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Chronic treatment with cyclosporine A in New Zealand rabbit: aortic and erectile tissue alterations

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Abstract Transplanted patients frequently present erectile impotence. In order to test any interference by cyclosporine A (CsA), which is commonly used in the post-transplantation management, we investigated the in vitro contractile and relaxant responses of corpus cavernosum and aorta from rabbits chronically treated with CsA. Male New Zealand White rabbits 6 months of age were treated with CsA (25 mg/kg per day s.c.) or solvent (corn oil) for 3 weeks. Descending thoracic aorta and erectile tissue were studied in vitro at the end of treatment. Isometric tension was recorded. In thoracic aorta, noradrenaline (0.1–30 mM) induced a concentration-dependent contraction with no difference between the two groups. Acetylcholine (30 nM–3 mM) produced relaxation (52 ± 4% at 1 mM) that was significantly reduced in comparison to controls (67 ± 4%, $P < 0.05$). ATP (3–10 mM) relaxation was not significantly different (maximal 78 ± 10% and 62 ± 12% in CsA-treated and controls). The relaxation produced by sodium nitrite was reduced in CsA-treated rabbits (at 10 mM and 0.1 mM concentrations). In erectile tissue, no significant variation in the response of isolated erectile tissue to the above drugs was observed between CsA-treated and control animals. These data indicate that chronic treatment with CsA in rabbits, despite alteration of the in vitro response of thoracic aorta, does not directly influence the function of penile tissue with relaxants.

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Introduction

Patients who are candidates of transplantation frequently present a very poor erectile function; even after successful transplantation, despite a maintained desire for sexual activity, a decrease of erectile rigidity and orgasmic ability is usually observed [2, 6, 14, 21, 27, 32, 34]. The etiology of such erectile impotence is considered to be multifactorial. Vascular, neurological, psychological and pharmacological factors are all believed to be implicated as causes of impotence [6, 34]. Some authors have indicated that cyclosporine A (CsA), a drug commonly used as an immunosuppressive to prevent rejection of transplanted organs, could be the cause of erectile failure [20, 34]. Nephrotoxicity and arterial hypertension after chronic treatment with the drug in humans and in animals [3, 12, 18, 23, 30, 33] have been observed, as well as attenuation of vascular relaxation [26]. CsA was also found to induce directly, or to potentiate, vascular contractility [36, 25]. Vascular effects of CsA mediated by hormonal changes, however, seem to be excluded [13]. Since CsA causes a sympathetic tone increase [20], this effect could explain the reduced response of cavernosal tissue to sexual stimuli [34].

Due to the vascular effects of CsA, it is possible to hypothesize that the drug may influence erection through direct vascular damage of cavernosal tissue. Great interest has been shown in the role of endogenous modulators of smooth muscle relaxation [8, 24, 15, 31] which may contribute to the pathophysiology of impotence [19, 29]. The use of animal models can be particularly helpful for detecting the evolution of penile responsiveness after pharmacological treatment. In the present study we have therefore investigated the

in vitro contractile and relaxant responses of corpus cavernosum obtained from rabbit chronically treated with CsA, in order to detect alterations of responses of corpus cavernosum induced by molecules acting via endothelium-dependent or -independent mechanisms. The tension changes produced by these drugs were also tested in thoracic aorta to assess a systemic vascular effect of chronically administered CsA.

Materials and methods

Ten male New Zealand white rabbits 6 months of age were divided into two groups of five animals each. The age of 6 months was preferred since at this age animals become sexually mature. A group of rabbits was assigned to CsA treatment and received the drug daily s.c. for 3 weeks at the dose of 25 mg/kg dissolved in corn oil. The other group of rabbits was treated daily with corn oil s.c. for the same time. Standard principles of laboratory animal care were followed. During the in vivo treatment, a sample of blood was taken weekly from an ear vein for serum determination. During the entire experimental section, all the animals were maintained in a 14L:10D light cycle and received a standard diet (4RF21 from Mucedola, Settimo Milanese, Italy) and water ad libitum. Forty-eight hours after the last administration of CsA the animals were sacrificed for the in vitro studies. The body weight was observed weekly during the period considered. Animals were anaesthetized with urethane (2 mg/kg i.p.) and sacrificed by cervical dislocation. The descending thoracic aorta and the penis were removed at the point of attachment to the ischium. The tissues were placed in saline solution (see later), maintained at 37 °C and gassed with a mixture of 95% O_2 and 5% CO_2. The aorta was cleaned of adjacent tissue and cut into transverse rings approximately 3 mm wide; each aortic preparation consisted of a chain of two rings tied by silk thread. From each animal, four two-ring aortic preparations were obtained and each preparation used to test one drug. The erectile tissue was dissected free from the tunica albuginea; from one animal six preparations were obtained. Four preparations were used for testing the relaxant drugs and two to determine the cumulative concentration-response curve of the contractile agent noradrenaline. The preparations were suspended in a 30-ml tissue bath containing modified Krebs-bicarbonate solution of the following composition (mmol/l): NaCl 116.0; KCl 3.2; $CaCl_2$ 1.2; $MgCl_2$ 1.2; NaH_2PO_4 1.2; $NaHCO_3$ 22.0; glucose 10.1; ascorbic acid 1.1, equilibrated with a 95% O_2–5% CO_2 gas mixture, pH 7.4, at 37 °C.

Isometric tension was recorded by means of force transducers (Type DY0, Basile, Comerio, Italy) connected to a chart recorder (Unirecord Basile, Comerio, Italy). Aortic and erectile tissue preparations were held at a resting tension of 35 and 20 mN, respectively, and allowed to equilibrate at optimal length for 60–90 min before experiments were started, the buffer being changed every 15 min. Preparations were pre-contracted with the approximate EC_{50} of noradrenaline, washed and equilibrated again for at least 60 min before experiments were started. This procedure was found to increase and stabilize any subsequent contractile response to noradrenaline. Noradrenaline EC_{50} (concentration inducing half maximal contraction) was determined for each rabbit. For relaxation studies, vasodilator drugs were cumulatively added to preparations precontracted with EC_{50} noradrenaline to steady-state tension. At the end of the experiments, EC_{50} noradrenaline was added to verify contraction stability. The spontaneous relaxation following this contraction was subtracted from the relaxation caused by the vasodilator agonists in the previous curve, to calculate the net effect of the agonists.

Creatinine, urea nitrogen, glucose, triglyceride and cholesterol concentrations were assayed in serum by enzymatic standard techniques on automatized Hitachi 747-200 equipment.

Drugs and reagents

CsA (Sandimmun, Sandoz) was a generous gift from Sandoz SpA (Milan, Italy). Adenosine 5′-triphosphate (ATP) sodium salt, adenosine, acetylcholine (ACh) bromide, sodium nitrite, nifedipine, A23187 and noradrenaline (NA) bitartrate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Prostaglandin E_1 (PGE1) (Alprostadil) was a kind gift from Upjohn SpA, Caponago (Italy).

Statistical analysis

The percentage relaxation caused by each drug was calculated by assigning the maximal contraction at steady state induced by noradrenaline EC_{50} as 100%. Data were expressed as means ± SEM. Differences between means were compared by Student's two-tailed t-test for unpaired data, and a probability level of 0.05 was accepted as significant.

Results

Body weight

The weight of the rabbits was determined weekly throughout the treatment period. Values are presented in Table 1. During the treatment the CsA-treated group showed a significant decrease in body weight after 2 and 3 weeks, in comparison to controls (Table 1).

Serum determinations

As shown in Table 1, serum creatinine as well as serum glucose concentrations were constantly higher in CsA-treated rabbits than in controls. However, the difference was statistically significant only at 14 days. No significant difference was detected in plasma triglycerides or urea nitrogen. Serum cholesterol was significantly increased in CsA-treated animals after 14 and 21 days of treatment.

Thoracic aorta functionality

The addition of NA to aorta preparations induced a concentration-dependent contraction in the concentration range 10 nM–30 mM. No difference was observed between the two groups (Fig. 1A). On NA-precontracted thoracic aorta, ACh produced a concentration-dependent relaxant effect (Fig. 1B) in the concentration range 30 nM–1 mM, followed by a reduction of the maximal relaxant tone at higher concentrations. Maximal relaxation obtained in thoracic aorta from the CsA group was significantly reduced in comparison to controls ($P < 0.05$) (Fig. 1B). ATP induced a concentration-dependent relaxant effect in the NA-precontracted preparations from both groups (Fig. 1C) in the concentration range 3–10 mM. The relaxant effect was similar in the CsA-treated and control groups. Adenosine was not able to produce any relaxant effect in either group in the concentration range considered (1–0.1 mM) (data not shown). The

Table 1 Body weight and serum determinations in the two groups of rabbits (five for each group) at different treatment times

	0 days		7 days		14 days		21 days	
	CsA	Control	CsA	Control	CsA	Control	CsA	Control
Body weight (g)	3600 ± 86	3581 ± 93	3452 ± 90	3574 ± 80	3274* ± 67	3493 ± 70	3230** ± 67	3557 ± 67
Creatinine (mmol/l)	80 ± 6	72 ± 4	100 ± 6	87.6 ± 3.5	118** ± 11	77 ± 2	126 ± 21	111 ± 7
Urea nitrogen (mmol/l)	6 ± 0.3	5.92 ± 0.5	5.98 ± 0.6	5.4 ± 0.3	6.9 ± 0.7	5.74 ± 0.4	11 ± 1.6	10.4 ± 0.9
Glucose (mmol/l)	6.3 ± 0.3	6.44 ± 0.3	6.74 ± 0.5	4.4 ± 0.45	6.72** ± 0.3	4.9 ± 0.2	8.8 ± 0.7	8.3 ± 0.9
Triglyceride (mmol/l)	0.84 ± 0.1	0.7 ± 0.1	0.72 ± 0.1	0.67 ± 0.1	0.63 ± 0.1	0.8 ± 0.1	1.33 ± 0.3	1.3 ± 0.1
Cholesterol (mmol/l)	0.84 ± 0.1	0.75 ± 0.1	1.67 ± 0.3	0.98 ± 0.1	3.14** ± 0.4	0.96 ± 0.1	3.81 ± 0.6	2.3 ± 0.6

* $P < 0.05$ and ** $P < 0.01$, significance vs control.

relaxation produced by sodium nitrite is presented in Fig. 1D. The relaxation in aorta of CsA-treated rabbits appeared to be reduced particularly at the lower concentrations (10 mM and 0.1 mM). Another drug acting through an endothelium-independent mechanism is the calcium-channel blocker nifedipine. The drug produced no relaxation in either group, nor did PGE_1 (data not shown).

Erectile tissue functionality

On initial incubation in saline solution, the preparations presented slight spontaneous contractions from baseline tone. The addition of NA induced a concentration-dependent contraction in the range 10 nM–10 mM (Fig. 2A). On NA-precontracted tissue, ACh produced a concentration-dependent relaxant effect in the two groups (Fig. 2B) in the range 10 nM–1 mM. No significant variation was observed between CsA-treated and control rabbits. ATP induced a concentration-dependent relaxant effect in the NA-precontracted preparations from the two groups (Fig. 2C). The relaxant effect obtained in the concentration range 0.1–10 mM was similar in the two groups, and able to reverse completely the contraction induced by EC_{50} NA. Adenosine induced a concentration-dependent relaxation of the preparations (Fig. 2D). The relaxant response was detected in the concentration range between 0.1 and 1 mM. The relaxation produced by sodium nitrite is presented in Fig. 2E. The relaxant effect was similar in the two groups in the concentration range 0.1–10 mM. Another drug acting through an endothelium-independent mechanism is the calcium-channel blocker nifedipine. The drug induced a concentration-dependent relaxation (1 nM–1 mM) that was similar in both groups of animals (data not shown). PGE_1 failed to induce any relaxation in erectile tissue from either group (data not shown).

Discussion

A prolonged in vivo treatment with CsA in rabbit, despite a reduction of thoracic aorta relaxant activity, induced no alteration of cavernosal tissue in vitro functionality. Vascular reactivity was considered both at endothelial and smooth muscle level. CsA treatment induced a significant reduction of ACh-induced relaxation in thoracic aorta, as well as a reduction of sodium nitrite relaxation. Since the impairment of the response to the two vasodilators was similar, we hypothesize that the location of the damage is not the endothelium, but the final target: the smooth muscle component. In agreement with these data are the findings of Rego et al. [26], who concluded that chronic CsA treatment (5–50 mg/kg per day) directly influences the vascular smooth muscle by inhibiting cGMP formation. The

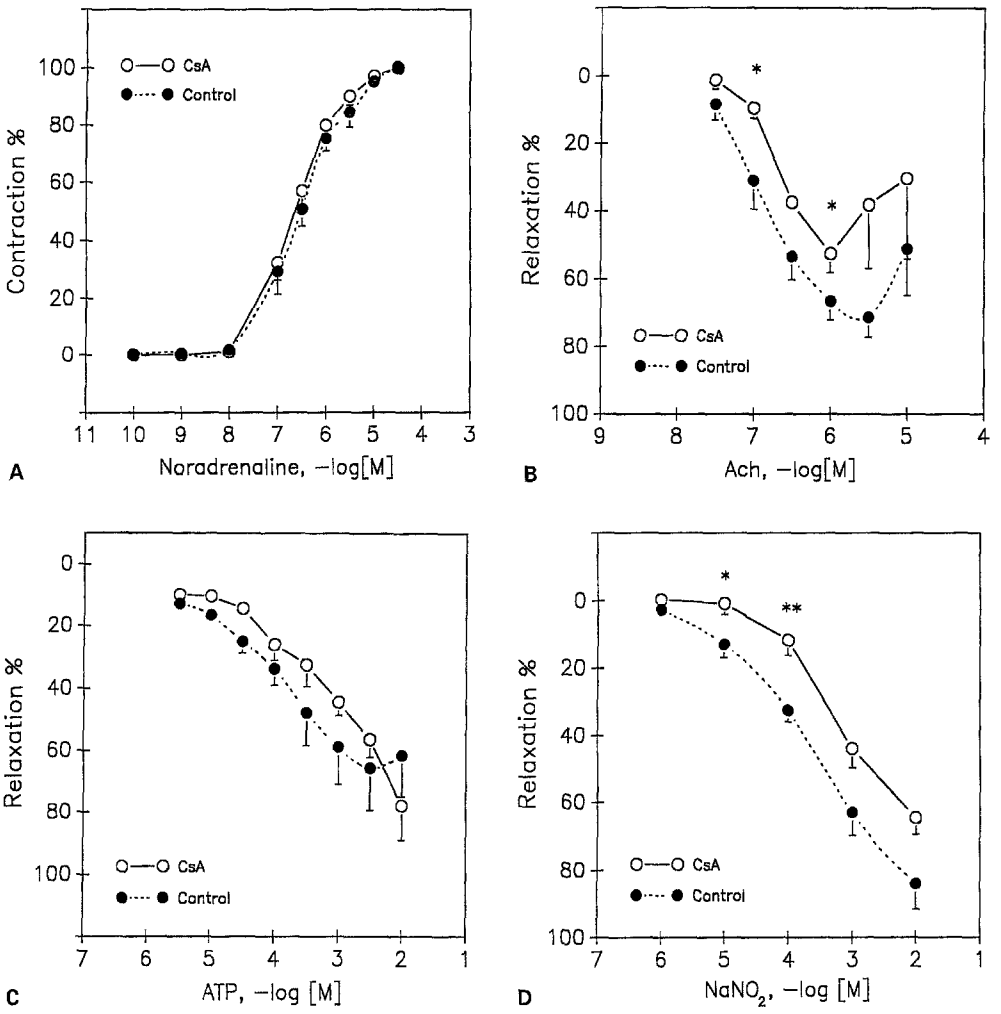

Fig. 1A–D Contraction induced by noradrenaline (**A**) in thoracic aorta obtained from rabbits treated with CsA or in controls. Responses are presented as percentage maximal response. Relaxing response of thoracic aorta to acetylcholine (ACh, **B**), to ATP (**C**) and sodium nitrite ($NaNO_2$, **D**), expressed as percentage EC_{50} NA contraction. Each point is a mean value ± SEM from five rabbits. $^*P < 0.05$, $^{**}P < 0.01$

ability of the endothelium from human coronary arteries to secrete a vasodilator substance (endothelium-dependent relaxing factor, EDRF) has not been affected by in vitro acute treatment with CsA (100–500 ng/ml) [22]. In vivo CsA administration in rat (5 mg/kg per day for 7–21 days) has failed to induce any direct damage of endothelial cell function and morphology [7]. On the contrary, other authors [1, 35] believe that CsA also acts by reducing the endothelium-dependent relaxation. These discrepancies are believed to derive from different experimental conditions [7]. In our work, the relaxant drug ATP, which acts partly via endothelium-dependent and partly by endothelium-independent mechanisms [9–11], showed no differences in effects in the aorta from the CsA-treated animals in comparison to controls. These results can be explained by considering that not all the vascular receptor components are equally sensitive to damage [10, 11]; also the lack of any variation in response to adenosine, PGE_1 and nifedipine confirms this hypothesis.

The reduced relaxation found with both endothelium-dependent and -independent drugs was suggested to be due to an increased release of endothelin caused by CsA [4, 5, 17]. We think that an altered release of endothelin is not involved in our experimental model, since the variation observed in the effect of ACh and sodium nitrite should also be observed with ATP.

Noradrenaline-induced contraction in thoracic aorta was no different in CsA-treated rabbits than in controls. In humans, CsA causes a sympathetic tone increase [34], which produces an arterial blood rise and may influence the physiological response of the cavernous smooth muscle. Despite these described effects, in our animal model, no evidence of changes in adrenergic receptor response due to CsA treatment was detected.

In cavernosal tissue preparation, the endothelial and smooth muscle components were tested, as in thoracic aorta, using various relaxing drugs. The response to ACh, which is usually an endothelium-dependent relaxation in this vascular area [28], in the present study was no differently affected by the treatment with CsA. Therefore this indicates that no alteration in the endothelial component of corpus cavernosum develops with CsA. This finding is confirmed also with ATP- and adenosine-induced relaxation. The direct smooth muscle relaxation induced by sodium nitrite or nifedipine was not found to be impaired by CsA treatment, suggesting also that the muscular compon-

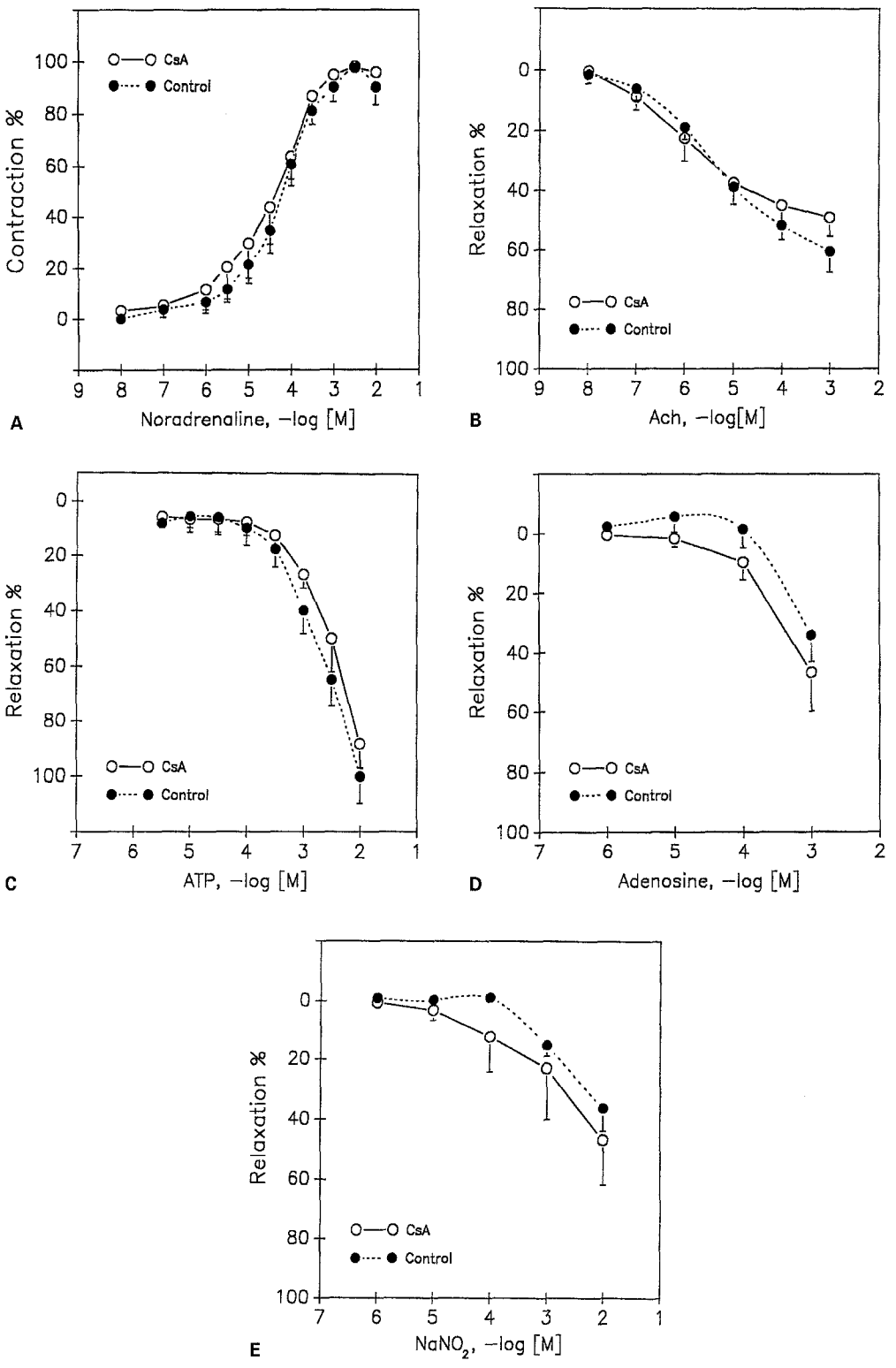

Fig. 2A–E Contraction induced by noradrenaline (**A**) in erectile tissue obtained from rabbits treated with CsA or in controls. Responses are presented as percentage maximal response. Relaxing response of erectile tissue to acetylcholine (ACh, **B**), to ATP (**C**), adenosine (**D**) and sodium nitrite ($NaNO_2$, **E**), expressed as percentage EC_{50} NA contraction. Each point is a mean value ± SEM from five rabbits. $^*P < 0.05$, $^{**}P < 0.01$

ent of the cavernous tissue is not affected by chronic treatment with the drug. Noradrenaline contraction was also not affected by CsA treatment, confirming the maintained muscular activity of the penile area.

Chronic treatment with CsA, at the doses used in the experiment, induced systemic toxic effects, revealed by a reduction of body weight. Analysis of chemical parameters in serum showed that creatinine levels were higher in rabbits treated with CsA, confirming a drug-induced renal impairment, as already described [33]. Urea nitrogen, however, was not significantly increased. Serum cholesterol was augmented in animals treated with

CsA, suggesting an interference with cholesterol metabolism, probably due to cholestasis or liver damage [16].

Our results show that chronic treatment with CsA in rabbit, despite alteration of the in vitro response of thoracic aorta, does not directly influence the response of penile tissue to relaxants. Although CsA may show species-dependent differences in its effects, from the present data it is possible to speculate that the impotence described to occur frequently in transplanted patients treated with CsA is not related to direct damage of corpus cavernosum induced by the drug, but is possibly due to other causes, organic or functional.

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