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A3 adenosine receptor allosteric modulator CF602 reverses erectile dysfunction in a diabetic rat model

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Abstract

Adenosine plays a major role in erection by binding to its receptors and activating pathways resulting in increased arterial blood flow and intracavernosal pressure (ICP). CF602, an allosteric modulator of the A3 adenosine receptor (A3AR), increases the binding affinity of the endogenous adenosine to the receptor. We examined the effect of CF602 on resolving erectile dysfunction (ED) in a diabetic ED rat model (streptozotocin-induced diabetic rats that were screened for ED using the apomorphine test). ED was assessed by measuring ICP and main arterial pressure (MAP) during electrostimulation of the cavernosal nerve. A single dose of CF602 or placebo was applied either topically (100 μl from a 100 nM or 500 nM solution) or orally (100, 200 or 500 μg/kg) prior to erectile function assessment. A significant dose-dependent improvement in the ICP:MAP ratio without a change in MAP was recorded with the topical and oral CF602 treatments. A significant increase in smooth muscle:collagen ratio, vascular endothelial growth factor and endothelial nitric oxide synthase was also observed in both administration modes. In conclusion, topical and oral treatment with CF602 significantly improved erectile function, supporting its further evaluation as a treatment for ED.

KEYWORDS
A3 adenosine receptor, erectile dysfunction, intracavernosal pressure, nitric oxide, VEGF

1 | INTRODUCTION

Erectile dysfunction (ED), which adversely impacts quality-of-life, is a growing problem, affecting 52% of men over 40 years old. ED is primarily a disease of vascular origin, although it can also be associated with neurologic, endocrinologic, or psychologic disorders, trauma or could be a side effect of medication or substance abuse (Evans & Hill, 2015). In diabetic men, where ED is prevalent, ED pathogenesis results from endothelial cell damage and vascular dysfunction due to poor blood sugar control that generally develops at an early age (Cignarelli et al., 2021). The damaged endothelial cells that line the corpus cavernosum lead to a decrease in endothelial nitric oxide synthase (eNOS), and ultimately to decreased nitric oxide (NO) secretion. NO plays a central role in smooth muscle relaxation and vascular inflow, which are key in normal penile erection. During ED, however, arterial inflow and smooth muscle cavernosal relaxation are impaired (McMahon, 2014; Shamloul & Ghanem, 2013).

NO elevation remains a target in ED therapy and PDE5 inhibitors (e.g., sildenafil and tadalafil) inhibit the pathways that counteract the NO synthesis. However, approximately 50% of diabetic patients do not respond to PDE5 inhibitors (Abdel-Hamida, Abo-Alyb, & Elsaied, 2014). Vascular endothelial growth factor (VEGF), a multifunctional protein known to upregulate eNOS production, has a protective effect on endothelial cells (Yamanaka et al., 2005). However, no VEGF products are currently indicated for ED. Other treatment options, including intracavernosal injections and penile prosthesis implantation, are complex and do not improve patient's quality-of-life (Hatzimouratidis & Hatzichristou, 2014; McMahon, 2019). Thus, clearly, additional treatment options are necessary.

Adenosine, a purine nucleoside, signals via the adenosine receptor family which entails four subtypes: A1, A2A, A2B and A3.

Adenosine plays a major role in erection and was found to increase arterial blood flow and intracavernosal pressure (ICP) in vivo and in vitro in a dose-dependent manner. Intracavernosal injection of adenosine in dogs led to increased arterial blood flow and ICP that resulted in full erection. This effect was counteracted by a non-selective adenosine receptor antagonist, indicating that the adenosine-specific effect is mediated via its receptors. In different species, in vitro studies with corpus cavernosal strips (CCS) and in vivo studies with Adora1$^{-/-}$, Ado-ra2a$^{-/-}$, Adora2b$^{-/-}$ and Adora3$^{-/-}$-deficient mice demonstrated that the A2B is responsible for the muscle relaxing effect of adenosine (Takahashi, Ishii, Lue, & Tanagho, 1992; Wen & Xia, 2012). Moreover, resistance to adenosine relaxation was observed in CCS derived from human subjects suffering from vasculogenic impotence due to endothelial A2B receptor dysfunction, a result which further supports the notion that the A2B receptor confers this effect (Faria, Magalhaes-Cardoso, Lafuente-de-Carvalho, & Correia-de-Sa, 2006). Agonists to A2A and A2B induce relaxation of mouse CCS, whereas antagonists to these receptors abrogate this response. A1 adenosine receptor agonist and antagonist did not induce a relaxation effect (Tostes et al., 2007).

The A3 adenosine receptor (A3AR) is overexpressed in pathological cells, and its activation by a specific agonist induces anti-inflammatory and protective effects via modulation of the PI3K-PKB/Akt signal transduction pathway (Cohen et al., 2011; Fishman et al., 2006; Headrick & Peart, 2005; Ochaion et al., 2008; Wahlman et al., 2018). This molecular mechanism is also involved with the adenosine effect on ED mediated via the A2B adenosine receptor (Wen et al., 2011; Wen & Xia, 2012). The highly specific A3AR agonists, piclidenoson and namodenoson, are currently in advanced clinical studies in inflammatory and liver diseases, respectively (NCT03168256, NCT02647762, NCT02128958 and NCT02927314). Interestingly, patients' anecdotal reports show an improvement in erectile function as a positive adverse effect to piclidenoson treatment (unpublished data). These findings were the rationale for the present study which investigated CF602, an allosteric modulator of A3AR for ED (Figure 1). CF602 is an imidazoquinolinamine allosteric enhancer of A3AR that enhances adenosine interaction with the receptor and raises its maximal effect by 45% (Ohana, Cohen, Rath-Wolfson, & Fishman, 2016). It acts as a dimmer switch that improves the binding affinity of the endogenous adenosine ligand to the receptor. As, in contrast to receptor agonists, an allosteric modulator has no effect on the unoccupied receptor, CF602 can specifically target areas where adenosine levels are increased, such as inflammatory and tumour sites, whereas normal cells and tissues where adenosine levels are low, are unaffected (Goblyos et al., 2006).

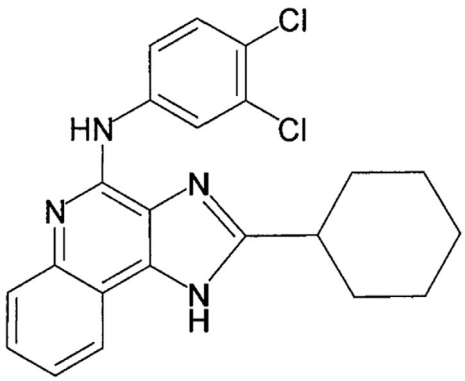

FIGURE 1 Chemical structure of CF602, an A3AR allosteric modulator

Our previous data showed the anti-inflammatory and protective effects of CF602 in three experimental animal models of rheumatoid arthritis, osteoarthritis and concanavalin A-induced liver inflammation. These effects are attributed to A3AR activation by the ligand, with a subsequent receptor down-regulation due to internalization and degradation (Cohen et al., 2014).

In the current study, we investigated the effect of CF602 on ED when given topically or orally in a rat diabetic ED model.

2 | METHODOLOGY

2.1 | Reagents

The allosteric modulator CF602 (N-(3,4-dichloro-phenyl)-2-cyclohexyl-IH-imidazo [4,5-c]quinolin-4-amine) was synthesized for Can-Fite BioPharma Ltd. at Haoyuan Chemexpress Co., Ltd. For the oral administration, a stock solution of 10 mM was prepared in dimethyl sulphoxide (DMSO; Sigma Aldrich) and further dilutions were prepared in phosphate-buffered saline (PBS). For the topical administration, a stock solution of 10 mM in Kollisolv® PEG E 400 (Sigma Aldrich) was prepared. Further dilutions were prepared in the same solvent.

2.2 | Animals

Healthy adult Sprague–Dawley male rats (weight average of 250 g) were purchased from Harlan laboratories. Animal handling was performed according to guidelines of the National Institute of Health and the Association for Assessment and Accreditation of Laboratory Animal Care. Animals were housed in polyethylene cages (3/cage) measuring 35 × 30 × 15 cm, with stainless steel top grill facilitating pelleted food and drinking water in plastic bottles; steam sterilized clean paddy husk (Harlan Sani-chip, cat #: 7090A; Harlan) were used as bedding material and were changed along with the cage at least twice a week. Animals were fed ad libitum a commercial rodent diet (Teklad Certified Global 18% Protein Diet cat #: 106S8216; Envigo) and had free access to autoclaved and acidified drinking water (pH between 2.5 and 3.5). Animals were housed under standard laboratory conditions, air conditioned and filtered (HEPA F6/6) with adequate fresh air supply (minimum 15 air changes/h). Animals were kept in a climate-controlled environment. Temperature range was 20–24°C and relative humidity range was 30%–70% with 12 h light and 12 h dark cycle. Studies were approved and in compliance with The Israel Animal Welfare Act and received the Israel Board for Animal Experiments approval (no. IL-20-12-530).

2.3 | Establishing a diabetic ED model in rats

Streptozotocin (STZ; Cayman Chemicals) (60 mg/kg in citrate buffer) was administered intraperitoneally. Animals with blood glucose level >290 mg/dl after 3 weeks were included in the study. These animals were screened for ED by the apomorphine test. Apomorphine (Sigma Aldrich) 150 μg/kg was injected subcutaneously and animals were observed for 30 min for penile erections. Erection was defined as the emergence of an engorged glands penis with distal shaft. Only animals with no erections were considered as having diabetic ED and included in the study.

2.4 | Erectile function assessment

Erectile function was determined by ICP and main arterial pressure (MAP) measurements. For the MAP measurements, animals were anaesthetised with ketamine/xylazine. A PE-50 catheter filled with 250 units/ml heparinized saline was inserted to the left carotid artery and connected to a pressure transducer (Biopac System MP 150) to monitor the arterial blood pressure. For ICP measurement, a low abdominal incision was used to expose the right major pelvic ganglion and identify the ipsilateral cavernous nerve. The right corporal body was cannulated with a 23-G butterfly needle to measure the ICP. The cavernous nerve was exposed and electro stimulation (20 Hz, 5 V, 60 s duration) was applied via a bipolar stainless steel hook electrode. The ratio of max ICP to MAP was calculated to normalize the variation in systemic blood pressure between the rats. Two modes of treatment were used, topical and oral. A single dose of 100 μl CF602 solution (100 or 500 nM) was topically applied to the surface of the penis 35, 50 and 65 min prior to erectile function assessment. In a different set of experiments, CF602 100 μg/kg, 200 μg/kg and 500 μg/kg were administered orally by gavage as a single dose 60 min prior to the erectile function assessment. Three different experiments were performed and each group included 10–12 animals. All experiments were double-blind and placebo-controlled.

2.5 | Immunohistochemical and histology analysis

After the ICP measurements, rats were sacrificed by CO_2 inhalation. The middle part of the penile tissue was exposed and dissected. Tissue samples were fixed for 48 h in 4% paraformaldehyde (Santa Cruz Biotechnology, Inc), processed with the Leica HistoCore tissue processor (Leica Biosystems) and embedded in paraffin. Sections ($n = 5$) were prepared from each animal at 4 μm. To detect the ratio of rat cavernosum muscle to collagen, cavernosum tissue sections were stained with Masson's trichrome staining according to manufacturer's instructions (Bio-Optica). The positive stained area was measured and expressed as the ratio of muscle to collagen. Immunostaining was performed using the Leica Bond max system (Leica Biosystems). Slides were baked for 30 min at 60°C, dewaxed and pretreated with epitope-retrieval solution (Leica Biosystems) followed by incubation with primary antibodies for VEGF or VEGF fluorescence (1:600; Proteintech catalogue number 19003-1-AP/1:100; Santa Cruz Biotechnology, Inc.) and eNOS or eNOS fluorescence (1:200; Abcam catalogue number ab5589/1:50; Abcam), α-SMA (1:600; Novusbio catalogue number NBP2-33006; Novus Biologicals) and A3AR (1:100 Santa Cruz catalogue number 13938; Santa Cruz Biotechnology, Inc.). Detection was performed using the Leica Bond Polymer Refine HRP kit (Leica Biosystems). All slides were counter-stained with haematoxylin.

Stained sections were examined and photographed using Olympus microscope (BX60, serial No. 7D04032) equipped with microscope's Camera (Olympus DP73, serial NO. OH05504; Olympus Corporation). For the fluorescence-stained sections, fluorescence microscope (E600; Nikon) equipped with Plan Fluor objectives connected to a CCD camera (DMX1200F; Nikon) was used.

Computerized image analysis of the positive-staining reaction was performed using the Image Pro Plus software (Media Cybernetics). Segmentation of the stained slides was performed based on morphology, brightness and colour using the RGB/HSI histogram option. For the VEGF, eNOS and α-SMA evaluation, positive stained area was measured relative to the whole slide area, expressed as % area. For the fluorescence-stained sections, the grade of the Ab fluorescence density was measured relative to the whole slide area. Quantification was performed using absolute numbers.

For the A3AR analysis sections, a semi-quantitative analysis of the immunohistochemical reaction of A3AR in the corpus cavernous was performed in a ×20 field using a scoring scale (Grade 0, no positive reaction; Grade 1, <5 immunopositive cells; Grade 2, 5–15 immunopositive cells; Grade 3, 15–25 immunopositive cells; Grade 4, 25–50 immunopositive cells; and Grade 5, >50 immunopositive cells).

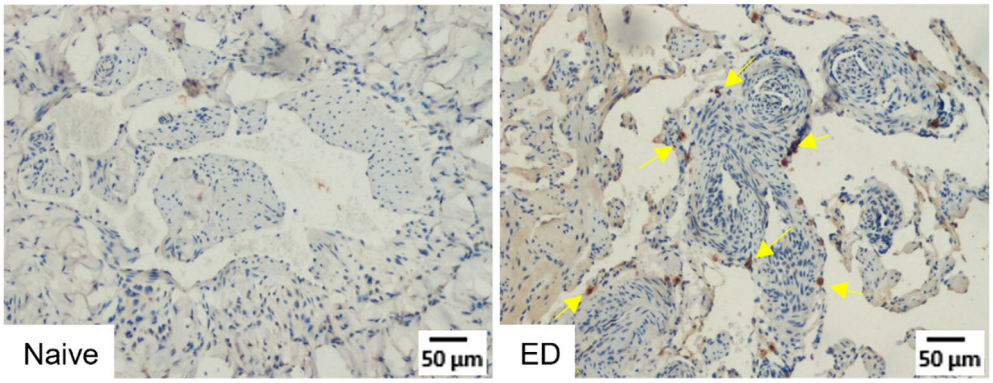

FIGURE 2 Representative pictures from penile sections from the naïve and the diabetic ED groups following immunostaining for A3AR. A definitive positive staining (dark brown-yellow arrows) is seen in the ED group.

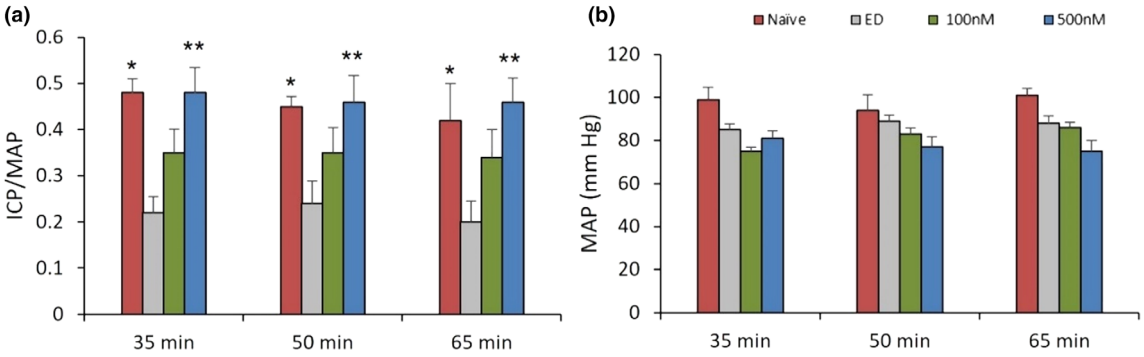

FIGURE 3 Treatment of ED with topically applied CF602 improved ICP: MAP ratio (a), whereas no significant changes in MAP (mm Hg) were detected (b). Naïve rats were not diabetic and did not receive any treatment; diabetic ED rats received placebo (ED group), or a single topical treatment with 100 μl CF602 (100 or 500 nM). Data are presented as mean ± SE. $^{*}p < 0.01$, $^{**}p < 0.05$ vs the ED group (ANOVA).

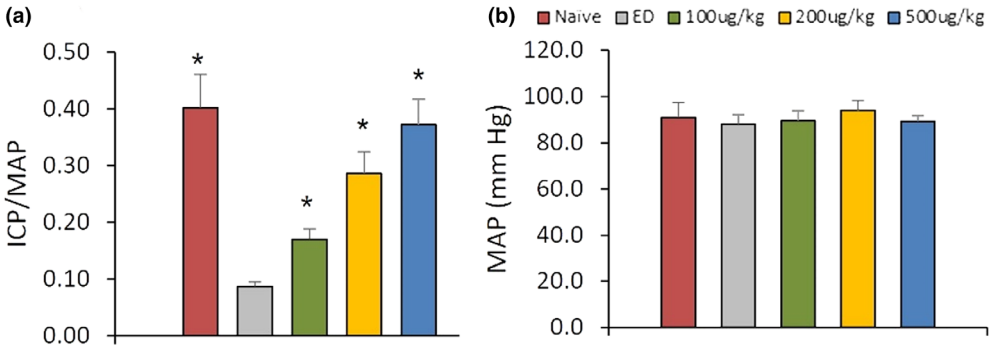

FIGURE 4 Treatment of ED with oral CF602 improved ICP:MAP ratio (a), whereas no changes in MAP (mm Hg) were detected (b). Naïve rats were not diabetic and did not receive any treatment; diabetic ED rats received placebo (ED group), 100, 200 or 500 μg/kg oral CF602. Data are presented as mean ± SE. $^{*}p < 0.01$ vs the ED group (ANOVA).

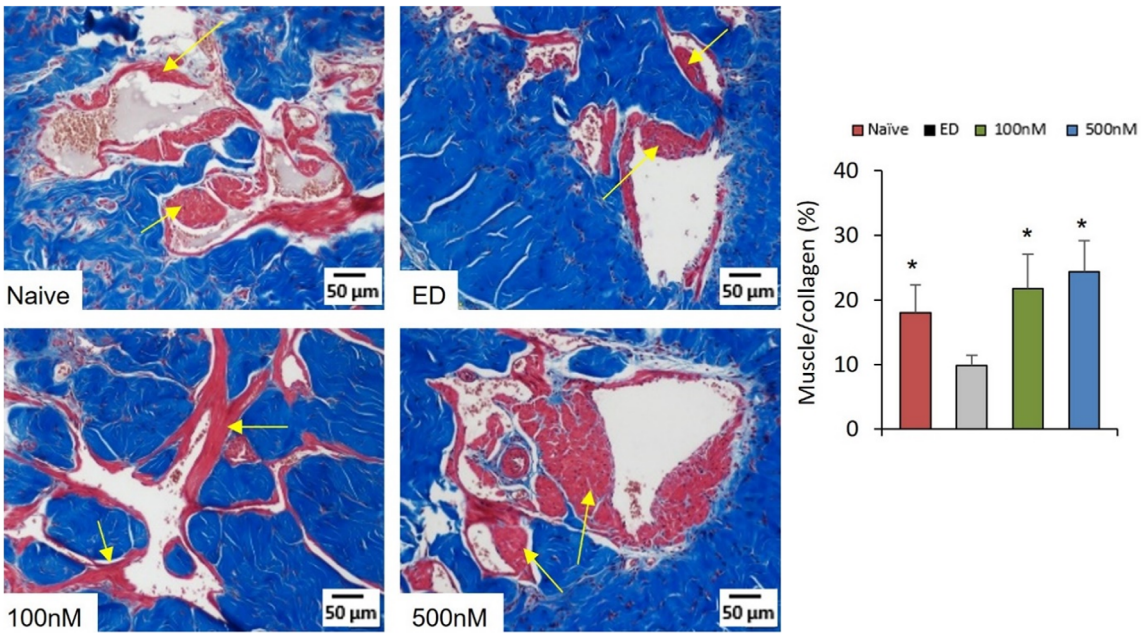

FIGURE 5 Representative pictures from a penile section stained for Masson's trichrome to assess the muscle:collagen ratio. Naïve rats were not diabetic and did not receive any treatment; diabetic ED rats received placebo (ED group) or a single topical treatment with 100 μl CF602 solution (100 or 500 nM). The area of smooth muscle is represented by red stain (yellow arrows) and the area of collagen by blue stain. The statistical analysis results are shown in the graph. Data are presented as mean ± SE. $^{*}p < 0.05$ vs the ED group (ANOVA). Magnification ×100.

2.6 | Statistical analysis

Data are expressed as mean ± standard error (SE). The statistical significance between the groups was assessed by *t* test for comparing two groups and by ANOVA when more than two groups were analysed. For all analyses, $p < 0.05$ was considered to be significant.

3 | RESULTS

3.1 | A3AR in the corpus cavernosum of diabetic ED rats

A3AR immunostaining in the corpus cavernosum was observed in diabetic ED, but not naïve rats (1.00 ± 0.40 vs 0.00 ± 0.00,

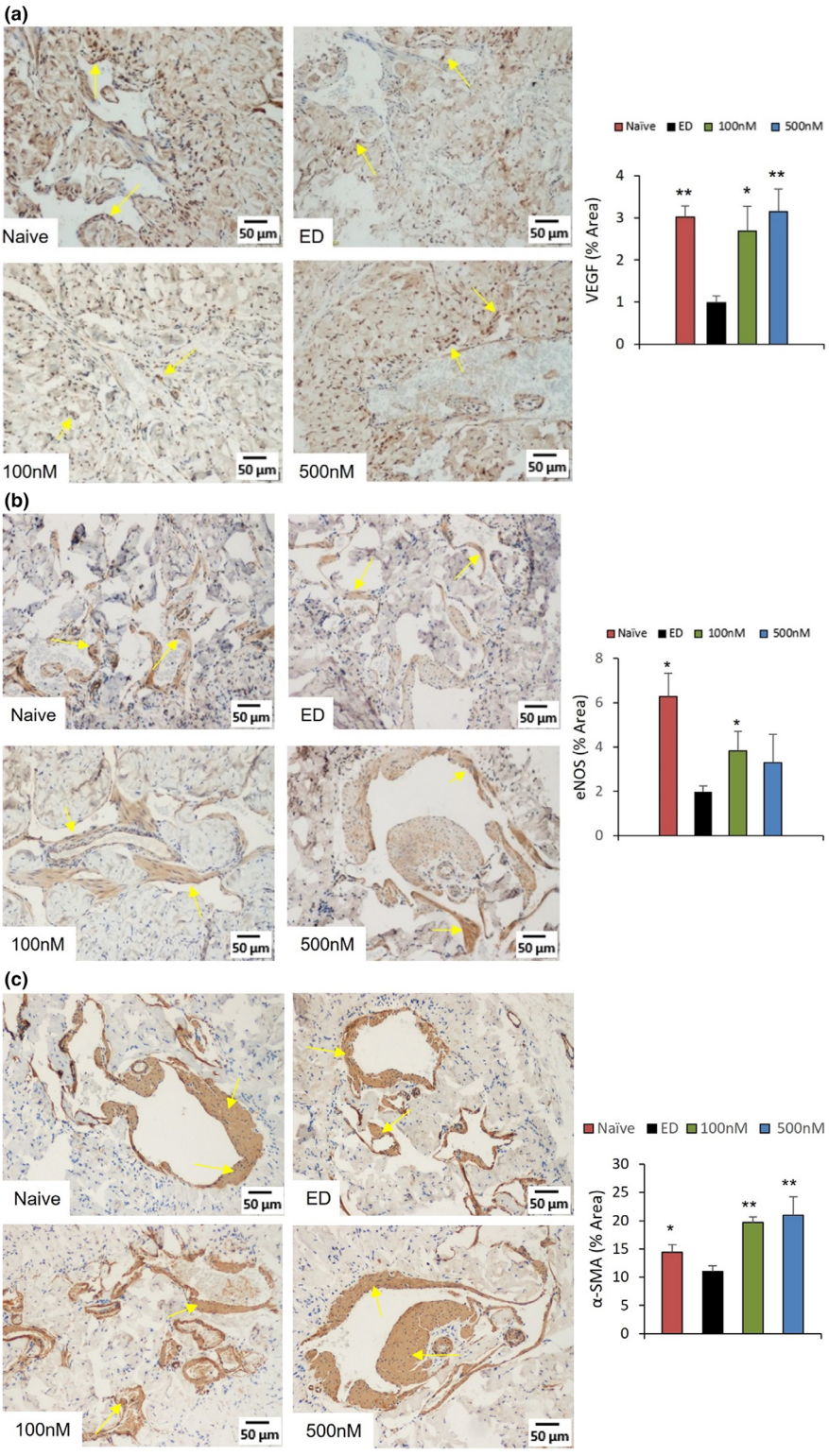

FIGURE 6 Representative pictures from penile sections immunostained for VEGF (a), eNOS (b) and α-SMA (c). Naïve rats were not diabetic and did not receive any treatment; diabetic ED rats received placebo (ED group) or a single topical treatment with 100 µl CF602 solution (100 or 500 nM). The positive staining (dark brown-yellow arrows) is predominantly in the endothelium lining of the cavernous sinusoid for all proteins. The statistical analysis results are shown in the graphs. Data are presented as mean ± SE. $^{*}p < 0.05$, $^{**}p < 0.005$ vs the ED group (ANOVA). Magnification ×100.

$p < 0.05$), demonstrating high A3AR expression in the former (Figure 2).

3.2 | CF602 effect on ICP and MAP

A single dose of 100 μl CF602 solution (100 or 500 nM) was topically applied 35, 50 and 65 min prior to erectile function assessment. CF602 treatment significantly improved the ICP: MAP ratio with a reverse to naïve values at 500 nM ($p < 0.005$) (Figure 3), whereas no significant changes in MAP were detected. Similar findings were observed when the rats received CF602 orally (a single dose of 100, 200 or 500 μg/kg 1 h prior to ICP measurement), with a reverse to naïve values at 500 μg/kg (Figure 4).

3.3 | CF602 improves cavernosal muscle and endothelial damage in the ED rats

Penile tissues were excised from the different groups and Masson's trichrome stained for muscle: collagen ratio evaluation. A marked reduction in muscle: collagen ratio was demonstrated in the diabetic ED group. CF602 treatment applied topically at a dose of 100 or 500 nM, significantly increased this ratio with a full reverse to naïve values at 500 nM (Figure 5).

Immunohistochemistry analysis showed a lower expression of eNOS, VEGF and α-SMA in the diabetic ED group compared with the naïve group. CF602 treatment (topically administered, 100 or 500 nM) was associated with an increase expression of these proteins (Figure 6).

Oral treatment with a single dose of 100 μg/kg CF602 prior to erectile function assessment induced a significant improvement in smooth muscle area ($p < 0.05$), eNOS expression ($p < 0.001$) and VEGF expression ($p < 0.001$) compared with the diabetic ED group (Figure 7).

4 | DISCUSSION

In this study, we demonstrated an increase in ICP:MAP ratio following topical or oral CF602 treatment in a diabetic ED rat model. Single

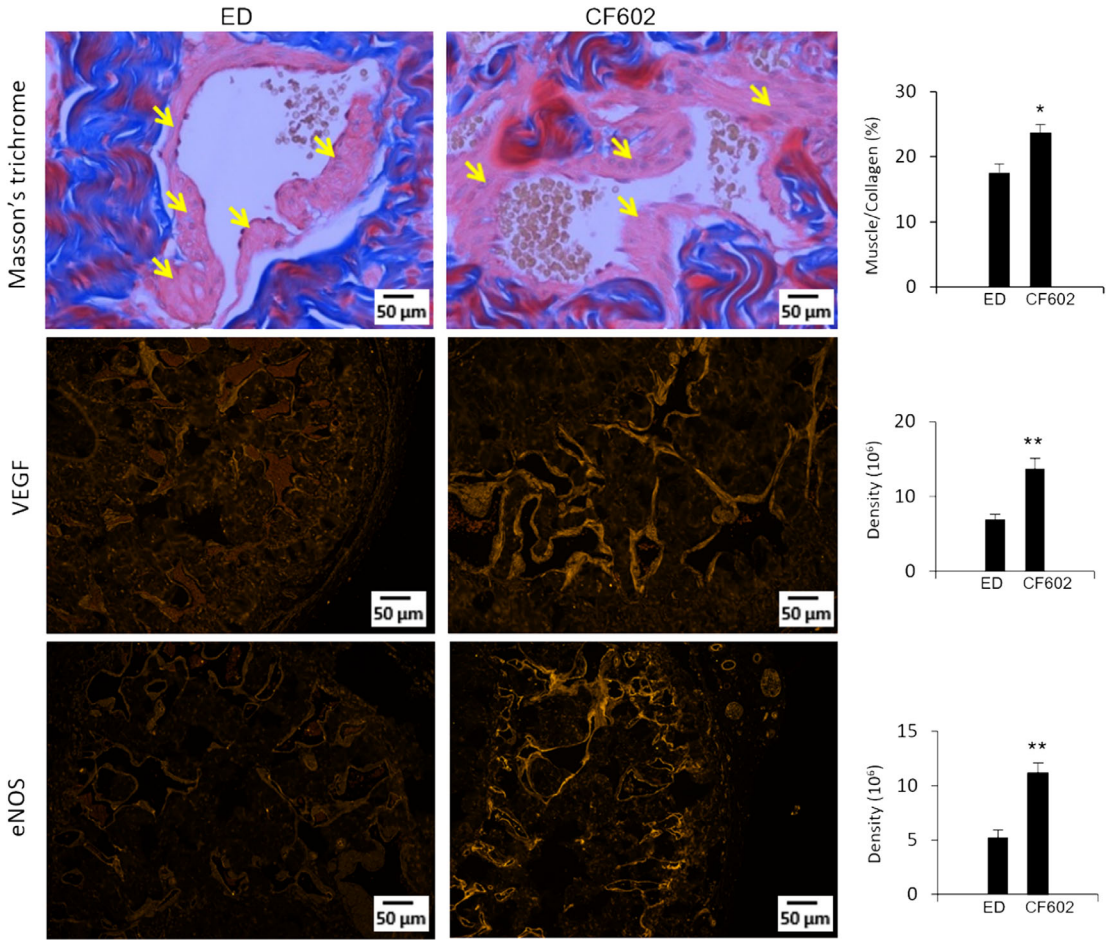

FIGURE 7 Representative pictures from penile sections of naïve and diabetic ED rats treated with placebo (ED group) or a single oral dose of CF602 100 μg/kg. Penile sections were stained with Masson's trichrome to assess muscle:collagen ratio and with immunofluorescence staining to assess VEGF and eNOS. The statistical analysis results are shown in the graph. Data are presented as mean ± SE. *$p < 0.01$, **$p < 0.001$ vs the ED group (student's t test). Magnification ×100.

dose of CF602 in each mode of administration showed a significant dose-dependent elevation in ICP: MAP ratio, an increase in smooth muscle:collagen ratio, as well as in eNOS, and VEGF expression. Interestingly, a full recovery of ICP: MAP ratio was shown with the high topical and oral dosages.

Our results suggest that CF602 has a pharmacologic effect on endothelial and smooth muscle cells, resulting in erectile function in the diabetic ED model. The effect of CF602 on penile tissue, as reported here, adds to the available evidence on the protective impact of CF602 on other organs including the joints and the liver. In preclinical models of rat adjuvant induced arthritis, monoiodoacetate-induced osteoarthritis, and concanavalin A-induced liver inflammation in mice, CF602 was shown to induce anti-inflammatory effects. These effects were mediated through deregulation of signalling proteins (PI3K, IKK, IκB, Jak-2 and STAT-1) leading to decreased levels of the nuclear factor NF-κB, which is known to have a key role in various processes including inflammation (Cohen et al., 2014).

Notably, our data show increased levels of ICP without deleterious effect on MAP. Maintaining blood pressure is a major concern for some ED patients, especially in patients suffering from diabetes and cardiovascular diseases. Preventing blood pressure elevation helps to prevent more complications associated with these conditions (Laffin & Bakris, 2015).

Our findings suggest that ICP:MAP increase is attributed to eNOS and VEGF elevation in the corpus cavernosum. Indeed, increased eNOS and VEGF levels are found with PDE5 inhibitors, an effect that is mediated by AKT-dependent eNOS phosphorylation and anti-oxidation activities which upregulate NO and improve endothelial function. Notably, in diabetic ED models, PDE5 treatment failed to increase eNOS and VEGF because of the impaired endothelial function due to chronic assault from blood sugar control (Goldstraw, Kirby, Bhardwa, & Kirby, 2007; Liu et al., 2010). A recent study also examined the role of pigment epithelium-derived factor (PEDF) in diabetic ED and found that plasma levels of PEDF were significantly higher in individuals with diabetic ED compared with diabetic individuals without ED and non-diabetic controls, as well as in diabetic ED preclinical models vs controls. The study demonstrated that PEDF overexpression suppressed ICP and eNOS phosphorylation in diabetic preclinical models, whereas blocking PEDF ameliorated this effect (Che et al., 2020).

To the best of our knowledge, this is the first reported study showing rapid remodelling of the endothelial and muscle tissue with a single dose of CF602, as well as ED reversal. We previously showed that 1 and 5 days of CF602 treatments significantly reversed ICP to normal values with the same mechanism of action (Cohen & Fishman, 2017). The current data could support the use of a single treatment in future human studies.

In studies investigating the PDE5 inhibitor, sildenafil, in the same diabetic ED model, sildenafil led to an increase in ICP: MAP ratio of two fold compared with the vehicle-treated group with daily administration for 10 weeks (Liu et al., 2010), whereas in the current study, an effect was observed with a single CF602 dose. Our data support the potential therapeutic effect of CF602 administration and the potential benefit to ED patients, particularly those not responding to PDE5 inhibitors.

CF602 treatment may also provide a benefit to patients taking anti-hypertensive medications or those at high risk for cardiovascular disease because of the minimal impact on MAP. This observation may stem from its mechanism of action as a positive allosteric modulator of the A3AR, which enhances the binding affinity of the endogenous adenosine ligand to the receptor. This mechanism of action also improves tolerability and reduces side effects by achieving the same level of efficacy with a lower dose of agonist/antagonist. Moreover, by keeping the natural ligand in place as the signalling molecule, the effects of off-target orthosteric agonists/antagonists are reduced.

CF602 is currently undergoing preclinical evaluation including toxicology assessment prior to its potential evaluation in clinical trials. The studies conducted thus far demonstrated that CF602 was safe and well tolerated (Can-Fite BioPharma Ltd., data on file).

5 | CONCLUSION

A full erectile recovery was achieved following a single dose of CF602 administered topically or orally, with restored muscle:collagen ratio and endothelial cell function. Our data suggest that CF602 could potentially offer an alternative treatment to PDE5 inhibitors, particularly to PDE5 non-responders. Therefore, further preclinical and potentially clinical evaluation is warranted.

ACKNOWELEDGEMENT

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CONFLICT OF INTEREST

Inbal Itzhak, Shira Cohen, Sari Fishman and Pnina Fishman are employees of Can-Fite Bio Pharma.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

Abdel-Hamida, I., Abo-Alyb, M., & Elsaied, M. (2014). Nonresponse to phosphodiesterase 5 inhibitors in erectile dysfunction. Part 1: Related factors. *Human Andrology*, *4*(2), 23–29.

Che, D., Fang, Z., Yan, L., Du, J., Li, F., Xie, J., Feng, J., Yin, P., Qi, W., Yang, Z., Ma, J., Yang, X., Gao, G., & Zhou, T. (2020). Elevated pigment epithelium-derived factor induces diabetic erectile dysfunction via interruption of the Akt/Hsp90beta/eNOS complex. *Diabetologia*, *63*(9), 1857–1871.

Cignarelli, A., Genchi, V. A., D'Oria, R., Giordano, F., Caruso, I., Perrini, S., Natalicchio, A., Laviola, L., & Giorgino, F. (2021). Role of glucose-

lowering medications in erectile dysfunction. *Journal of Clinical Medicine*, *10*(11), 2501. https://doi.org/10.3390/jcm10112501

Cohen, S., Barer, F., Bar-Yehuda, S., IJzerman, A. P., Jacobson, K. A., & Fishman, P. (2014). A(3) adenosine receptor allosteric modulator induces an anti-inflammatory effect: In vivo studies and molecular mechanism of action. *Mediators of Inflammation*, *2014*, 708746. https://doi.org/10.1155/2014/708746

Cohen, S., & Fishman, P. (2017). CF602 improves erectile dysfunction in diabetic rats. *Journal of Urology*, *195*, e1138.

Cohen, S., Stemmer, S. M., Zozulya, G., Ochaion, A., Patoka, R., Barer, F., Bar-Yehuda, S., Rath-Wolfson, L., Jacobson, K. A., & Fishman, P. (2011). CF102 an A3 adenosine receptor agonist mediates anti-tumor and anti-inflammatory effects in the liver. *Journal of Cellular Physiology*, *226*(9), 2438–2447. https://doi.org/10.1002/jcp.22593

Evans, J. D., & Hill, S. R. (2015). A comparison of the available phosphodiesterase-5 inhibitors in the treatment of erectile dysfunction: A focus on avanafil. *Patient Preference and Adherence*, *9*, 1159–1164. https://doi.org/10.2147/PPA.S56002

Faria, M., Magalhaes-Cardoso, T., Lafuente-de-Carvalho, J. M., & Correia-de-Sa, P. (2006). Corpus cavernosum from men with vasculogenic impotence is partially resistant to adenosine relaxation due to endothelial A(2B) receptor dysfunction. *The Journal of Pharmacology and Experimental Therapeutics*, *319*(1), 405–413. https://doi.org/10.1124/jpet.106.107821

Fishman, P., Bar-Yehuda, S., Madi, L., Rath-Wolfson, L., Ochaion, A., Cohen, S., & Baharav, E. (2006). The PI3K-NF-kappaB signal transduction pathway is involved in mediating the anti-inflammatory effect of IB-MECA in adjuvant-induced arthritis. *Arthritis Research & Therapy*, *8*(1), R33. https://doi.org/10.1186/ar1887

Goblyos, A., Gao, Z. G., Brussee, J., Connestari, R., Santiago, S. N., Ye, K., Ijzerman, A. P., & Jacobson, K. A. (2006). Structure-activity relationships of new 1H-imidazo[4,5-c]quinolin-4-amine derivatives as allosteric enhancers of the A3 adenosine receptor. *Journal of Medicinal Chemistry*, *49*(11), 3354–3361. https://doi.org/10.1021/jm060086s

Goldstraw, M. A., Kirby, M. G., Bhardwa, J., & Kirby, R. S. (2007). Diabetes and the urologist: A growing problem. *BJU International*, *99*(3), 513–517. https://doi.org/10.1111/j.1464-410X.2006.06588.x

Hatzimouratidis, K., & Hatzichristou, D. (2014). How to treat erectile dysfunction in men with diabetes: From pathophysiology to treatment. *Current Diabetes Reports*, *14*(11), 545. https://doi.org/10.1007/s11892-014-0545-6

Headrick, J. P., & Peart, J. (2005). A3 adenosine receptor-mediated protection of the ischemic heart. *Vascular Pharmacology*, *42*(5–6), 271–279. https://doi.org/10.1016/j.vph.2005.02.009

Laffin, L. J., & Bakris, G. L. (2015). Update on blood pressure goals in diabetes mellitus. *Current Cardiology Reports*, *17*(6), 37. https://doi.org/10.1007/s11886-015-0591-y

Liu, G., Sun, X., Dai, Y., Zheng, F., Wang, D., Huang, Y., Bian, J., & Deng, C. (2010). Chronic administration of sildenafil modified the impaired VEGF system and improved the erectile function in rats with diabetic erectile dysfunction. *The Journal of Sexual Medicine*, *7*(12), 3868–3878. https://doi.org/10.1111/j.1743-6109.2010.01844.x

McMahon, C. G. (2014). Erectile dysfunction. *Internal Medicine Journal*, *44*(1), 18–26. https://doi.org/10.1111/imj.12325

McMahon, C. G. (2019). Current diagnosis and management of erectile dysfunction. *The Medical Journal of Australia*, *210*(10), 469–476. https://doi.org/10.5694/mja2.50167

Ochaion, A., Bar-Yehuda, S., Cohen, S., Amital, H., Jacobson, K. A., Joshi, B. V., Gao, Z. G., Barer, F., Patoka, R., Del Valle, L., Perez-Liz, G., & Fishman, P. (2008). The A3 adenosine receptor agonist CF502 inhibits the PI3K, PKB/Akt and NF-kappaB signaling pathway in synoviocytes from rheumatoid arthritis patients and in adjuvant-induced arthritis rats. *Biochemical Pharmacology*, *76*(4), 482–494. https://doi.org/10.1016/j.bcp.2008.05.032

Ohana, G., Cohen, S., Rath-Wolfson, L., & Fishman, P. (2016). A3 adenosine receptor agonist, CF102, protects against hepatic ischemia/reperfusion injury following partial hepatectomy. *Molecular Medicine Reports*, *14*(5), 4335–4341. https://doi.org/10.3892/mmr.2016.5746

Shamloul, R., & Ghanem, H. (2013). Erectile dysfunction. *Lancet*, *381*(9861), 153–165. https://doi.org/10.1016/S0140-6736(12)60520-0

Takahashi, Y., Ishii, N., Lue, T. F., & Tanagho, E. A. (1992). Effects of adenosine on canine penile erection. *Journal of Urology*, *148*(4), 1323–1325. https://doi.org/10.1016/s0022-5347(17)36901-x

Tostes, R. C., Giachini, F. R., Carneiro, F. S., Leite, R., Inscho, E. W., & Webb, R. C. (2007). Determination of adenosine effects and adenosine receptors in murine corpus cavernosum. *The Journal of Pharmacology and Experimental Therapeutics*, *322*(2), 678–685. https://doi.org/10.1124/jpet.107.122705

Wahlman, C., Doyle, T. M., Little, J. W., Luongo, L., Janes, K., Chen, Z., Esposito, E., Tosh, D. K., Cuzzocrea, S., Jacobson, K. A., & Salvemini, D. (2018). Chemotherapy-induced pain is promoted by enhanced spinal adenosine kinase levels through astrocyte-dependent mechanisms. *Pain*, *159*(6), 1025–1034. https://doi.org/10.1097/j.pain.0000000000001177

Wen, J., Grenz, A., Zhang, Y., Dai, Y., Kellems, R. E., Blackburn, M. R., Eltzschig, H. K., & Xia, Y. (2011). A2B adenosine receptor contributes to penile erection via PI3K/AKT signaling cascade-mediated eNOS activation. *FASEB Journal*, *25*(8), 2823–2830. https://doi.org/10.1096/fj.11-181057

Wen, J., & Xia, Y. (2012). Adenosine signaling: Good or bad in erectile function? *Arteriosclerosis, Thrombosis, and Vascular Biology*, *32*(4), 845–850. https://doi.org/10.1161/ATVBAHA.111.226803

Yamanaka, M., Shirai, M., Shiina, H., Tanaka, Y., Enokida, H., Tsujimura, A., Matsumiya, K., Okuyama, A., & Dahiya, R. (2005). Vascular endothelial growth factor restores erectile function through inhibition of apoptosis in diabetic rat penile crura. *Journal of Urology*, *173*(1), 318–323. https://doi.org/10.1097/01.ju.0000141586.46822.44

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