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EFFECT OF N, N'-BIS(METHYLISATIN-β-THIOSEMICARBAZONE)-2-METHYLPIPERAZINE AGAINST VIRUS-INDUCED ENCEPHALITIS IN MICE[1])

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Summary. — N,N'-bis(methylisatin-β-thiosemicarbazone)-2-methylpiperazine (TSKI-VI) proved to be significantly effective against lethal vaccinia, pseudorabies and Mengo virus-induced encephalitis in different strains of mice when administered subcutaneously (s.c.) in doses of 20 mg/kg body weight, twice daily, for a period of five days. The strongest effects occurred in vaccinia virus-infected mice, and the degree of protection was both dose- and virus-dependent. Titres of vaccinia virus in brains of infected mice were slightly lower in TSKI-VI or methisazone-treated mice as compared to virus controls.

Key words: vaccinia virus; pseudorabies virus; mengo virus; antiviral compound; isatin-β-thiosemicarbazone derivative; mouse

Introduction

The antiviral effect of some derivatives of isatin-β-thiosemicarbazone Mannich bases against vaccinia virus has been demonstrated both in vitro and in vivo (Zgórniak—Nowosielska *et al.*, 1973, 1976, 1978; Borysiewicz *et al.*, 1973; Borysiewicz and Tadeusiewicz, 1976; Borysiewicz and Lucka—Sobstel, 1978; Borysiewicz and Witaliński, 1979). The most active of these compounds was N,N'-bis(methylisatin-β-thiosemicarbazone)-2-methylpiperazine (TSKI-VI). The results in vitro and in vivo against vaccinia virus seemed to warrant further in vivo investigations.

The present paper deals with the protective effect of TSKI-VI against DNA and RNA virus-induced encephalitis in different mouse strains.

Materials and Methods

Compounds. N, N'-bis-(methylisatin-β-thiosemicarbazone)-2-methylpiperazine (TSKI-VI), synthesized by B. Lucka-Sobstel and A. Zejc (Lucka-Sobstel and Zejc, 1973), Dept. of Pharmaceutical Chemistry, Medical Academy, Cracow, was used. 1-Methylisatin-3-thisoemicarbazone (Methisa-

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Table 1. Effect of s.c. treatment with TSKI-VI on viral infections in mice

Virus	Route of inoculation and dose of virus (LD_{50})		Dose of compound per injection (mg/kg)	Treatments per day[1])	Survivors/total Treated	Control	Rate of protection and significance[2]) (%)	
Vaccinia	i.c.	10	20	2	20/20	0/20	100.0	+
	i.c.	10^3	20	2	8/10	0/10	80.0	+
Pseudorabies	s.c.	45	20	2	5/20	0/20	25.0	+
Mengo	i.p.	10	20	1	0/10	0/10	0.0	−
	i.p.	10	10	2	7/20	1/20	31.6	+
	i.p.	10	20	2	8/20	2/20	33.3	+

[1]) Treatment was begun immediately prior to or, in case of vaccinia virus, 3 hr after infection and continued for five days.
[2]) According to the onesided fourfold table test at the 95 % level.

one) obtained from Dr. D. J. Bauer, Wellcome Laboratories, London, served as a control compound. For the experiments on vaccinia and pseudorabies viruses, stock suspensions of the compounds were prepared as described (Zgórniak-Nowosielska *et al.*, 1976). In the experiments on Mengo virus, suspensions in 0.1 % methyl-cellulose (Tylose MH 300, Schuchard, Munich, F.R.G.) were made up immediately prior to their subcutaneous (s.c.) administration.

Viruses. The IHD strain of neurovaccinia virus (genus *Orthopox virus*) from the State Institute of Hygiene, Warsaw (Poteć and Zgórniak-Nowosielska, 1979), $Mengo_m$ virus (genus *Cardiovirus*), characterized previously (Veckenstedt, 1974), and pseudorabies virus (herpesvirus suis), strain XXV, of pig brain origin and adapted to chick embryo fibroblasts (Cserey-Pechány *et al.*, 1951, were used. The titres of virus stocks, prepared from mouse brain suspensions, assayed in mice) were $10^{5.5}$ intracerebral (i.c.) LD_{50}/0.02 ml for vaccinia virus, $10^{7.5}$ intraperitoneal (i.p.) LD_{50}/0.1 m for Mengo virus, and $10^{3.5}$ s.c. LD_{50}/0.1 ml for pseudorabies virus.

Mice. BALB/c mice of both sexes, approximately 3 weeks old and weighing 10 g each, were used in experiments on vaccinia virus. Male ABD2F_1 (AB/Jena × DBA 2/Jena) hybrids, 4—6 weeks old and selected to 17—22 g of weight, obtained from the SPF Mouse Production Unit of the Central Institute of Microbiology and Experimental Therapy, Jena, were used in experiments on Mengo virus. Male outbred mice Lati:CFLP (Breeding Farm Lati, Gödöllö, Hungary) selected to 25 g of weight were used with pseudorabies virus.

Assay of antiviral effectiveness. Groups of 10 or 20 mice each were injected i.c., i.p. or s.c. as indicated, with virus 3 hr prior to (vaccinia virus), or shortly after (Mengo and pseudorabies viruses) first administration of the compound. Animals were treated s.c. with different doses of the drug as indicated below for five consecutive days. Before treatment, the mice were weighed to allow accurate administtation of the compound according to their body weight (mg per kg of body weight = mg/kg). The virus control groups of mice received 0.9 % saline (vaccinia and pseudorabies viruses) or compound diluent (Mengo virus) along the same scheme of s.c. injections as the treated mice. Toxicity control mice were injected s.c. with 20 mg/kg of TSKI-VI or methisazone at times corresponding to drug treatment. Surviving animals were recorded daily for 10 (pseudorabies virus) or 14 days.

Dose-response experiments were done on vaccinia virus as reported by Poteć and Zgórniak-Nowosielska (1979).

Vaccinia virus titration. BALB/c mice (6 in each group) infected i.c. with 10^3 LD_{50} of vaccinia virus, treated s.c. twice daily with 20 mg/kg of TSKI-VI or 20 mg/kg of methisazone and virus control mice were killed daily for 5 days post infection (p.i.). The brains were removed, homogenized in phosphate buffered saline (PBS) with 10 % bovine albumin and stored at −20°C until assayed for infectious virus by plaque titration in chick embryo fibroblasts (CEF). Virus titre was expressed in PFU per 0.1 g of brain tissue.

Statistical evaluation of animal experiments was done according to a "2-step procedure" of in vivo testing (Veckenstedt and Horn, 1974, 1976; Horn *et al.*, 1977).

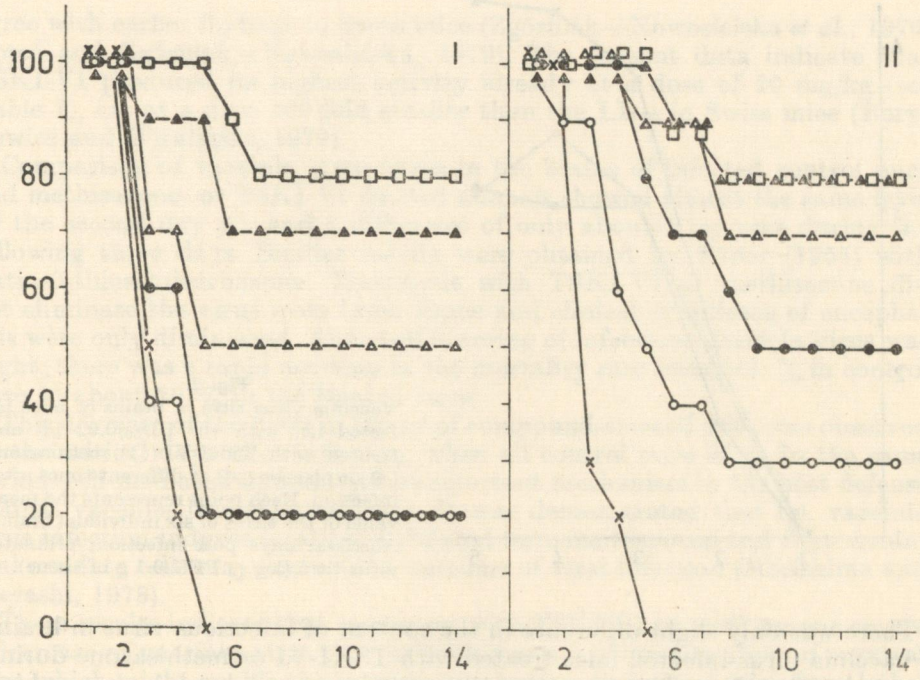

Fig. 1.

Dose-response curves of TSKI-VI (I) and methisazone (II) on the outcome of lethal vaccinia virus infection in mice .Ten animals each in treatment and control groups were inoculated i.c. with 10^3 LD_{50} of the virus. Treatment (s.c.) was initiated 3 hr after i.c. inoculation .Doses: 1.25 (○). 2.5 (●), 5 (△), 10 (▲), or 20 (□) mg/kg body weight twice daily for five consecutive days. Virus control mice were injected with placebo (×) in the same manner.
Abscissa: days post infection; ordinate: % survivors.

Results

Mice infected with lethal doses of vaccinia, pseudorabies or Mengo virus were significantly protected from death when treated s.c. with TSKI-VI twice daily at five consecutive days (Table 1). Single injections of the full daily dose of compound failed to prevent death in mice infected with Mengo virus. The strongest effects occurred in vaccinia virus-infected mice, even when the infectious dose was as high as 10^3 LD_{50}.

Results of dose-response experiments on vaccinia virus are presented in Fig. 1. The protective effect of TSKI-VI was similar to that of methisazone. Protection rate increased along with the dose of the compound. With 20 mg/kg, both compounds produced the same rate of protection, but with 2.5 mg/kg methisazone was comparatively more effective. Control mice died 3 to 5 days p.i. No signs of toxicity were observed in compound-treated control mice.

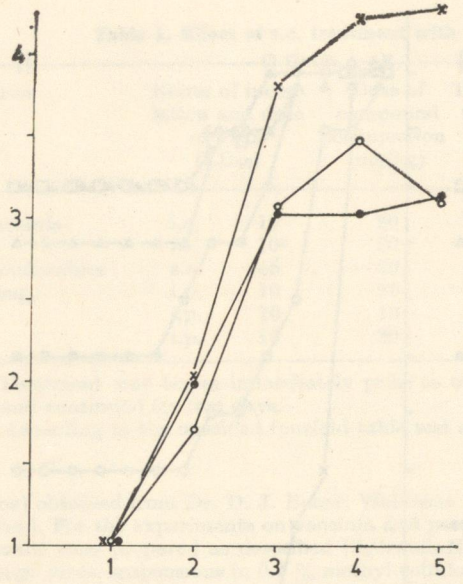

Fig. 2.
Vaccinia virus titre in brains of mice infected i.c. with 10^3 LD_{50}/0.02 ml and treated with TSKI-VI (○), methisazone (●)or placebo (×) at different times after infection. Each point represents the mean value of the titres of six individual brains. Abscissa: days post infection; ordinate: virus titre (log $_{10}$ PFU/0.1 g of tissue).

There was only slight difference in the content of infectious virus in brains of vaccinia virus-infected mice treated with TSKI-VI or methisazone during a period of five days. The respective titres on the second day p.i. were as high as in virus control mice, but differed by about 1 log unit on the following three days (Fig. 2).

Discussion

The present results offered evidence that repeated s.c. administration of TSKI-VI produced significant protection from lethal encephalitis in mice infected with vaccinia, pseudorabies or Mengo viruses.

The studies on vaccinia virus were performed in BALB/c mice. Earlier findings in vaccinia virus-infected Swiss mice were thus confirmed and it was shown that protection in BALB/c mice was even higher (Zgórniak—Nowosielska *et al.*, 1976; Poteć and Zgórniak—Nowosielska, 1979). Degree of protection depended on the dose of virus, reaching 100 % with 10 LD_{50} and 80 % with 10_3 LD_{50}, even after i.c. inoculation.

The effect of TSKI-VI against experimental pseudorabies and Mengo virus infection was lower, but indicated the ability of the compound to exert a protective activity against unrelated viruses causing different pathogenesis. Recently, it was demonstrated that a representative of isatinisothiosemicarbazones produced significant protection against rapidly developing Mengo virus encephalitis in mice (Veckenstedt and Zgórniak—Nowosielska, 1979).

With TSKI-VI, a dose-response relationship was observed in the range of 2.5—20 mg/kg, while with methisazone the respective range was 1.25 to 10 mg/kg, pointing to a slightly higher activity of methisazone. These results

agree with earlier findings in Swiss mice (Zgórniak—Nowosielska *et al.*, 1976; Poteć and Zgórniak—Nowosielska, 1979). The present data indicate that TSKI-VI produced its highest activity already at a dose of 20 mg/kg (see Table 1), i.e. at a dose 100-fold smaller than the LD_{50} in Swiss mice (Borysiewicz and Witaliński, 1979).

Comparison of vaccinia virus titres in the brains of infected control mice and methisazone- or TSKI-VI-treated animals showed almost the same level on the second day p.i. and a difference of only about 1 log unit during the following three days. Similar results were obtained by Bauer (1955) with isatin-3-thiosemicarbazone. Treatment with TSKI-VI or methisazone did not eliminate the virus from brain tissue and clinical symptoms of encephalitis were only diminished. Although lowering of infectious vaccinia virus was slight, there was a rapid decrease in the mortality rate from 100 % in control mice to about 20 % in the treated mice.

This decrease of the virus in brains of compound-treated mice was observed on the critical days after infection, when all control mice died. In the same period, cell-mediated immunity, an important mechanism in the host defense against vaccinia infection occurred. It was demonstrated that i.c. vaccinia virus infection induces local cell-mediated immune response and that meningeal exudate cells may control the outcome of viral infection (Morishima and Hayashi, 1978).

These results indicate that treatment with TSKI-VI or methisazone causes a decrease of vaccinia virus titre in brain tissue and prolongation of survival time which might enable the immune mechanism to protect mice from lethal vaccinia virus-induced encephalitis.

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