific activity was 10^8 IU/mg of protein and it only contained 5.5 ng of pyrogen per 10^8 IU.

Experimental procedure

Seven colostrum-deprived newborn calves were used in this experiment. All of them were isolated immediately after birth in individual containers in a decontaminated area, as previously described (Schwers *et al.*, 1983). They were fed daily two to three times with fresh cow milk. They were inoculated orally, 2 to 24 hours after birth, with 100 ml of a suspension containing approximately 10^8 PFU/ml of rotavirus 81/36F in minimum essential medium (MEM). Two calves (T3 and IFN2) received on day 1 one meal composed of a mixture of milk and colostrum.

Hu-IFN $\alpha 2$ in phosphate buffer saline was intramuscularly injected daily at a dose of 10^8 IU/kg body weight, under a total volume of 1 ml per injection; this dose was shown to be well tolerated in older calves and seemed the minimal dose able to protect against experimental viral infection (Wérenne *et al.*, 1983, 1985). A daily treatment schedule was chosen as interferon at this dose is completely cleared from the circulation after 24 hours (Schwers *et al.*, 1982; Wérenne *et al.*, 1985).

Four calves (T1 to T4) served as untreated controls. One calf (IFN 1) was treated on days - 1, 0, 1 and 2 and two calves (IFN 2 and 3) on days 0, 1 and 2, day 0 representing the day of inoculation. Plasma samples for determination of circulating antiviral activity were collected 0, 2, 5, 8 and 24 hours after at least one interferon injection for each calf, before and 24 hours after the other interferon injections and then twice a day until day 10.

Serum samples were taken on days 0, 8 and 15, except for calves T2 and IFN 1. Stool samples were collected twice daily for ten days after inoculation.

Bacteriological examinations

Meconium samples taken before inoculation and stool samples from the beginning of diarrhoea were controlled for the presence of enteropathogenic *Escherichia coli* or salmonella.

Counterimmunoelectrosmoporesis and virus isolation

Each stool sample was tested for the presence of rotavirus by counterimmunoelectrosmoporesis (CIEOP), following the method of Middleton *et al.* (1976); antirotavirus antibodies were detected by the same technique, as previously described (Schwers *et al.*, 1983). Virus isolation on MA 104 cells was performed on at least one stool sample per calf and per day, following a previously described method (Babiuk *et al.*, 1977). Positive results were controlled by CIEOP.

Interferon titration

Circulating antiviral activity was determined by the classical technique of reduction of cytopathic effect of vesicular stomatitis virus (VSV) in GBK cells (Stewart, 1979).

Results

Clinical observations

Diarrhoea appeared in the control calves 36 to 72 hours after inoculation (fig. 1), excepted for calf T3, which received on day 1 a mixture of milk and colostrum: in this calf, the onset of diarrhoea was delayed until day 6. Diarrhoea persisted for at least 48 hours. One calf (T2) became severely depressed and dehydrated and died on day 5: even if he did not present any symptom of septicaemia, *E. coli* was recovered from blood at necropsy. The remaining animals recovered spontaneously.

From the three treated calves, only one (IFN 3) presented a transient diarrhoea for less than 12 hours on day 4, while the others remained clinically normal. No toxicity was observed for interferon.

Circulating interferon

In control calves, multiple peaks of interferon activity were observed in plasma (fig. 2), except for calf T2, in which interferon activity remained at a constant level from day 1 until the death of the animal.

In the interferon treated animals, interferon appeared in the circulation very rapidly after each injection and was completely cleared within 24 hours (fig. 2). Two further weak peaks of antiviral activity were observed in these three calves around days 5 and 9.

Bacteriological examinations

No enteropathogenic *E. coli* or salmonella was recovered from meconium or stool samples from the seven calves.

Virus excretion

All the inoculated calves excreted rotavirus, as shown by CIEOP and virus isolation on MA 104 cells (fig. 1). Virus excretion began 24 to 48 hours after inoculation in the four control animals and in

two from the three treated calves; in calf IFN 1, for which interferon treatment began 24 hours before inoculation, the beginning of virus excretion was delayed until day 5.

Moreover, although the CIEOP technique is not quantitative, the level of viral excretion appeared

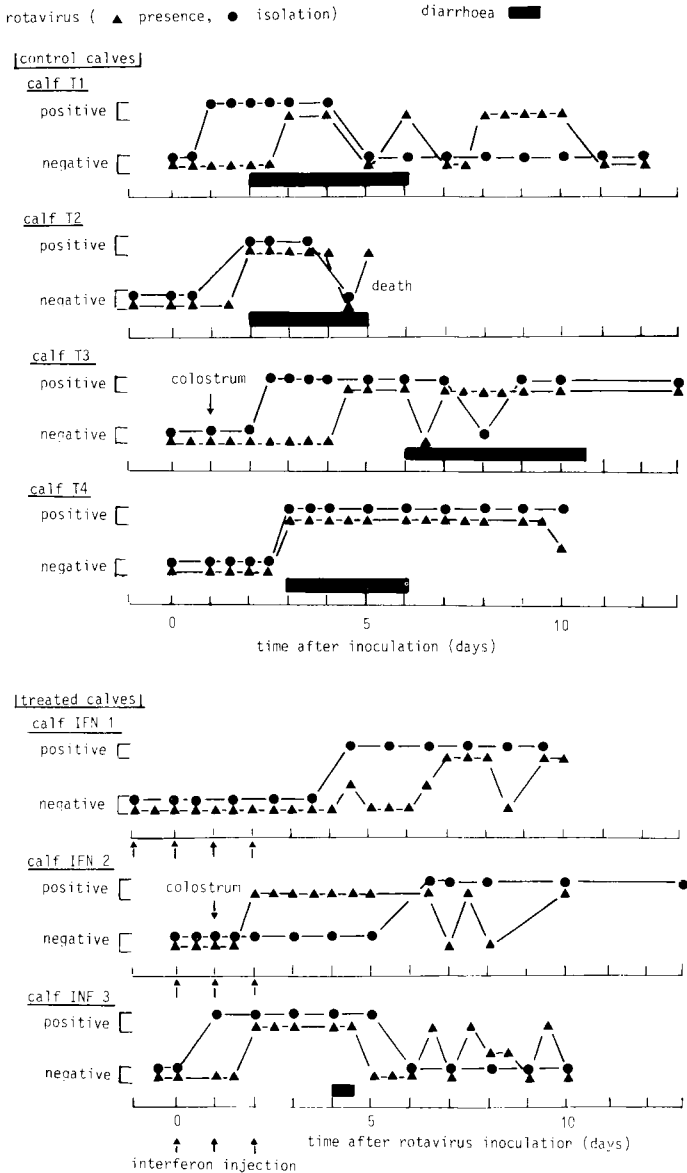


Fig. 1. — Virus excretion in control and treated calves.

lower in treated than in control calves, precipitation bands being often at the limit of detection.

Anti-rotavirus antibodies

None of the animals possessed anti-rotavirus antibodies at birth. Seroconversion was observed on day 8 (T3, T4) or 15 (T1) for all the control calves, except calf T2, which died on day 6; in treated animals, specific antibodies were detected respectively on day 15 (IFN 2) and 22 (IFN 3), while for calf IFN 1, no serum was obtained later than day 8.

Discussion

Bacterially produced Hu-IFN $\alpha 2$ seems to be able to control rotavirus diarrhoea in colostrum-deprived newborn calves, as only one of the three treated calves presented a transient diarrhoea for a few hours, while all the control animals suffered from diarrhoea for at least two days.

This confirms the significant role of endogenous interferon in the control of rotavirus pathogenicity in newborn calf (Mc Kimm-Breschkin and Holmes, 1982; Schwers *et al.*, 1983; Vanden Broecke *et al.*, 1984).

Interferon treatment did not inhibit completely virus replication, as infectious virus was still

excreted in faeces and as seroconversion was observed in the two treated calves tested for anti-rotavirus antibodies. However, interferon seemed to reduce the level of rotavirus excretion in stools. Unfortunately, bovine rotavirus isolation in cell culture usually needs one or two blind passages before obtaining a characteristic cytopathic effect (Babiuk *et al.*, 1977), and direct titration of viral antigen in faeces with a technique like ELISA could provide no information concerning the excretion of infectious virus particles: it is therefore difficult to evaluate the epidemiological consequences of interferon treatment.

Although Hu-IFN $\alpha 2$ was active in all the treated animals, individual variations were observed in the sensitivity to interferon: with the same treatment schedule, one calf (IFN 2) was completely protected while a second one (IFN 3) suffered from a transient diarrhoea. Such differences in individual sensitivity were already noticed in a preliminary study on the effect of Hu-IFN $\alpha 2$ against experimental vaccinia virus infection in calves (Wérenne *et al.*, 1983, 1985).

A daily interferon dose of 10^6 IU/kg body weight was perfectly well tolerated in newborn calves: no side effects were observed.

The kinetics of plasmatic interferon in the control animals was similar to that observed in a previous study using another bovine rotavirus strain (Vanden Broecke *et al.*, 1984), with multiple peaks of endogenous interferon; only calf T2 presented a constant level of antiviral activity until its death on day 5: we have no explanation to suggest for that very peculiar type of kinetics.

As already observed in older calves (Schwers *et al.*, 1982; Wérenne *et al.*, 1985), interferon appeared quite rapidly in the circulation of the three Hu-IFN $\alpha 2$ treated animals and was completely cleared from it within 24 hours after each injection, justifying a daily treatment schedule. After the end of treatment, around days 5 and 9, these animals presented two further peaks of antiviral activity, probably due to endogenous interferon production.

This is the first report of an efficient protection against an economically important viral disease in cattle obtained by another mean than the classical approach of vaccination. Moreover, interferon appears active when administered at the time of infection. If such preliminary results are confirmed, interferon might become a useful antiviral drug in veterinary medicine, at least if it becomes available in large amounts and is not too expensive.

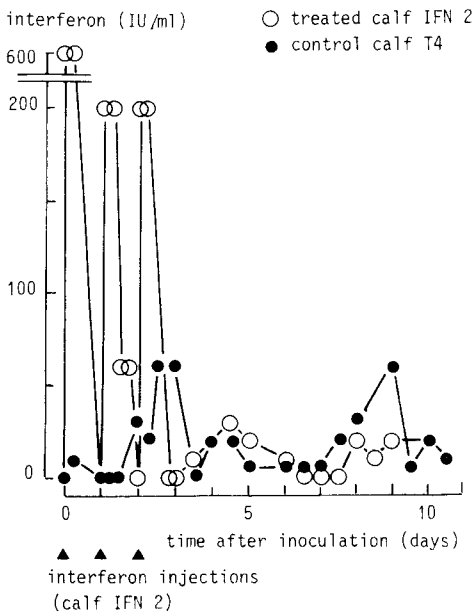


Fig. 2. — Kinetics of circulating interferon activity in one control and one treated calf.

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Summary

Seven colostrum-deprived newborn calves were orally inoculated within 24 hours after birth with bovine rotavirus. Three of them were intramuscularly injected with bacterially produced human interferon (Hu-IFN α 2). The four control animals presented a severe diarrhoea for at least 48 hours, while only one of the treated calves suffered from a transient diarrhoea for a few hours. Hu-IFN α 2 seems therefore able to control rotavirus diarrhoea in newborn calves, although it did not inhibit virus excretion and seroconversion in the treated animals. Moreover, the administration of endogenous interferon appeared to be well tolerated by newborn calves. The efficacy of human α 2 interferon for the treatment of this important virus infection of cattle seems thus well established.

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