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Adenosine Deaminase Enzyme Therapy Prevents and Reverses the Heightened Cavernosal Relaxation in Priapism

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Abstract

Introduction—Priapism featured with painful prolonged penile erection is dangerous and commonly seen in sickle cell disease (SCD). The preventive approaches or effective treatment options for the disorder are limited because of poor understanding of its pathogenesis. Recent studies have revealed a novel role of excess adenosine in priapism caused by heightened cavernosal relaxation, and therefore present an intriguing mechanism-based therapeutic possibility.

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Aim—The aim of this study was to determine the therapeutic effects of adenosine deaminase (ADA) enzyme therapy to lower adenosine in priapism.

Methods—Both ADA-deficient mice and SCD transgenic (Tg) mice display priapism caused by excessive adenosine. Thus, we used these two distinct lines of mouse models of priapism as our investigative tools. Specifically, we treated both of these mice with different dosages of polyethylene glycol-modified ADA (PEG-ADA) to reduce adenosine levels in vivo. At the end points of the experiments, we evaluated the therapeutic effects of PEG-ADA treatment by measuring adenosine levels and monitoring the cavernosal relaxation.

Main Outcome Measures—Adenosine levels in penile tissues were measured by high-performance liquid chromatography, and cavernosal relaxation was quantified by electrical field stimulation (EFS)-induced corporal cavernosal strip (CCS) assays.

Results—We found that lowering adenosine levels in penile tissues by PEG-ADA treatment from birth in ADA-deficient mice prevented the increased EFS-induced CCS relaxation associated with priapism. Intriguingly, in both ADA-deficient mice and SCD Tg mice with established priapism, we found that normalization of adenosine levels in penile tissues by PEG-ADA treatment relieved the heightened EFS-induced cavernosal relaxation in priapism.

Conclusions—Our studies have identified that PEG-ADA is a novel, safe, and mechanism-based drug to prevent and correct excess adenosine-mediated increased cavernosal relaxation seen in two independent priapic animal models, and suggested its therapeutic possibility in men suffering from priapism.

Keywords

Adenosine Signaling; Priapism; Novel Therapies; Pharmacologic Treatment of Priapism

Introduction

Priapism is a condition of persistent penile erection lasting at least 4 hours in the absence of sexual excitation [1,2]. Although uncommon in the general population [3], it was recognized as a serious complication of sickle cell disease (SCD) as early as 1934 [4]. Ischemic priapism is the most common type and is highly associated with SCD. In general, about 40% of men with SCD display priapism [5–7]. The disorder is a urological emergency requiring a prompt and an accurate diagnosis and treatment because it is associated with erectile tissue damage and erectile disability [8–11]. However, the reactive treatments rarely restore normal erectile function, and preventive approaches to limit abnormal erection tendencies are limited because of the poor understanding of the pathophysiology of priapism.

In recent years, numerous studies have focused on the functional role of nitric oxide in priapism and erectile dysfunction (ED) [12,13]. Unexpectedly, a recent report indicates that endothelial nitric oxide synthase (eNOS)-deficient mice and eNOS/nNOS-double-deficient mice display priapic activity associated with dysregulated phosphodiesterase type 5 (PDE5) activity [14]. This surprising finding suggests that besides NO, some other factors may contribute to priapism [15,16]. For example, adenosine, like NO, is a potent vasodilator and neurotransmitter that elicits many physiological effects by engaging membrane receptors [17], and has long been implicated in regulating penile tumescence [18–20]. For example, earlier studies in multiple animal species, including humans [21], showed that intracavernous injection of adenosine resulted in tumescence and penile erection [18,19,22–24]. Theophylline, an adenosine receptor antagonist, inhibited adenosine-induced penile tumescence [23]. More recently, Tostes et al. presented evidence that adenosine-induced relaxation in mouse corpus cavernosum is mediated through activation of both A_{2A} and A_{2B} adenosine receptors [20]. These findings suggest that adenosine may contribute to penile erection through the activation of adenosine receptors.

Consistent with these reports, a recent study demonstrates that ED in men, in some cases, may be caused by endothelial A_{2B} adenosine receptor dysfunction [25]. However, the role of adenosine signaling in priapism was not recognized prior to recent studies reported by Mi et al. [26].

Adenosine deaminase (ADA) is a purine metabolic enzyme that catalyzes the conversion of adenosine to inosine. As a result of ADA deficiency, mice exhibit a marked increase in adenosine concentrations, particularly in the penis, which has the highest level of adenosine among all the tissues examined. These mice display features of priapism seen in humans, including spontaneous prolonged penile erection caused by excess adenosine-mediated increased vascular relaxation. This finding revealed adenosine as a causative factor contributing to priapism, and indicated that ADA-deficient mice represented a novel and important animal model to study the role of adenosine signaling in priapism. Subsequently, the significance of excess adenosine in priapism was extended and confirmed by a well-accepted animal model of priapism, SCD transgenic (Tg) mice [27–31]. Overall, these findings identified an essential role of excess adenosine signaling in priapism by increasing cavernosal relaxation in two animal models of this condition, and suggested potential therapies in priapism by targeting on this pathway.

To extend our discovery to therapeutic possibility in priapism, we took advantage of a safe drug, polyethylene glycol–modified ADA (PEG–ADA), to lower adenosine levels in both ADA-deficient mice and SCD Tg mice. The PEG modification of ADA serves to significantly prolong the serum half-life of the enzyme. Enzyme therapy is possible in the case of ADA deficiency because the enzyme does not require any cofactors and because the substrate, adenosine, is readily available in the circulation. PEG–ADA has been successfully used to treat and prevent elevated adenosine-mediated damage in multiple organs in both ADA-deficient humans and mice [32–35]. Here, we show that chronic reduction of accumulation of penile adenosine levels by PEG–ADA enzyme therapy prevents and corrects excess adenosine-mediated increased cavernosal relaxation in two independent priapic animal models: ADA-deficient mice and SCD Tg mice. Thus, these studies provide the direct evidence for the utility of PEG–ADA enzyme therapy for priapism in mice by attenuating the heightened cavernosal relaxation, and suggest its therapeutic possibility in men suffering from priapism.

Materials and Methods

Mice

ADA-deficient mice were generated and genotyped as previously described [33,36,37]. Control mice, designated ADA⁺, were littermates that were heterozygotes for the null *Ada* allele. Heterozygous mice do not display a phenotype. All mice were on a mixed 129sV/C57BL/6J background and were backcrossed at least 10 generations on the C57BL/6 background. All phenotypic comparisons were performed among littermates. SCD Tg mice, expressing exclusively human sickle hemoglobin, were purchased from The Jackson Laboratory (Bar Harbor, ME, USA) [29,30]. For SCD mice study, wild-type mice C57BL/6 were used as controls. All mice were maintained and housed in accordance with National Institutes of Health guidelines and with the approval of the Animal Care and Use Committee at the University of Texas Health Science Center at Houston.

ADA Enzyme Therapy

PEG–ADA was generated by the covalent modification of purified bovine ADA with activated PEG as described previously [38–40]. Different dosages of PEG–ADA were delivered weekly by intraperitoneal injection to reduce adenosine levels. Specifically, the ADA-deficient mice were maintained on high-dose (HD) enzyme therapy at 5 U/week for at least 8 weeks to allow

for normal penile development. At 8 weeks of age, the dose of PEG-ADA was gradually tapered down over a period of 8 weeks to a low dosage of 0.625 U/week (2.5 U for 2 weeks, 1.25 U for 2 weeks, 0.625 U for 4 weeks). This dosing protocol was designated a “low-dose” (LD) PEG-ADA treatment regimen. Another group of mice were tapered down to an LD of PEG-ADA for 6 weeks, and then returned to high dosage of PEG-ADA for 2 weeks (2.5 U for 2 weeks, 1.25 U for 2 weeks, 0.625 U for 2 weeks, and 5 U for 2 weeks). This dosing protocol was designated an “LD-HD” (LD + HD) PEG-ADA treatment regimen. Some ADA-deficient mice were treated from birth with a high dosage of PEG-ADA at 5 U/week for 16 weeks. This dosing protocol was designated an “HD” PEG-ADA treatment regimen. For all experiments, ADA⁺ mice treated with or without HD of PEG-ADA at 5 U/week were used as the controls.

For SCD Tg mice, at 8 weeks of age, a group of mice were injected with 2.5 U PEG-ADA weekly for 8 weeks. This dosing protocol was designated SCD with PEG-ADA treatment regimen (SCD + PEG-ADA). Other SCD mice were injected with normal saline for 8 weeks; it was designated as SCD without PEG-ADA treatment regimen (SCD). Age-matched C57Bl6 treated with or without PEG-ADA at 2.5 U/week were used as the controls.

Quantification of Penile Adenosine Levels

The mice were anesthetized, and the penises were rapidly removed and frozen in liquid nitrogen. Adenine nucleosides were extracted from frozen penises using 0.4 N perchloric acid, and adenosine was separated and quantified using reversed-phase high-performance liquid chromatography (HPLC) as described previously [36,37].

Physiological Function Experiments

The mice were anesthetized, and the penises were surgically removed and placed in Krebs solution as described previously [26]. The erectile tissue was partially dissected free from the tunica, and two strips of tissue were obtained from each corpus cavernosum. The contractility of each isolated corpus cavernosal strip (CCS) was measured using an isometric force transducer (AD Instruments Inc., Colorado Springs, CO, USA). The strips were mounted in a 10-mL organ culture system containing Krebs solution at 37°C continuously bubbled with a mixture of 95% O₂ and 5% CO₂ (pH 7.4) and stretched to a resting tension of 0.1 mN. The contractile responses of the strips were obtained by adding 10 μM phenylephrine to the bath, and force changes were recorded in response to electrical field stimulation (EFS). The particular EFS parameters of 0–30 V and 30 Hz, with a pulse width of approximately 0.5 ms, were used in these experiments. Available evidence indicates that to mimic normal penile erection, these electrical field strength parameters stimulate only neurons but not muscle [41–43].

Statistical Analysis

All data were expressed as the mean ± SEM. Data were analyzed for statistical significance using GraphPad Prism 4 software (GraphPad Software, San Diego, CA, USA). Student's *t*-tests (paired or unpaired as appropriate) were applied in two-group analysis. Differences between the means of multiple groups were compared by one-way analysis of variance, followed by a Tukey's multiple comparisons test. A value of *P* < 0.05 was considered significant and was the threshold to reject the null hypothesis.

Results

PEG-ADA Enzyme Therapy from Birth Lowers Adenosine Levels to Normal in the Penile Tissues of ADA-Deficient Mice

ADA-deficient mice are a well-accepted animal model to study the adverse affects of enhanced adenosine signaling. Recently, we have unexpectedly found that ADA-deficient mice display features of priapism seen in humans, spontaneous prolonged penile erection, caused by excess adenosine-mediated increased cavernosal relaxation, indicating ADA-deficient mice are valuable animal models of priapism [26]. On the basis of these findings, we hypothesized that we could prevent the priapism by attenuating increased cavernosal relaxation in these mice if we could block the accumulation of adenosine. To test this hypothesis, we treated ADA-deficient mice with a high dosage regimen of PEG-ADA (HD) from birth to 8 weeks of age in an effort to maintain normal adenosine levels, and allow for normal penile development. At 8 weeks of age, half the group continued to receive HD PEG-ADA therapy (5 units/week) to prevent the accumulation of adenosine. For the other half, the dose of PEG-ADA was gradually tapered down over a period of 8 weeks (for details, see Figure 1A and Materials and Methods) to a low dosage of 0.625 units/week. By this means, accumulated adenosine in penile tissues led to priapic phenotype as shown before [26,44]. At 16 weeks of age, all the mice were sacrificed. We found that the adenosine levels in the penises of mice receiving the HD regimen of PEG-ADA were similar to those in penile tissues of the ADA⁺ controls. However, adenosine levels in the penile tissues of the mice on the LD regimen of PEG-ADA were remarkably higher than those of HD-treated mice and the controls (Figure 1B). We also observed that the adenosine levels in the controls with HD PEG-ADA treatment were decreased compared with control ADA⁺ mice without PEG-ADA treatment. Thus, PEG-ADA treatment since birth is a safe approach to effectively lower adenosine levels to normal levels in penile tissues.

Chronic Reduction of Penile Adenosine Accumulation by PEG-ADA Enzyme Therapy Prevents the Increased Cavernosal Relaxation in ADA-Deficient Mice Since Birth

Realizing that the increased excess adenosine-induced cavernosal relaxation is the major underlying mechanism for priapism seen in ADA-deficient mice, we chose to determine the preventative effect of PEG-ADA enzyme therapy by measuring the relaxation of isolated CCSs in response to EFS as previously described [26]. This method is commonly used to stimulate the cavernosal nerves, and results in smooth muscle relaxation in the corpus cavernosum, corresponding to normal physiological erection [14,41–43,45]. After 16 weeks of PEG-ADA treatment, we found that EFS-induced relaxation of CCSs showed a voltage-dependent response in both the control and mutant mice. In particular, the CCSs of ADA-deficient mice with LD PEG-ADA treatment displayed increased sensitivity to EFS and achieved maximal relaxation with 5 V and 30 Hz stimulation. However, the CCSs from the ADA-deficient mice receiving the HD treatment reached the maximal relaxation with 20 V and 30 Hz stimulation as did the controls (Figure 2). For subsequent experiments, we chose 5 V and 30 Hz for 60 seconds to stimulate the CCSs from the different mice. We found that HD-treated mutant mice showed significantly decreased intensity and duration of CCS relaxation in comparison to the mice on the LD regimen (Figure 3). Taken together, these results demonstrate that the HD PEG-ADA treatment from birth attenuates excess adenosine-enhanced cavernosal relaxation, a major underlying mechanism for priapism, in ADA-deficient mice on the LD regimen. Thus, these studies suggest that PEG-ADA is likely a safe and mechanism-based drug to prevent excess adenosine-mediated increased cavernosal relaxation associated with priapism since birth in these mice.

PEG-ADA Enzyme Therapy Reverses the Excess Adenosine-Induced Cavernosal Relaxation in Adult ADA-Deficient Mice

Subsequently, we propose to determine whether PEG-ADA enzyme therapy can reverse the increased cavernosal relaxation seen in adult ADA-deficient mice. For this purpose, we chose to treat a group of ADA-deficient mice with the LD enzyme therapy regimen to allow adenosine elevated and generate priapism features as described previously (for details, see Figure 1A and Methods) [44]. To assess the therapeutic possibility of PEG-ADA treatment on priapism seen in adult ADA deficient, half of the LD-treated mice were switched to the HD regimen of PEG-ADA enzyme therapy (5 units/week) for two more weeks (LD + HD) (Figure 1A). HPLC results showed that adenosine levels in penile tissue of LD + HD-treated mice were significantly reduced by HD PEG-ADA therapy compared with LD-treated mice (Figure 1B). Next, we found that the HD PEG-ADA treatment reversed the increased cavernosal relaxation in response to EFS seen in LD-treated ADA-deficient mice. Specifically, HD PEG-ADA treatment corrected the increased sensitivity to EFS and achieved maximal relaxation with 20 V and 30 Hz stimulation similar to those seen in the controls (Figure 2). In particular, physiological functional assays demonstrated that CCSs of LD + HD-treated mice exhibited a marked decrease in the intensity and duration of CCS relaxation in response to EFS at 5 V and 30 Hz for 60 seconds compared with CCSs of LD-treated mice (Figure 3). These results indicate that PEG-ADA enzyme therapy can reduce elevated adenosine levels to normal and can relieve the increased cavernosal relaxation associated with priapism in adult ADA-deficient mice.

PEG-ADA Enzyme Therapy Reduces Adenosine Levels to Normal in SCD Tg Mice

Our previous studies have shown that adenosine levels in penile tissue of 8-week-old SCD Tg mice are higher than that in controls, and that the increased level of adenosine contributes to priapic features caused by increased cavernosal relaxation [26]. To explore the significance of PEG-ADA therapy in the treatment of priapism, we extended our studies to SCD Tg mice, a well-accepted animal model of priapism [27–31]. Briefly, 8-week-old SCD Tg mice with established features of priapism were treated with a relatively HD of PEG-ADA (2.5 U/week) in order to reduce the elevated levels of adenosine associated with penile tissue of SCD Tg mice (Figure 4A). After 8 weeks of PEG-ADA enzyme therapy, the adenosine concentration in the penises of SCD Tg mice was significantly reduced (Figure 4B). Thus, PEG-ADA enzyme therapy is a safe and an effective drug to lower adenosine levels in penile tissues in SCD Tg mice as seen in ADA-deficient mice.

Chronic Reduction of Adenosine Levels by PEG-ADA Enzyme Therapy Corrects the Increased Cavernosal Relaxation in SCD Tg Mice

To determine whether the reduction in adenosine levels to normal by PEG-ADA enzyme therapy corrected the increased cavernosal relaxation seen in SCD Tg mice, we performed physiological functional assays as described earlier. Similar to LD + HD-treated ADA-deficient mice, the CCSs of SCD Tg mice treated with PEG-ADA achieved maximal relaxation with 20 V and 30 Hz compared to SCD Tg mice that did not receive PEG-ADA treatment that showed maximal relaxation with 10 V and 30 Hz (Figure 5). Furthermore, PEG-ADA enzyme therapy also resulted in a significant reduction in the intensity and duration of EFS-induced CCS relaxation in SCD Tg mice (Figure 6). Thus, these results demonstrate that reduction of penile adenosine levels by chronic PEG-ADA enzyme therapy ameliorates the increased cavernosal relaxation, a major cause of priapism, in SCD Tg mice.

Discussion

An unexpected priapic phenotype associated with ADA-deficient mice prompted us to hypothesize that increased adenosine contributes to priapism. This hypothesis was confirmed and significantly extended by analysis of SCD Tg mice, a well-accepted animal model of

priapism [26]. We have demonstrated that excessive adenosine-induced cavernosal relaxation via A_{2B} adenosine receptor signaling is an underlying mechanism responsible for priapism in these two distinct lines of mice with priapism. Our discovery immediately suggests that targeting on adenosine signaling pathway represents a mechanism-based therapeutic possibility for the treatment and prevention of priapism. Here, we report that chronic reduction of adenosine by PEG-ADA in penile tissues prevented and/or reversed increased cavernosal relaxation, a major underlying mechanism responsible for priapism in both ADA-deficient mice and SCD Tg mice. Thus, our studies have revealed previously unrecognized application of PEG-ADA as a safe, an effective, and a mechanism-based drug to treat and prevent the elevated cavernosal relaxation in two independent animal models of priapism.

Priapism is a dangerous and painful condition that, without intervention, will develop penile vascular damage and fibrosis, eventually resulting in ED. For example, Pryor et al. revealed that 90% of patients result in ED if the priapism episode lasts more than 24 hours [46]. Recently, Bennett and Mulhall demonstrated that no patient is able to generate a functional erection if the patient experiences priapism more than 36 hours [47]. In contrast, 44% of patients are able to maintain penetration quality erections after 24–36 hours of priapism episode [47]. Overall, the rate of ED seen in priapism patients is high, and therefore it is urgent to find a way to prevent and treat priapism, and avoid the erectile tissue damage, fibrosis, and loss of erectile function in SCD patients. This highlights the need for basic research aimed at understanding the molecular mechanisms of the disease. Such efforts will lead to better treatment and prevention of priapism.

Multiple factors may contribute to priapism. Previous research indicates dysregulated nitric oxide signaling may lead to priapism. Champion et al. found decreased PDE5 expression and activity in the penises of genetically altered mouse models lacking eNOS and in SCD Tg mice. Each of these mice display a priapism-like phenotype [14]. These observations imply that pathological changes in PDE5 may contribute to priapism. In addition to NO, soluble guanylyl cyclase is also activated by carbon monoxide (CO) which is produced by the enzyme heme oxygenase. An inducible isoform of heme oxygenase (HO-1) is activated under hypoxic conditions to regulate vascular smooth muscle tone [15,16]. Jin et al. recently reported that the synthesis and activity of HO-1 are increased in experimentally induced low-flow priapism in rat penile tissues [48], suggesting that hypoxia-mediated CO production may contribute to low-flow priapism. In addition, Munarriz et al. presented an interesting finding that in the rabbit corpus cavernosum, ischemic priapism is associated with decreased binding affinity of adrenergic ligands for their receptors; therefore, resulting in decreased contractile ability [49]. These findings imply that imbalance of vasoconstrictors and vasodilators may be an underlying mechanism for priapism. Intriguingly, our current studies revealed that excess adenosine, one of best-known molecule to be induced under hypoxic/ischemic conditions, is a causative factor contributing to priapism by increasing cavernosal relaxation in both ADA-deficient mice and SCD Tg mice [26,44]. Adenosine is well known for its potent direct vascular relaxation effect. We have revealed that increased cGMP mediated via A_{2B}R is an underlying mechanism responsible for excess adenosine-mediated priapism in these mice [26,44]. Based on the fact that adenosine is highly induced under ischemic and hypoxic conditions, and the study reported by Lin et al. showing that PDE5 is down-regulated in ischemic corpus cavernosal smooth muscle cells in vitro [50], it is also possible that increased adenosine caused by ischemia may be a potential factor to down-regulate PDE5 via A_{2B}R, and can lead to elevated cGMP production in priapism associated with SCD Tg mice or eNOS-deficient mice. It is also possible that excess adenosine signaling may also contribute to priapism via regulation of HO-1 expression/activity and adrenergic receptor affinity to its ligand. These will be important issues to address in the near future.

Although the therapeutic strategies for priapism are limited, several studies shed light on the potential of prevention and treatment of priapism based on our current progress in understanding of the pathogenesis of priapism as described earlier [49,51–53]. For example, Burnett et al. showed that chronic reduction of PDE5 by its inhibitor is successfully used to treat several patients in decreasing the priapic episode by normalization of dysregulated PDE5 activity [51,54]. In addition, Munarriz et al. presented a finding on the use of high dosage of intracavernosal phenylephrine in the management of ischemic priapism in two patients [49]. Notably, our published studies provide the in vivo evidence that direct injection of PEG–ADA into ADA-deficient mice quickly corrects the spontaneously prolonged penile erection by lowering adenosine-mediated cavernosal relaxation. These findings suggest that PEG–ADA is likely a potential therapy to treat priapism under urgent conditions [26,44]. In the current study, we test the therapeutic possibility of chronic reduction of adenosine by PEG–ADA enzyme therapy in priapism. Here, we demonstrate that chronic reduction of adenosine levels by PEG–ADA treatment since birth in ADA-deficient mice prevents the increased cavernosal relaxation, a major cause of priapism. Additionally, we discovered that PEG–ADA enzyme therapy corrects excess adenosine-induced cavernosal relaxation, the underlying mechanism contributing to priapism in ADA-deficient mice and SCD Tg mice. We believe our current studies demonstrate that PEG–ADA is a novel and an effective mechanism-based drug to prevent and treat excess adenosine-induced cavernosal relaxation in these two animal models of priapism.

Early studies hinted at a potential role for adenosine signaling in penile erection [18,55–57]. However, attention was diverted from adenosine signaling to NO by the discovery of PDE5 inhibitors, such as sildenafil (i.e., Viagra), that promote penile erection [12]. Attention has recently returned to adenosine signaling with reports indicating that impaired A_{2B}R signaling is associated with ED in men [58], and our study showing that excess adenosine contributes to priapism via A_{2B}R signaling in both ADA-deficient mice and SCD Tg mice [26]. Taken together, substantial evidence now suggests a general role for adenosine signaling in normal penile erection as a vasorelaxant and neuromodulator. From this perspective, it is not surprising that impaired adenosine signaling is associated with ED [58], and excessive adenosine signaling is associated with priapism [26]. Thus, adenosine signaling represents a potentially important therapeutic target for the treatment of priapism and ED. Notably, the discovery of excess adenosine as the causative factor for priapism in mice opens up the possibility of treating and even preventing this painful and dangerous disorder with PEG–ADA enzyme therapy to reduce adenosine or specific antagonists to block A_{2B}R signaling. Finally, the role of adenosine signaling in sickle cell anemia has not been previously identified; it will be very interesting to determine whether this signaling pathway plays a role in sickle cell anemia in the future.

Clinical Significance

Our preclinical studies suggest that PEG–ADA is likely a novel mechanism-based therapy for priapism in men by attenuation of increased cavernosal relaxation. Over 150 patients worldwide have received PEG–ADA enzyme therapy for the treatment of ADA deficiency (SCID), and approximately 90 patients were alive as of 2006 when the most recent survey was reported [59,60]. Approximately one-third of the 90 individuals currently alive have received PEG–ADA for 10–19 years [34,59–61]. PEG–ADA has generally been well tolerated, and no allergic or hypersensitivity reactions have been reported. PEG–ADA is effective and life-saving for ADA-deficient individuals. More importantly, we also treated SCD Tg mice for over 2 months with PEG–ADA. We have not observed any adverse effects of PEG–ADA use in these mice. Thus, PEG–ADA treatment may be safe for humans with SCD and priapism. We believe our animal studies are important, and point the way to future clinical studies in men suffering from priapism.

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Figure 1.

Average adenosine level in the penises of adenosine deaminase (ADA)-deficient mice following different treatment regimens of polyethylene glycol (PEG)-ADA enzyme therapy. (A) Different dosages of PEG-ADA treatment in both the controls and ADA-deficient mice. (B) Adenosine level in penile tissues. Data are expressed as means $\pm$ SEM ($n = 4-5$). * $P < 0.05$ vs. control mice. ** $P < 0.05$ vs. ADA-deficient mice with low-dose (LD) PEG-ADA therapy. HD = high dose; LD + HD = LD regimen followed with HD regimen of PEG-ADA therapy to reverse priapic feature.

Figure 2.

Corpus cavernosal strip relaxation was achieved by electrical field stimulation (EFS) using different voltages. Low-dose (LD) mice displayed hypersensitivity to EFS and achieved maximal relaxation at 5 V. High-dose (HD) and LD + HD polyethylene glycol (PEG)–adenosine deaminase (ADA) enzyme therapy prevented and reversed the hypersensitive response to EFS, and achieved maximal relaxation at 20 V. (n = 4–5). LD + HD = LD regimen followed with HD regimen of PEG–ADA therapy to reverse priapic feature. * $P < 0.05$ vs. control mice. ** $P < 0.05$ vs. ADA-deficient mice with LD PEG–ADA therapy.

Figure 3.

Polyethylene glycol (PEG)-adenosine deaminase (ADA) enzyme therapy prevents and reverses increased cavernosal relaxation seen in ADA-deficient mice. (A) Representative recordings of electrical field stimulation (EFS)-induced corporal cavernosal strip (CCS) relaxation (5 V and 30 Hz for 60 seconds) in ADA-deficient mice with different regimens of PEG-ADA enzyme therapy. Δ means EFS stop. (B-D) CCS relaxation was induced by EFS at 5 V in ADA-deficient mice with different regimens of PEG-ADA enzyme therapy. PEG-ADA enzyme therapy prevented and reversed the increased cavernosal relaxation seen in ADA-deficient mice. Data are expressed as the mean $\pm$ SEM ($n = 4-5$). * $P < 0.05$ vs. control mice. ** $P < 0.05$ vs. ADA-deficient mice with LD PEG-ADA therapy. (B) Average EFS-induced

relaxation. (C) Average duration of relaxation. (D) The area under baseline (the combination of force and duration) following EFS-induced relaxation.

Figure 4. Adenosine levels in the penises of sickle cell disease (SCD) transgenic (Tg) mice with or without polyethylene glycol (PEG)–adenosine deaminase (ADA) enzyme therapy. (A) Different dosages of PEG–ADA treatment in both the controls and SCD Tg mice. (B) Adenosine levels in penile tissues. Data are expressed as mean $\pm$ SEM ($n = 5-6$). * $P < 0.05$ vs. control mice. ** $P < 0.05$ vs. SCD Tg mice without PEG–ADA therapy.

Figure 5.

Sickle cell disease (SCD) transgenic (Tg) mice displayed hypersensitivity to electrical field stimulation (EFS) and achieved maximal relaxation at lower voltage. Polyethylene glycol (PEG)–adenosine deaminase (ADA) enzyme therapy corrected the hypersensitive response to EFS. (n = 5–6). * $P < 0.05$ vs. control mice. ** $P < 0.05$ vs. SCD Tg mice without PEG–ADA therapy.

Figure 6.

Polyethylene glycol (PEG)-adenosine deaminase (ADA) enzyme therapy corrects excess adenosine-induced cavernosal relaxation seen in sickle cell disease (SCD) transgenic (Tg) mice. (A) Representative recordings of electrical field stimulation (EFS)-induced corporal cavernosal strip (CCS) relaxation (10 V and 30 Hz for 60 seconds) in SCD Tg mice. Δ means EFS stop. (B–D) CCS relaxation was induced by EFS at 10 V in SCD Tg mice with or without PEG-ADA enzyme therapy. PEG-ADA enzyme therapy corrected the enhanced cavernosal relaxation in SCD Tg mice. Data are expressed as mean $\pm$ SEM (n = 5–6). * P < 0.05 vs. control mice. ** P < 0.05 vs. SCD Tg mice without PEG-ADA therapy. (B) Average EFS-induced

relaxation. (C) Average duration of relaxation. (D) The area under baseline following EFS-induced relaxation.