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Short communication

Short-term prophylaxis with deoxyspergualin prevents testicular autoimmunity in mice

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Abstract

The effects of the treatment with the immunosuppressant deoxyspergualin on the development of experimental autoimmune orchitis were studied. The results showed that C3H/He mice immunized with testicular germ cells and treated daily with either 0.3 or 3 mg/kg body weight deoxyspergualin during days 15–20 post-immunization developed experimental autoimmune orchitis lesions with a significantly lower incidence and severity than did the control mice treated under the same experimental conditions with phosphate buffered saline (PBS). The effects of deoxyspergualin were clearly dose-dependent, and the higher dose of the drug also markedly reduced the degree of delayed type hypersensitivity responses against testicular germ cells. These data suggest that deoxyspergualin may be worthy of consideration for the prevention/treatment of human immunoinflammatory orchitis.

Keywords: Autoimmunity; Deoxyspergualin; Immunotherapy; Orchitis

1. Introduction

We have previously described a new model of experimental autoimmune orchitis that can be induced in susceptible C3H/He mice by two injections of syngeneic testicular germ cells given at a 14-day interval without the aid of any adjuvants (Itoh et al., 1991a). Delayed type hypersensitivity responses against testicular germ cells are simultaneously elicited (Itoh et al., 1991b). We have also shown that the so-induced experimental autoimmune orchitis is a CD4Th1-dependent immunoinflammatory disease, as judged by the possibility of transfer via CD4 T cells (Itoh et al., 1991a,b), and is prevented by eliminating the action of type 1 cytokine interferon-γ with monoclonal antibody (Itoh et al., 1998).

Testicular germ cell-induced experimental autoimmune orchitis is characterized by inflammatory cell infiltration followed by disturbance of spermatogenesis (Itoh et al., 1991a,b, 1998). The inflammatory infiltrates first appear from day 20 and propagation of the inflammation with spermatogenesis is prominent from day 30. By day 40, the mice develop histologic lesions fully characteristic of experimental autoimmune orchitis which may persist for as long as 4 months (Itoh et al., 1991a,b).

Because the lymphocytic infiltration of the testis, the disturbance of spermatogenesis and the occurrence of delayed type hypersensitivity responses against testicular antigens can also be observed in cases of human immunologic infertility (Anderson and Hill, 1998), testicular germ cell-induced orchitis may be used as in vivo tool for studying immunoinflammatory orchitogenic pathways and immunotherapeutic approaches for the treatment of the human disease counterpart.

Following this line of research, we have now studied the effects of the immunosuppressant, 15-deoxyspergualin (DSP, C17H37N7O3-3HCL; M.W.496.9) (reviewed by Thompson, 1992), on the treatment of testicular germ cells-induced experimental autoimmune orchitis. The results demonstrated that when deoxyspergualin (0.3 or 3 mg/kg body weight, daily) is given during days 15 to 20 after

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immunization, it is capable of reducing both the incidence and severity of experimental autoimmune orchitis lesions in a clearly dose-dependent fashion. At the highest dose, deoxyspergualin also markedly suppressed the degree of delayed type hypersensitivity responses against testicular germ cells.

2. Materials and methods

2.1. Animals

Male C3H/He mice were purchased from SLC (Hamamatsu, Japan). All animals were maintained under standard laboratory conditions (nonspecific pathogen-free) with free access to food and water. They were allowed 1 week to adapt to their new environment before initiation of the experiments. At the end of the study, the mice were anesthetized and then killed by cervical dislocation.

2.2. Preparation of TGC

Testes were excised from syngeneic mice, teased with scissors into cold Hanks' balanced salt solution (HBSS) and passed through a stainless steel mesh. The testicular germ cells were harvested by centrifugation at 400 × *g* for 10 min and washed three times in cold phosphate buffered saline (PBS) pH 7.4, after counting viability, using trypan blue dye exclusion. The testicular germ cell suspension contained >99% germ cells at all stages of spermatogenesis, while the remaining 1% were Sertoli cells and Leydig cells.

2.3. Induction of experimental autoimmune orchitis

To induce experimental autoimmune orchitis, 10-week-old male mice were injected subcutaneously twice with 1×10^7 testicular germ cells in 200 ul PBS on days 0 and 14. Control mice were injected twice with 200 μl PBS alone on days 0 and 14 (Table 1, Fig. 1).

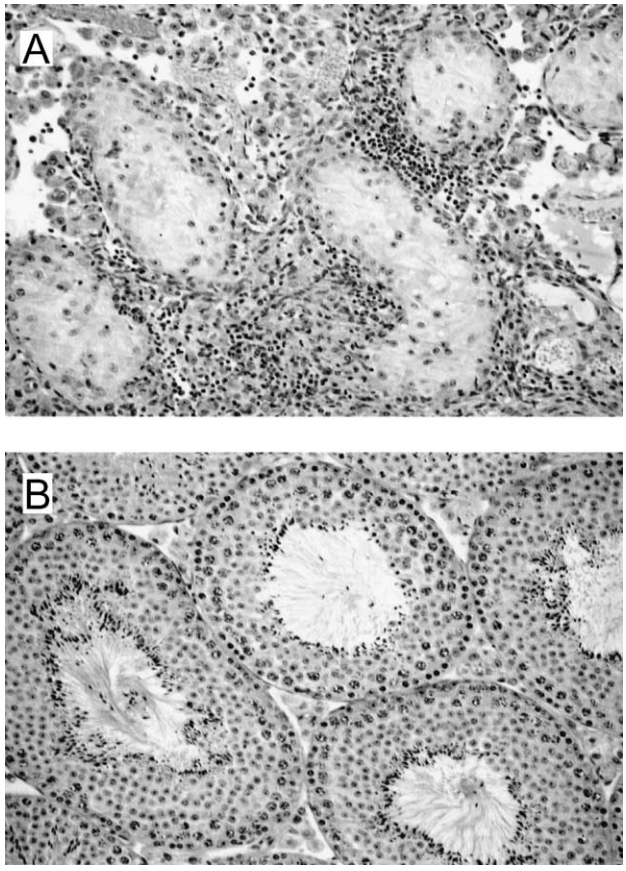

Fig. 1. Prevention of experimental autoimmune orchitis by deoxyspergualin. (A) A testis section of a control mouse challenged twice with testicular germ cells on days 0 and 14 and treated daily with PBS during days 15–20. Note severe infiltration of lymphocytes and spermatogenic disturbance (× 100). (B) A testis section of a mouse treated with 3 mg/kg body weight deoxyspergualin during days 15–20 post-immunization with testicular germ cells. Normal spermatogenesis was preserved with no lymphocytic infiltration.

2.4. Treatment with deoxyspergualin

Deoxyspergualin was purchased from Behring, Milan, Italy. The drug was dissolved in sterile PBS. A stock

Table 1
The effects of deoxyspergualin (DSP) on experimental orchitis (EAO) autoimmune lesions and related delayed type hypersensitivity (DTH) responses

Group	Numbers of mice	Immunization Day 0	Immunization Day 14	Treatment Day 15 20	Treatment Day 40	EAO score 0	1	2	3	4	5	Mean±S.E.M.	EAO incidence (%)	DTH on Day 40 (mean±s.e.m.)
A	10	PBS	PBS	PBS alone	killed	10	0	0	0	0	0	0±0	0/10 (0)	6±1
B	13	TGC	TGC	PBS alone	killed	0	0	1	3	5	4	3.9±0.3	13/13 (100)	44±3
C	12	TGC	TGC	0.3mg/kgbw DSP	killed	7	0	0	2	3	0	1.5±0.5	5/12 (42)	40±3
D	12	TGC	TGC	3.0mg/kgbw DSP	killed	10	1	1	0	0	0	0.3±0.2	5/12 (17)	16±3

B vs. C: $P<0.0001$; B vs. D: $P<0.0001$; C vs. D: $P<0.05$

EAO incidence: B vs. C: $P<0.0001$; B vs. D: $P<0.05$; C vs. D: n.s.

DTH on Day 40: B vs. C: n.s.; B vs. D: $P<0.0001$; C vs. D: $P<0.0001$

Mice were subcutaneously injected twice with 1×10^7 testicular germ cells (TGC) on days 0 and 14.
Mice were intraperitoneally injected with phosphate buffered saline (PBS) containing DSP on days 15–20.
Histopathological stage of each mouse was scored on day 40. If the stages in testes of both slides differed, advanced stage in one of the pair was scored as the disease severity in each mouse.
EAO incidence=(numbers of mice with EAO lesions/number of mice examined) × 100.
Delayed footpad reaction. The degree of reaction was expressed as the increased thickness at 24 h after local injection of 1×10^6 TGC (× 10 mm^2).

solution of 10 mg deoxyspergualin/ml was made and stored at −40 °C. Before use, deoxyspergualin was freshly thawed and diluted in PBS. The drug was given (i.p. in a final volume of 100 ul) daily from days 15 to 20 post-immunization at the dose of either 0.3 or 3.0 mg/kg body weight/day. Control mice were treated under similar experimental conditions with PBS (Table 1).

2.5. Histological examination

On day 45, the mice were killed and testes and epididymides were removed, fixed with Bouin's solution and embedded in plastic (Technovit 7100; Kulzer & Co., Wehrheim, Germany) without cutting the organs to avoid artificial damage to the testicular tissue. About 5-um-thick sections were obtained at 50-um intervals and stained with Gill hematoxylin III and 2% eosin Y solution for light microscopical observation that was carried blind by an observer unaware of the treatment of the mice.

2.6. Histopathological assessment of experimental autoimmune orchitis lesions

As we described elsewhere (Itoh et al., 1995), experimental autoimmune orchitis lesions can be divided into six stages (Stages 0, I, II, III, IV, and V) according to the spread of inflammatory cells in the testis. Briefly, 0 = no inflammation; I = focal inflammation in tunica albuginea; II = focal inflammation adjacent to tubuli recti; III = inflammation surrounding tubuli recti; IV = spreading inflammation around seminiferous tubules; and V = widespread inflammation involving seminiferous tubules and tubuli recti. For statistical evaluation, stages 0, I, II, III, IV, and V were scored as 0, +1, +2, +3, +4, and +5, respectively. In cases where stages of right and left testes differed, the most severe lesion determined the EAO stage of the mouse.

2.7. Delayed type hypersensitivity responses to testicular germ cells

Degrees of delayed type hypersensitivity responses against testicular germ cells were determined by delayed footpad reaction. Just before injection with test antigens, footpad thickness was measured, using a dial thickness gauge micrometer (Mitsutoyo, Tokyo, Japan). About 1×10^6 testicular germ cells in 50 μl of PBS were injected into the hind footpads on day 39. After 24 h, the footpad thickness was measured. The degree of reaction was expressed as increased thickness of the footpad ($\times 10^2$ mm).

3. Results

Experimental autoimmune orchitis developed in all the control mice by day 40 after primary immunization. Severe orchitis lesions were frequently observed in these mice (see Table 1). Simultaneously, strong delayed type hypersensitivity responses against testicular germ cells were observed. (Table 1) In contrast, deoxyspergualin prophylaxis favourably influenced the course of the disease, reducing both the incidence and severity of the orchitis lesions in a clear dose-dependent fashion (Table 1). In addition, relative to the control mice, the animals treated with the highest dose of the drug exhibited a marked reduction of delayed type hypersensitivity responses against testicular germ cells (Table 1).

4. Discussion

Deoxyspergualin is one of the active metabolites of spergualin, an antibiotic originally obtained from culture filtrates from a strain of *Bacillus laterosporus* (reviewed by Thompson, 1992). This drug, previously studied as an anti-tumor agent, was subsequently found to be capable of suppressing allo- and autoimmune reactions in experimental models (Thompson, 1992). It has been shown by us and others that deoxyspergualin possesses an immunopharmacological mode of action different from that of other immunosuppressants, such as cyclosporin A, tacronimus, or glucocorticoids, which seems to primarily depend on its capacity to interfere with antigen presentation from macrophages through binding with its endogenous ligand, Heat shock protein 70 (Hoeger et al., 1994; Nicoletti et al., 1996). Also, both in vitro and ex vivo studies have shown that deoxyspergualin may suppress the production of interferon-γ (Nicoletti et al., 1993) and interleukin-1β (Nicoletti et al., 1996).

The results presented herein demonstrated that even when administered for as a short time as six consecutive days from days 15 to 20 after challenge with testicular germ cells, deoxyspergualin was capable of inhibiting the development of experimental autoimmune orchitis as shown by the significantly reduced incidence and milder severity of orchitis observed in deoxyspergualin-treated mice as compared to controls. The effect of deoxyspergualin was dose-dependent and the highest dose of the drug also markedly reduced the degree of delayed type hypersensitivity responses against testicular germ cells.

Although the precise mode of action by which deoxyspergualin prevented the development of experimental autoimmune orchitis has not been ascertained, the CD4Th1-dependent nature of the disease suggests that the drug may have interfered with the function of orchitogenic CD4Th1 cells, perhaps by altering (auto)antigen presentation to these cells from macrophages and/or by inhibiting T cell production of interferon-γ which is involved in testicular germ cell-induced experimental autoimmune orchitis (Itoh et al., 1998).

These data add murine testicular germ cell-induced experimental autoimmune orchitis to the list of experimental immunoinflammatory diseases that benefit from deoxysper-

gualin application, among others, rodent models of type 1 diabetes (Nicoletti et al., 1992, 1993; Di Marco et al., 1996), autoimmune thyroiditis (Nicoletti et al., 1994), systemic lupus erythematosus (Ito et al., 1990), rheumatoid arthritis (Takagishi et al., 1990), uveoretinitis (Mochizuki and Kawashima, 1990), nephritis (Nikolic-Paterson et al., 1995), multiple sclerosis (Jung et al., 1994), and myocarditis (Kodama et al., 1995). Beneficial effects of deoxyspergualin were also observed in patients with proliferative glomerulonephritis (Hotta et al., 1999). If the present findings can be transferred to the clinical setting, deoxyspergualin may be a suitable candidate for the prevention or early treatment of cases of human infertility of immunologic origins. However, additional studies are required to demonstrate its lack of toxicity on testicular germ cell viability and function. In addition, since prophylactic interventions may be difficult to carry out in humans, the treatment of mice with established experimental autoimmune orchitis lesions will provide clues to better understand the potential utility of this drug in human orchitis.

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