Activation of adenosine $A_{2A}$ receptors by polydeoxyribonucleotide increases vascular endothelial growth factor and protects against testicular damage induced by experimental varicocele in rats

In rat experimental varicocele, polydeoxyribonucleotide (PDRN) induces vascular endothelial growth factor (VEGF) production, thereby enhancing testicular function. This may point to a new therapeutic approach in human varicocele. (Fertil Steril® 2011;95:1510–3. ©2011 by American Society for Reproductive Medicine.)

Key Words: Adenosine $A_{2A}$-receptor, apoptosis, PDRN, spermatogenesis, varicocele, VEGF

Varicocele is a major cause of male infertility (1–4), and several factors seem to be involved in the spermatogenesis alterations: back flow of noxious substances from the kidney or adrenal glands, increase in the testicular temperature, and tissue hypoxia induced by venous stasis (5). Hypoxia promotes angiogenesis by up-regulating the expression of several angiogenic factors, including vascular endothelial growth factor (VEGF) (6–7).

Vascular endothelial growth factor, which plays a crucial role in testis morphogenesis by causing neovascularization and cord formation (8), acts by binding to tyrosine-kinase-receptor-1 (flt-1) and 2 (flk/KDR), which are located on the surface of Leydig and Sertoli cells (9). It is interesting that increased expression of VEGF in the testis has been observed in rat experimental varicocele (10), and a study has indicated that intratesticular injection of VEGF improves varicocele-impaired spermatogenesis and reduces apoptosis (11).

Adenosine, which is known to be released in hypoxic tissues, may represent a therapeutic target (12); indeed, activation of adenosine $A_{2A}$-receptors stimulates VEGF release (13). Polideoxyribonucleotide (PDRN), which is obtained from trout sperm by an extraction process (14), acts through stimulation of the $A_{2A}$-receptor to induce VEGF production during pathologic conditions of low tissue perfusion (14). This evidence prompted us to investigate the effect of PDRN on experimental varicocele.

Letteria Minutoli, M.D.a
Salvatore Arena, M.D.b
Giulio Bonvissuto, M.D.c
Alessandra Bitto, M.D.a
Francesca Polito, Ph.D.a
Natasha Iriera, Ph.D.a
Francesco Arena, M.D.d
Eugenia Fragala, M.D.c
Carmelo Romeo, M.D.d
Piero Antonio Nicotina, M.D.e
Carmine Fazzari, M.D.e
Herbert Marinii, M.D.f
Alessandra Implatini, M.D.b
Silvia Grimaldi, M.D.b
Noemi Cantone, M.D.b
Vincenzo Di Benedetto, M.D.b
Francesco Squadrito, M.D.a
Domenica Altavilla, Ph.D.a
Giuseppe Morgia, M.D.c

Department of Clinical and Experimental Medicine and Pharmacology, University of Messina, Messina, Italy
Department of Pediatric Surgery, Unit of Pediatric Surgery, University of Catania, Catania, Italy
Department of Medical and Surgical Pediatrics, University of Messina, Messina, Italy
Department of Human Pathology, University of Messina, Messina, Italy
Department of Biochemical, Physiological, and Nutritional Sciences, Section of Physiology and Human Nutrition, University of Messina, Messina, Italy

Received March 9, 2010; revised June 21, 2010; accepted July 5, 2010; published online August 24, 2010.
L.M. has nothing to disclose. S.A. has nothing to disclose. G.B. has nothing to disclose. A.B. has nothing to disclose. F.P. has nothing to disclose. N.I. has nothing to disclose. F.A. has nothing to disclose. E.F. has nothing to disclose. C.R. has nothing to disclose. P.A.N. has nothing to disclose. C.F. has nothing to disclose. H.M. has nothing to disclose. A.I. has nothing to disclose. S.G. has nothing to disclose. N.C. has nothing to disclose. V.D.B. has nothing to disclose. F.S. has nothing to disclose. D.A. has nothing to disclose. G.M. has nothing to disclose.

Supported by departmental funding.
Reprint requests: Giuseppe Morgia, M.D., Professor and Chairman Department of Urology, School of Medicine Policlinico Hospital, University of Catania, Catania, Italy (E-mail: gmorgia@policlinico.unict.it).
Male Sprague-Dawley rats aged 7 weeks and weighing 200 to 225 g were used. During the experiments, the animals were housed one per cage, maintained under controlled environmental conditions (12-hour light/dark cycle, temperature approximately 23°C), and provided with standard laboratory food and water ad libitum. After induction of anesthesia with an intraperitoneal (IP) injection of sodium pentobarbital (50 mg/kg), varicocele was induced as previously described elsewhere (10). Sham-operated rats underwent to the same vertical midline incision, and the suture was also placed, but it was not tied. All animal procedures were in accordance with the declaration of Helsinki and with the Guide for the Care and Use of Laboratory Animals.

Twenty-eight days after the creation of varicocele, the animals were randomized to the following daily IP treatment: vehicle (1 mL/kg); PDRN (8 mg/kg); 3,7-dimethyl-propargilxanthine (DMPX, 0.1 mg/kg), a specific adenosine A$_{2A}$-receptor antagonist; PDRN plus DMPX; varicocelectomy alone; or varicocelectomy plus PDRN. The PDRN was a gift of Mastelli Srl (Sanremo, Italy), and the DMPX was obtained from Sigma (Milano, Italy). The vehicle of both PDRN and DMPX was a 0.9% NaCl solution.

Twenty-one days after randomization, all animals were anesthetized to allow a laser Doppler measurement of blood flow in the left spermatic vessels. Afterward, they were killed by anesthetic overdose, and the left testis was harvested to evaluate the VEGF, Bax, Bel-2, and inhibin β-B expression by use of Western blot analysis, the histologic changes by techniques previously shown elsewhere (15), and spermatogenic activity by means of Johnsen’s score (16).

All data are expressed as mean ± standard error of the mean (SEM). Data were analyzed by one-way analysis of variance (ANOVA) followed by post hoc evaluation. *P* < .05 was considered statistically significant.

The testis from the sham-operated rats showed normal morphology (Fig. 1A), but the testis harvested 28 days after varicocele induction showed severe edema and moderate venular ectasia and lobular necrosis (see Fig. 1B). An additional 3 weeks of vehicle treatment further worsened the testis damage (see Fig. 1C). By contrast, administration of PDRN was effective in inhibiting the histologic changes caused by the varicocele (see Fig. 1D). Administration of DMPX alone did not modify the histologic damage, and it abrogated the beneficial effects of PDRN (see Figs. 1E and 1F). Varicocelectomy succeeded in preserving testicular morphology (see Fig. 1G). Varicocelectomy plus PDRN did not further improve the histologic damage that had been induced by the varicocele (see Fig. 1H).

Varicocele caused a statistically significant impairment in spermatogenesis 4 weeks after the ligation of left renal vein (4.52 ± 0.54). An additional 3 weeks of vehicle treatment further worsened spermatogenesis (2.63 ± 0.46). In contrast, administration of PDRN statistically significantly increased the Johnsen’s score (8.78 ± 0.41). Administration of DMPX did not modify the altered spermatogenesis, and it abated the beneficial effects of PDRN (3.43 ± 0.72).

Varicocelectomy reverted the altered spermatogenesis (8.66 ± 0.36). Combining varicocelectomy with PDRN treatment did not cause any additional alteration to the Johnsen’s score (8.83 ± 0.48).

Varicocele produced an increase in VEGF expression and reduced inhibin β-B expression in the left testis (see Figs. 1I–1L). Administration of PDRN further enhanced VEGF and inhibin β-B proteins (see Figs. 11–1L). Treatment with DMPX reduced the VEGF expression and did not modify the reduction in inhibin β-B expression; it abrogated the stimulation of VEGF and inhibin β-B caused by PDRN (see Figs. 11–1L). Varicocelectomy resulted in a significantly reduced expression of the growth factor and a higher expression of inhibin β-B (see Figs. 11–1L). Varicocelectomy plus PDRN caused a slight but not statistically significant increase in VEGF and inhibit β-B expression (see Figs. 11–1L).

Varicocele produced a marked increase in Bax expression and decreased the Bel-2 protein (see Figs. 1M and 1N). In contrast, administration of PDRN reduced Bax protein and enhanced Bel-2 expression (see Figs. 1M and 1N). Administration of DMPX slightly modified the changes in the apoptotic machinery induced by varicocele (see Figs. 1M and 1N). The concomitant administration of DMPX plus PDRN abrogated the effects of PDRN on the apoptotic proteins (see Figs. 1M and 1N). Furthermore, varicocelectomy resolved the alteration in the apoptotic machinery (see Figs. 1M and 1N), but varicocelectomy plus PDRN did not affect the changes induced by the surgical treatment.

Varicocele rats showed a marked increase in blood flow when compared with the sham-operated rats (see Figs. 1O and 1P). Administration of DMPX and PDRN (alone or in combination) did not affect the increased blood flow (see Figs. 1Q–1S). The enhancement in perfusion was shown by the intense red color of spermatic vessels. In contrast, varicocelectomy produced a statistically significant reduction in blood-flow perfusion, as documented by the low intensity red color of spermatic vessels (see Fig. 1T); varicocelectomy plus PDRN did not change the effect of the surgical treatment (see Fig. 1U).

Our main finding was that PDRN treatment was as effective as varicocelectomy in curing the experimental varicocele. Although PDRN failed to influence the reflux in the left spermatic vein that creates a varicocele in the pampiniform plexus, it succeeded in ameliorating the histologic damage and the poor spermatogenesis in the rats with experimental varicocele. Indeed, it has been demonstrated that spermatogenesis can be recovered 3 to 4 weeks after varicocelectomy (11), thus supporting our experimental findings. It was surprising that the surgical treatment did not show any superiority in comparison with PDRN in terms of histologic protection and improvement in spermatogenesis; additionally, PDRN did not modify the efficacy of varicocelectomy, thus suggesting that this medical option might be combined with the surgical treatment to accelerate the recovery of depressed testicular function.

It has been suggested that systemic hypoxia increases the expression of VEGF (17) in the testis and that it occurs in testes with varicocele (18). It could be therefore hypothesized that VEGF expression rises as a compensatory mechanism to counteract the stasis and the consequent hypoxic-ischemic state that may impair testicular function. However, VEGF might have either a paracrine effect on testicular microvasculature, providing an adequate microenvironment in the seminiferous, tubular, and interstitial compartments of the testes, or an autocrine effect on the activity of testis cell types (19). To better define the multiple actions of PDRN in the testis, we also characterized the apoptosis machinery in our experimental model. Increased germ cell apoptosis has been found in the ejaculate of men with varicocele (20), and creation of varicocele in rats causes a marked activation of...
Our data allow us to hypothesize that the curative effects of PDRN are likely related to its ability either to induce VEGF (22, 23), which causes an augmented oxygen supply, or to balance the apoptosis machinery, as evidenced by the reduction of proapoptotic Bax and increased levels of antiapoptotic protein Bcl-2.

Finally, we characterized the molecular mechanism by which PDRN causes increased VEGF production. The concomitant administration of DMPX reverted all the beneficial effects of PDRN on VEGF production, apoptosis, testicular damage, and spermatogenesis.

Adenosine modulates cellular and organ function via occupancy of four specific cell surface receptors (A1, A2A, A2B, and A3) (24). Activation of adenosine A2A-receptors stimulates VEGF release through the signaling pathways activated by hypoxia (13). Besides influencing testicular function through VEGF production, the A2A-receptor modulates directly the function of Sertoli and germinative cells. It has also been proposed that adenosine may regulate cell proliferation and maturation of seminiferous tubules through interaction with A2A-receptors (25). This would suggest that the beneficial effect of PDRN in experimental varicocele might be also mediated by a direct activation of adenosine A2A on Sertoli and germinative cells.
Consequently, we studied the testicular expression of inhibin ⢠, a marker of germinative cell function. Inhibins, glycoproteins mainly produced by Sertoli cells, are markedly reduced during varicocele (26). We found that PDRN statistically significantly increased the varicocele-induced reduction of inhibin ⢠; the blockade of the A₂A-receptor by DMPX reverted this effect, thus unmasking this additional mechanism of action. Indeed, we did not find any reduction in the number of Sertoli cells 4 weeks after varicocele induction. Therefore, both PDRN and surgical treatment re-create a microenvironment where the Sertoli cells may normally function. In agreement with this hypothesis, it has been demonstrated (27, 28) that varicocelectomy increases inhibin expression.

Our experiment has demonstrated that PDRN produces a safe induction of VEGF. This may represent a new therapeutic option for accelerating recovery from depressed testicular function in varicocele.

REFERENCES