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The inhibitory effect of the imidazoquinolinamine S-28828 on the pathogenesis of a type II adenovirus in turkeys

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Abstract

In this study we show that a type I-IFN inducing compound, S-28828, modulated the pathogenesis of an avian type II adenovirus in turkeys. By itself, S-28828 induced a strong reaction in the spleen characterized by hyperplasia of the red and white pulps as well as an increase in lymphoid cell aggregations. Oral administration of S-28828 before the time of virus inoculation suppressed significantly ($P < 0.05$) the replication of hemorrhagic enteritis virus (HEV) in turkeys. Two doses of 5 or 50 mg of S-28828 administered at 2 days before and at the day of virus inoculation inhibited HEV-induced pathological and histopathological lesions. Virus-induced apoptosis and reduced IgM-surface expression of B cells were suppressed by low dose S-28828 treatment. These results are of interest because mammalian adenoviruses were shown to be resistant to antiviral effects of type I IFN, the major effector cytokine induced by S-28828.

Keywords: Adenovirus; Turkeys; Imidazoquinolinamine

1. Introduction

S-28828 is a synthetic compound belonging to the family of imidazoquinolinamines and has very potent antitumor and antiviral activities (Miller et al., 1985; Borden et al., 1991; Harrison et al., 1991; Sidky et al., 1992; Bernstein et al., 1993; Miller et al., 1995). Imidazoquinolinamines have been shown to be effective antiviral agents against herpes simplex virus (HSV) type 2 genital infections (Bernstein and Harrison, 1989; Harrison et al., 1991; Bernstein et al., 1993), cytomegalovirus (CMV) infection in guinea pigs (Chen et al., 1988) and arbovirus infection in mice (Kende et al., 1988). Drug treatment reduced vaginal viral replication, completely protected against primary disease and reduced recurrent genital HSV disease (Harrison et al., 1988; Bernstein et al., 1995). Interferon (IFN)-α plays an important role in the overall biological activity of imidazoquinolinamines in mammals (Weeks and Gibson, 1993; Reiter et al., 1994). Other cytokines such as tumor necrosis factor (TNF)-α, interleukin-6 (IL-6), IL-8

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and chemokines have also been shown to be induced by imidazoquinolinamines (Weeks and Gibson, 1993; Reiter et al., 1994).

S-28828 was recently used in turkeys to induce high levels of type I IFN in vivo after oral application (Rautenschlein et al., 1998a). In vitro stimulation of various turkey immune cell populations with S-28828 also stimulated the release of type I IFN in culture supernatants (Rautenschlein et al., 1998a). S-28828 induced circulating IFN in a dose-dependent manner while 100 mg/kg body weight (BW) induced the highest IFN levels in the serum. Repeated daily treatment of turkeys with 100 mg/kg body weight of S-28828 induced a refractory stage in IFN-producing cells and the circulating levels of IFN went down after every treatment (Reiter et al., 1994; Rautenschlein et al., 1998a).

Wild-type adenoviruses have been shown to be resistant to antiviral effects of type I IFN (Kitajewski et al., 1986; Anderson and Fennie, 1987; Reich et al., 1988; Gutch and Reich, 1991). The effects of imidazoquinolinamines on adenoviruses have not been examined, because they were thought to be resistant to imidazoquinolinamine induced type I IFN effects.

One important viral disease in turkey flocks is the infection with hemorrhagic enteritis virus (HEV). HEV together with the marble spleen disease virus (MSDV) of pheasants and the splenomegaly virus of chickens belongs to the genus of avian adenovirus type II. In turkeys, infection with HEV results in an acute disease characterized clinically by depression of the birds and ruffled feathers. Further, splenomegaly and intestinal hemorrhage are typical pathological lesions and immunosuppression can be observed (Domermuth and Gross, 1991). Although hemorrhagic enteritis (HE) has been recognized since 1937 (Pomeroy and Fenstermacher, 1937), the pathological mechanisms of HE and immunosuppression are not fully known. Complete records of losses from HE have not been kept, however, estimates within the USA exceeded $3 million per year prior to development of a vaccine. Losses due to immunosuppression and subsequent secondary bacterial infections may actually have been much higher and do still occur despite vaccination (Pierson and Domermuth, 1997).

Our preliminary data indicate that cytokines may play an important role in the pathogenesis of hemorrhagic enteritis (HE) in turkeys as they do in wild-type mammalian adenovirus infections (Mistchenko et al., 1994; Rautenschlein and Sharma, 1999).

The objective of this research was to study the effect of S-28828 on HEV. S-28828-treated and untreated turkeys were exposed to HEV and virus-induced pathological lesions and virus replication in tissues were compared.

2. Material and methods

2.1. Turkeys

Specific-pathogen-free (SPF) turkey eggs lacking maternal antibodies against HEV were obtained from the National Animal Disease Center, Ames, IA. Because of the non-availability of SPF-turkeys for all the experiments, commercial turkeys were used as well. Commercial turkey eggs positive for maternal antibodies against HEV were kindly donated by Willmar Poultry Co., Willmar, MN. All turkeys were hatched and reared for the duration of the study in Horsfall–Bauer-type isolation units. The birds were provided with food and water ad libitum.

2.2. Viruses

Virulent HEV was kindly provided by K. Nazerian (Avian Disease and Oncology Laboratory, East Lansing, MI). It was propagated in SPF turkeys and titrated in MDTC-RP19 cells following previously published procedures (Nazerian and Fadly, 1982; Rautenschlein et al., 1998b). HEVp30, a cell culture adapted strain of HEV was prepared by passaging HEV thirty times in MDTC-RP19 cells (Sharma, 1994) and titrated in MDTC-RP19 cells (Nazerian and Fadly, 1982; Rautenschlein et al., 1998b).

2.3. Imidazoquinolinamine

S-28828 is 1-*n*-butyl-ethoxymethyl-1*H*-imidazo[4,5-c]quinolin-4-amine-hydrochloride, syn-

thesized by 3M Pharmaceuticals, was dissolved at 50°C in distilled water immediately before administration or stored in solution at −20°C.

2.4. Monoclonal antibodies

The following monoclonal antibodies against chicken mononuclear cells were used for surface staining in immunohistochemistry: anti μ chain of chicken IgM cross-reacting with turkey (Suresh and Sharma, 1995) was a gift from N. Dren, Hungarian Academy of Science, Budapest, Hungary; C9 reacted against HEV positive cells (Rautenschlein, 1998).

2.5. IFN assay

The IFN assay was performed following described procedures (Sekellik and Marcus, 1986; Karaca et al., 1996). Briefly, secondary turkey embryo fibroblasts (TEFs) were plated over night at a cell density of 4×10^5 cells/ml in Leibovitz's L-15 and McCoys 5A medium (1:2) supplemented with antibiotics (50 U/ml penicillin and 50 μg/ml streptomycin; Sigma, St. Louis, MO) and 1% fetal bovine serum (FCS; Sigma). The TEFs were incubated with serially diluted test samples at 37°C for 16–24 h. The test culture fluids were replaced with medium containing vesicular stomatitis virus (VSV) (4×10^4 $TCID_{50}$/well) and incubated for 48 h. Virus-induced cytopathic effect (CPE) was determined after fixation of the cells with methanol for 5 min and crystal violet staining for 10 min. The IFN activity of the test samples is expressed in units/ml (U/ml). One unit is defined as the highest dilution of the sample that caused 100% protection against VSV-induced CPE (Karaca et al., 1996).

2.6. In situ apoptotis detection

Formalin-fixed spleen tissue was paraffin embedded. After deparaffinization, the apoptotic cells in spleen sections (4 μm) were stained by the TUNEL method following a modified procedure published by the supplier of the ApopTac® Kit (Oncor, Gaithersburd, MD). Briefly, paraffin-embedded sections were deparaffinized in xylene and rehydrated in descending grades of ethanol. After washing in phosphate-buffered saline (PBS; 50 mM sodium phosphate, pH 7.4, 200 mM NaCl), the tissues were digested for 30 min at 37°C with 20 μg/ml Proteinase K (Sigma, St. Louis, MO). After four washings in distilled water, the endogenous peroxidase in the spleen sections was quenched with 2.0% H_2O_2 in PBS for 5 min at room temperature (RT). After rinsing the specimen twice with PBS for 5 min each time, the tissues were equilibrated with 1 X equilibration buffer provided by the manufacturer. Further, the sections were incubated for 1 h at 37°C with working strength terminal deoxynucleotidyl transferase (TdT) enzyme in a humidified chamber. After stopping the reaction with working strength stop/wash buffer provided by the manufacturer for 10 min at RT, the tissue sections were washed with PBS. The sections were incubated for 30 min with 50 μl per section of anti-digoxigenin-peroxidase (150 U/ml; Boehringer Mannheim). Following three washings with PBS, slides were tested for peroxidase activity with 3,3′-diaminobenzidine-tetrahydrochloride (DAB; Sigma) 0.5 mg/ml in Tris–HCl buffer (0.05 M, pH 7.6) containing H_2O_2 0.01%. Slides were counterstained with haematoxylin and mounted under a coverslip with Permount (Fisher). The number of apoptotic cells was counted at ×400 in ten microscopic fields.

2.7. Immunohistochemistry and H&E staining

By immunohistochemical staining, the number of HEV-positive cells as well as the distribution of IgM + cells was determined (Rautenschlein et al., 1998b). Briefly, tissue sections (4 μm) were fixed in acetone, air-dried and incubated for 1 h at RT with monoclonal antibodies. After rinsing the slides in PBS (0.01 M NaCl, pH 7.4), the tissue sections were incubated for 1 h with goat-anti-mouse immunoglobulin peroxidase conjugate (Sigma) containing bovine serum albumin 0.2%. After rinsing, slides were tested for peroxidase activity with DAB 0.5 mg/ml in Tris–HCl buffer (0.05 M, pH 7.6) containing H_2O_2 0.01%. Control slides were treated as described above, except that the first incubation step was omitted. The slides were counterstained with haematoxylin, dehy-

drated and mounted with Permount. The tissues were examined for surface labeling of specific cell markers and intracellular labeling for HEV. The HEV-positive cells were counted in ten randomly chosen fields at a magnification of $\times 400$.

Further, formalin hyp fixed spleen tissues were stained with H&E for histopathological examination. Lymphoid cell hyperplasia of the white pulp was determined by comparing the ratio between red and white pulp areas of the spleens between control birds and infected or drug-treated birds. Follicle-like lymphocyte aggregations were counted in five to ten randomly chosen microscopical fields of spleen sections at a magnification of $\times 100$ and the group average $\pm$ S.D. was calculated.

2.8. Statistics

The experiments were analyzed by the Analysis of Variance (ANOVA), Kruskal Wallis test and the chi-square test. $P < 0.05$ was considered to be significant.

2.9. Experimental design

In a preliminary experiment (Experiment 1), turkeys were inoculated with different dosages and inoculation schedules of S-28828. Groups of six birds received either 5 or 50 mg/kg of S-28828 orally on days 1 and 3 or 100 mg/kg of S-28828 on days 3, 4 and 5. The negative control group of three birds was inoculated orally with diluent on day 1. Serum samples were collected daily from each group after 2 h of each drug-inoculation and tested for circulating IFN by the VSV protection assay. At days 6–7, birds were sacrificed and pathological lesions and the spleen body weight ratios were determined. Further, spleen sections were stained with H&E and the pathohistological lesions were observed.

The response of turkeys given S-28828 alone or in combination with HEV or HEVp30 was examined in two experiments (Experiments 2 and 3).

In Experiment 2, three groups of 20 2-week-old SPF turkeys each were orally inoculated at day 0 (Group 1), 1 (Group 2) or 2 (Group 3) with one dose of S-28828 (100 mg/kg BW/dose). This dose was previously shown to induce the highest circulating IFN levels (Rautenschlein et al., 1998a). Twenty control turkeys received PBS orally at day 0 (Group 4). Ten turkeys from each of the four groups were inoculated at day 1 with HEVp30 (10^4 $TCID_{50}$/bird) orally. HEVp30 was chosen for this experiment because of its low viral replication in turkeys in order to be able to identify numerical differences in the number of virus-positive cells following S-28828 treatment. Serum samples were collected at 2 h after each S-28828 inoculation and tested for circulating IFN levels. The birds from each group were sacrificed at 3 and 4 days post infection (PI) and the spleens harvested, snap frozen in liquid nitrogen and examined for the presence of HEV antigen by immunohistochemistry.

In Experiment 3, two groups each of 15 and one group of 12, 5-week-old commercial turkeys, free of anti-HEV antibodies detectable by ELISA (KPL, Gaithersburg, MD), were used. The two groups of 15 turkeys were inoculated orally each with 5 or 50 mg of S-28828/kg BW at day -2 and 0. The control group of 12 turkeys was each given PBS orally at day -2 and 0. At day 0, six control turkeys and nine turkeys from each group inoculated with S-28828 received 10^3 $TCID_{50}$/bird of HEV orally. At days 3 and 4 PI, when the peak of HEV-induced lesions was expected (Domermuth and Gross, 1991; Saunders et al., 1993; Rautenschlein et al., 1998a), three to five birds per group were examined for pathological lesions and spleen to body weight (BW) ratios. Spleen sections were examined by immunohistochemistry for HEV and IgM surface expression. A piece of spleen tissue was examined for histopathological lesions and apoptosis.

3. Results

3.1. Effect of S-28828 on turkeys

Previous results demonstrated that 100 mg/kg BW of S-28828 induced the highest levels of circulating IFN in the serum (Rautenschlein et al., 1998a). In Table 1, different inoculation schedules and doses were compared not only in their IFN

Table 1
Effect of S-28828 treatment on turkey spleens (Experiment 1)[a]

Dose of S-28828 (mg/kg BW)	Number of birds positive for IFN/total number of birds per group (average of IFN levels in U/ml) S-28828 administered at day				Pathological and histopathological spleen changes (days 6–7)		
	1	3	4	5	Spleen/BW ratio	Lymphoid hyperplasia of red and white pulp	Number of lymphocyte aggregations/field[b]
0	0/3	0/3	0/3	0/3	1.2 ± 0.2[c]	0/3[d]	2.5 ± 0.8
5	5/6 (300)	1/6 (30)	0/6	0/6	1.2 ± 0.2	5/6	6.8 ± 2.7*
50	6/6 (3000)	2/6 (200)	0/6	0/6	1.7 ± 0.2*	6/6	9.6 ± 2.1*
100	0/6	6/6 (8000)	3/6 (250)	2/6 (75)	1.4 ± 0.3	ND	ND

[a] Turkeys were orally administered with different doses of S-28828/kg body weight (BW). Three birds per group were sacrificed at days 5 and 6 of the experiment. The spleen to BW ratios were determined. Spleens were fixed in formalin and H&E stained tissue sections were evaluated microscopically.

[b] Number of follicle-like lymphocyte aggregations/microscopic field ($100\times$).

[c] Average of three to six turkeys per groups $\pm$ S.D.

[d] Number of turkeys positive/number of turkeys per group.

* Groups are significantly different from the other groups but not from each other ($P<0.05$; ANOVA).

inducing ability but also in their effect on the spleen. Spleen is the major target tissue of HEV and is also susceptible to the effects of S-28828. Birds received either twice 5 or 50 mg/kg BW of S-28828 orally on days 1 and 3, or three times 100 mg/kg BW of S-28828 on days 3, 4, and 5 of the experiment. All dosages of S-28828 tested induced circulating type I IFN in 80–100% of the turkeys 2 h after the first administration. Average serum titers of 300–8000 U of IFN/ml were induced in a dose dependent manner (Table 1). Re-administration of S-28828 induced circulating IFN in only 17–50% of the treated birds. Others and we have shown previously that repeated stimulation reduces the susceptibility of cells to produce type I IFN (Reiter et al., 1994; Rautenschlein et al., 1998a). Treatment of turkeys with 50 mg/kg (Table 1) and 100 mg/kg (data not shown) BW of S-28828 induced splenomegaly at 5 days after the first S-28828 administration ($P < 0.05$). At earlier and later time points, S-28828 did not cause grossly detectable spleen changes at any dosage tested (data not shown). Spleen sections from S-28828 treated turkeys were evaluated for histopathological changes. Five and 50 mg/kg BW of S-28828 induced mild to moderate lymphoid cell hyperplasia of the white pulp as determined by comparing the ratio between red and white pulp areas of the spleens between control and S-28828 treated birds (Table 1, Fig. 1c). The average number of follicle-like lymphocyte aggregations per microscopical field of spleen sections at a magnification of $\times 100$ increased significantly ($P < 0.05$) following treatment with 5 and 50 mg/kg BW of S-28828, respectively (Table 1).

3.2. Effect of S-28828 on HEV replication

In order to determine the effect of S-28828 on HEV replication, turkey were treated with S-28828 at different time points (days 0, 1, or 2) with a single high dose of S-28828 or PBS. At day

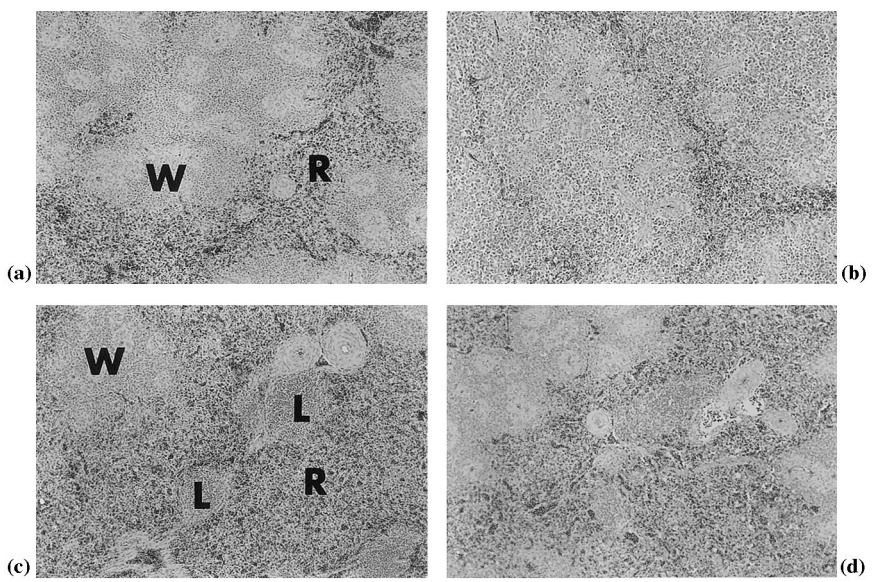


Fig. 1. Effect of S-28828 and S-28828 + HEV on spleen histopathology. Five-week-old commercial turkeys lacking anti-HEV antibodies, were given 5 mg of S-28828/kg BW orally or PBS at days −2. At day 0, the birds received the same dosages of S-28828 or PBS. HEV (10^3 $TCID_{50}$/bird) was given orally at day 0. Tissue sections were evaluated microscopically at days 3–4 PI ($200 \times$). (a) control spleen showing a clear distinction between red (R) and white (W) pulp; (b) HEV-infected spleen 4 days PI with hyperplasia of the white pulp and destruction of the normal spleen architecture (c) S-28828-treated turkey 5 days after the first S-28828 administration (5 mg/kg BW) with clearly separated red (R) and white (W) pulp areas and increased number of lymphoid cell aggregations (L); and (d) HEV-infected and S-28828-treated turkey (5 mg/kg BW) 4 days PI showing a conserved spleen architecture.

Table 2
Influence of S-28828 on HEVp30 replication in the spleen (Experiment 2)[a]

HEV inoculation	S-28828 treatment at day	Average number of HEV+cells/40 fields (statistical rank value)[b]
−	–	0 (1)*
+	–	6.6 (3.4)**
+	d0	0.2 (1.2)*
+	d1	1.6 (2.4)*·**
+	d2	8.1 (3.9)**

[a] Groups of 20 turkeys received orally a single dose of S-28828 (100 mg/kg BW) or PBS at days 0, 1 or 2 or day 0, respectively. Ten turkeys of each group were inoculated with 10^4 $TCID_{50}$/bird HEVp30 at day 1. At 3 and 4 days PI, the turkeys were necropsied and the spleens were snap-frozen. HEVp30 replication was detected by immunohistochemistry in tissue sections, $n = 10$.

[b] Groups marked with * are significantly different to groups marked with ** ($P < 0.05$; Kruskal Wallis test).

1, turkeys were exposed to a mild strain of HEV (HEVp30; Sharma, 1994). Table 2 shows that when turkeys inoculated with HEVp30 were treated with S-28828 one day before virus inoculation, HEV replication was significantly suppressed in comparison to S-28828-untreated groups or groups that received S-28828 at later time points during the infection ($P < 0.05$). Birds given S-28828 and HEV on the same day had a reduced number of HEV-positive spleen cells in comparison with infected birds that did not receive the drug although the data were not significantly different ($P > 0.05$) because of high individual variation.

3.3. Effect of moderate and low dose S-28828 treatment on HEV pathogenesis

After demonstrating that the mild strain of the avian adenovirus type II (HEVp30) is susceptible to the immunomodulatory effects of S-28828, the effect of low and moderate dosages of S-28828 on virulent HEV was determined.

HEV infection by itself induced mild to severe hyperplasia of the white pulp with destruction of

Table 3
Effect of S-28828 on HEV-induced lesions in the spleen at 3–4 days PI (Experiment 2)[a]

Inoculum	Mottling of spleen (%)	HEV-histopathological spleen lesions[b] (% birds positive)	Loss of IgM spleen cell surface determinants[c]	Apoptosis rate[d]	Number of birds positive for HEV/number of birds per group
PBS	0/6 (0)	0	−	29 ± 7.8	0/6 (0)
HEV	6/6* (100)	67*	+	67 ± 40*	6/6* (100)
S-28828 (5 or 50 mg/kg BW)	0/12 (0)	0	−	21 ± 1.3	0/12 (0)
HEV+S-28828 (50 mg/kg BW)	5/9 (56)	10	−	33 ± 17.0	2/9 (22)
HEV+S-28828 (5 mg/kg BW)	2/9 (22)	11	−	23 ± 9.9	3/9 (33)

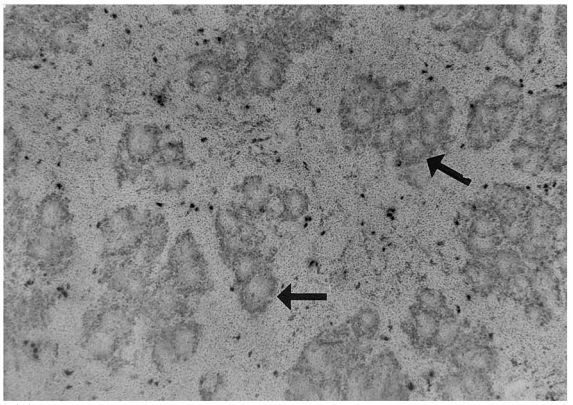
[a] Groups of commercial turkeys received 5 and 50 mg/kg BW of S-28828 or PBS at days −2 and 0. Six to ten birds of each group received 10^3 $TCID_{50}$/bird of HEV orally at day 0. At days 3–4, birds were sacrificed, spleen to BW ratios determined, and the spleens were fixed in formalin and the histopathological lesions were determined. Spleen sections were further evaluated for the staining of IgM+ cell populations.

[b] HEV histopathological spleen lesions such as hyperplasia of the white pulp, inclusion bodies, apoptotic bodies and necrosis.

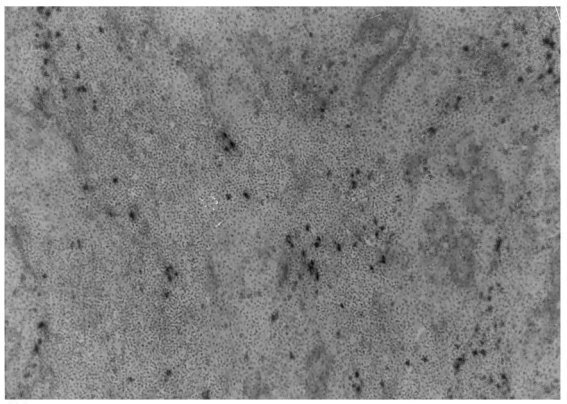
[c] HEV induced loss in the number of IgM+ cells in the spleen as well as reduction in the staining intensity of the remaining IgM+ cells (Suresh and Sharma, 1995; Rautenschlein et al., 1998b); +, loss of IgM+ cell surface staining in the spleen; −, IgM+ cell surface staining was comparable to spleen sections from virus-free control turkeys.

[d] Average per group ± S.D.

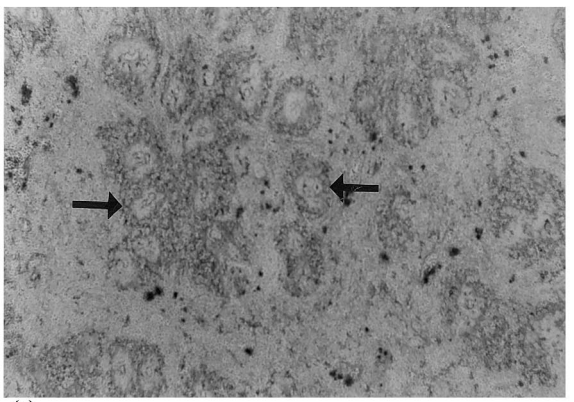
* Significantly different to the other groups ($P < 0.05$; chi-square test or ANOVA).



(a)



(b)



(c)

Fig. 2.

the spleen architecture (Pierson and Domermuth, 1997; Table 3, Fig. 1). The majority of cells in the white pulp of HEV-infected turkeys were lymphoid and macrophage like cells (Domermuth and Gross, 1991; Rautenschlein et al., 1998b). A few to numerous inclusion bodies were detected in the spleen sections of HEV infected turkeys at 3 and 4 days PI (data not shown).

When virus-free turkeys were treated with either dose of S-28828, no HEV-specific lesions or enhanced apoptosis rates were observed (Table 3, Fig. 1). S-28828 treatment of HEV-infected turkeys resulted in a spleen cell reaction specific to S-28828 such as mild to moderate lymphoid cell hyperplasia of the white pulp, an increase in the number of lymphoid cells and follicle-like lymphoid cell aggregations in the red pulp (Fig. 1).

As shown previously, HEV infection led to the destruction of the B-cell areas in the spleen resulting in diminished surface staining of IgM + cells by immunohistochemistry (Table 3, Fig. 2) (Suresh and Sharma, 1995; Rautenschlein et al., 1998b). Treatment of HEV-infected turkeys with S-28828 prevented the HEV-induced destruction of B cells and the HEV-induced reduction in the expression of IgM on their cell surface (Table 3, Fig. 2).

Infection with virulent HEV is expected to induce a high incidence of apoptosis in spleen cells infected with HEV (Rautenschlein et al., 2000). In HEV-infected turkeys, the number of apoptotic cells was significantly enhanced ($P < 0.05$). Low dosages of S-28828 prevented the significant increase in the apoptosis rate following HEV infection in comparison to virus-free control birds ($P > 0.05$; Table 3). Two out of nine HEV-infected birds treated with 50 mg/kg BW of S-28828 had an enhanced apoptosis rate with an average

Fig. 2. Effect of S-28828 on the distribution of IgM + cells in the spleens of HEV-infected and uninfected turkeys. Five-week-old commercial turkeys lacking anti-HEV antibodies, were given 5 mg of S-28828/kg BW orally or PBS at days −2. At day 0, the birds received the same dosages of S-28828 or PBS. HEV (10^3 $TCID_{50}$/bird) was given orally at day 0. Spleen sections were evaluated for the surface staining of IgM + cell populations by immunohistochemistry (100 ×) at days 3–4 PI. (a) Control turkey with clearly stained B cell areas (arrows) and IgM-secreting cells (very dark stained cells); (b) HEV-infected turkey 4 days PI; and (c) S-28828-treated and HEV-infected turkey at 4 days PI with clearly stained B cell areas (arrows).

of 45 and 71 apoptotic cells per microscopic field, respectively, in comparison to an average of 29 apoptotic cells in spleens from non-infected virus-free control birds. But overall, the average number of apoptotic cells in infected birds, which were treated with 50 mg/kg BW of S-28828 was not different form the apoptotic cell numbers in spleens from control birds, which did not receive the drug.

All the HEV-infected turkeys that were not treated with S-28828 had detectable HEV replication in spleen cells at 3 and 4 days PI (Table 3). As noted with HEVp30, when virulent HEV-infected turkeys were treated with S-28828, HEV replication was significantly suppressed ($P < 0.05$) (Table 3). Only three and two birds out of nine were positive for HEV by immunohistochemistry after treatment with 5 and 50 mg/kg BW of S-28828, respectively. The increased incidence of HEV-positive spleen cells in birds treated with 50 mg/kg BW of S-28828 coincided with an enhanced apoptosis rate. On the other hand, the HEV-positive birds treated with 5 mg/kg BW of S-28828 did not show enhanced apoptosis rates in their spleen cells.

4. Discussion

This is the first study demonstrating that an acute adenovirus infection can be modulated by the immunomodifier S-28828. The incidence of infection with HEV, HEV replication and virus-induced lesions were significantly lower in S-28828 treated turkeys than in untreated turkeys ($P < 0.05$). Previous studies have shown that the biological activity of imidazoquinolinamine is, in large part, mediated through the production of cytokines by monocyte/macrophage cells (Weeks and Gibson, 1993; Reiter et al., 1994). Infections with viruses such as CMV, HSV, and arbovirus have been shown to be modulated by imidazoquinolinamines (Bernstein and Harrison, 1989; Harrison et al., 1991; Bernstein et al., 1993; Miller et al., 1995). Treatment with imidazoquinolinamine reduced vaginal viral replication, completely protected against primary disease and reduced recurrent genital HSV disease (Chen et al., 1988; Harrison et al., 1988). Also, imidazoquinolinamine induced variable enhancement of cell-mediated cytolytic acitivity against HSV-2 targets. Imidazoquinolinamine accelerated HSV-2 specific in vitro IL-2 production and peripheral blood mononuclear cell proliferation (Harrison et al., 1988, 1991). Now we show that S-28828 induced IFN in turkeys and reduced in vivo replication and pathologic response of turkeys to HEV. We speculated that inhibition of viral replication likely was responsible for reduced pathological lesions, B cell destruction and apoptosis. Previous studies had indicated that HEV replication correlated with the apoptosis rate in spleen cells (Rautenschlein et al., 2000). However, the possibility cannot be excluded that S-28828 directly reduced the apoptosis rate independent of the reduction in viral replication. Whether HEV persists longer because of the reduced apoptosis rate following S-28828 treatment needs to be examined.

The mechanism by which S-28828 reduced adenovirus replication and modulated HEV pathogenesis in turkeys was not investigated. Type I IFN may have induced the major immunomodulatory effects on HEV pathogenesis but it may not have been the only effector cytokine involved (Weeks and Gibson, 1993; Reiter et al., 1994). Previous studies in mammals indicated that other cytokines were also induced by imidazoquinolinamine administration (Weeks and Gibson, 1993; Reiter et al., 1994). It is also possible that the effect of S-28828 on HEV may not be mediated by cytokines but by other drug effects not identified so far.

Mammalian adenoviruses have been shown to resist direct antiviral effects of type I IFN. Recently it was demonstrated that adenovirus E1A early gene products antagonize interferon regulatory factor-3 mediated activation of IFN genes (Juang et al., 1998). Adenovirus-associated RNAs block activation of latent protein kinase R (Kitajewski et al., 1986). Avian adenoviruses such as HEV may be different than mammalian adenoviruses. HEV may be susceptible to type I IFN bioactivities induced by S-28828. No homologues to mammalian adenovirus genes such as E1A were detected in HEV (Pitcovski et al., 1998). The HEV-viral genomes that are rich in open reading

frames may encode functional genes replacing E1 but data to establish the presence of gene products homologous to the E1 regions in HEV are lacking.

This study shows that it is possible to prevent avian adenoviruses infections with S-28828 if given before or at the time of virus inoculation. Lower dosages of S-28828 are possibly more beneficial for these effects, because dosages of 50 mg/kg BW or higher may induce some toxic effects. For instance, treatment of HEV-infected turkeys with 50 mg/kg BW of S-28828 had an enhanced apoptosis rate in two of nine treated birds. The reason for the enhanced apoptosis is not know. The incidence of an enhanced apoptosis rate in these two birds coincided with the appearance of HEV. Neither HEV-positive birds inoculated with 5 mg/kg of S-28828 had elevated levels of apoptosis in their spleens nor did a dosage of 50 mg/kg BW of S-28828 alone induce apoptosis in uninfected turkeys. Based on these data we speculate that the enhanced apoptosis observed in HEV-infected birds, which were treated with 50 mg/kg BW of S-28828, may possibly be induced though a synergistic effect of a moderate dose of S-28828 and HEV on the immune response. The mechanisms of this effect are not known. Low dosages do not synergize with HEV to induce apoptosis instead they prevent HEV-induced apoptosis in infected spleens. Previous studies in mammals demonstrated that high doses of imidazoquinolinamine were associated with some toxicity possibly due to the very high IFN levels (Harrison et al., 1988), while low doses were not (Chen et al., 1988). A dose of 5 mg/kg BW of S-28828 did not cause detectable macroscopical lesions or apoptosis, but was strong enough to modify HEV pathogenesis. Whether mammalian adenoviruses will be susceptible to the modulating effect of imidazoquinolinamine needs to be established.

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