Arginase Alteration in the Reproductive System of Alloxan-Diabetic Dogs

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Abstract. This study was conducted to evaluate possible alteration in the activity of arginase, an important enzyme of cell proliferation and vascular smooth muscle contraction regulator in diabetics, that may be correlated with low fertility in diabetic patients. In this investigation, 6 apparently healthy adult male dogs were selected and divided in two groups, diabetics and non-diabetics. Diabetes mellitus was induced in one group by intravenous (IV) injection of alloxan (100 mg/kg). Dogs with a fasting blood glucose (FBS) of more than 200 mg/dl were considered to be diabetic. Four weeks following induction of diabetes mellitus, the animals in both groups were anesthetized by an IV injection of sodium thiopental. Livers and whole reproductive systems, including the testes, penis, urethra, and prostate, were dissected. The epididymides, corpus cavernosum, corpus spongiosum, penile urethra, and vas deferens were also dissected and removed from the reproductive system. Arginase activity and total protein were measured by the urea and Lowry’s methods respectively in above mentioned sections. Plasma testosterone was determined by the radioimmunoassay method. The results showed significantly (P<0.05) increased arginase specific activity (ASA) in the liver, epididymis, prostate, corpus cavernosum and corpus spongiosum of the diabetic dogs. In the reproductive system of the diabetic dog, the maximum and minimum ASA was seen in the corpus cavernosum and testes, respectively (105.12 ± 8.76 vs. 25.0 ± 0.55). No such variation was observed in the ASA of normal dogs (39.0 ± 5.47 vs. 25.0 ± 5.47). There was no significant difference in plasma testosterone level between the groups. In conclusion, diabetes increased the ASA in liver, prostate, epididymis, corpora cavernosa, and corpora spongiosum of the male dogs and may contribute to erectile dysfunction or low fertility in diabetics.

Key words: Arginase, Diabetes mellitus, Dog, Reproductive system

associated with an increased level of certain amino acids in the ejaculate and may contribute to reduced fertilization rates. However, the mechanisms of altered spermatogenesis in diabetic men are poorly understood [7].

Erectile dysfunction affects a significant portion of the male population and is associated with diabetes, cardiovascular disease, smoking of cigarettes, and depression [8]. DM is the most common cause of erectile dysfunction in men [9, 10]. Arginase II (extrahepatic arginase) has also been reported to be a potential enzyme target for treatment of male and female arousal disorders. The results of hemodynamic experiments conducted in vivo suggest a role for arginase II in regulating both male and female sexual arousal [11]. Additionally, the gene expression, protein level, and catalytic activity of arginase II are elevated in the diabetic corpus cavernosum [12].

The importance of arginase may be in the production of ornithine for the synthesis of the polyamines putrescine, spermidine, and spermine, which are required for normal cellular proliferation [13, 14] and differentiation [15]. Arginase activity has been identified in the prostate and has been suggested to be in synthesis of polyamines in accessory sex glands. Polyamines in turn may mediate the action of androgens [5]. Arginase activity has been reported in different parts of the reproductive systems of cattle [16], rams [17], and ewes [18]. The existence of multiple forms of arginase in eukaryotes suggests a complex regulatory role for this enzyme in the metabolism, development, and maintenance of these organisms. Mammalian arginase is well characterized [19, 20]; however, little is known about the role of the extrahepatic arginase found in erythrocytes the mammary gland, kidney, salivary gland, gastrointestinal tract and reproductive system [21]. Arginase is available in abundance in the mammary gland where the urea cycle is not active [22]. Increased expression of arginase II in the corpus cavernosum of diabetic patients has been suggested to contribute to erectile dysfunction [12]. Kim et al. [23] demonstrated that arginase is present in human penile corpus cavernosal tissue and the competitive inhibition of this enzyme by an arginine analogue, BEC [S-(2-boronoethyl)-L-cysteine], caused significant enhancement of nitric oxide (NO)-dependent smooth muscle relaxation in this tissue. Recent studies have demonstrated that potent boronic acid, an inhibitor of arginase, enhances NO-dependent smooth muscle relaxation in the rabbit and human penile corpus cavernosum [23].

The purposes of this investigation were to evaluate arginase specific activity in tissue from different parts of dog reproductive system and to discover possible alterations in arginase activity in the reproductive system of diabetic patients that may contribute to low fertility and sexual dysfunction.

**Materials and Methods**

**Animals**

Six apparently healthy and sexually mature male mixed breed dogs (3 years old and 17–20 kg body weight) were selected for use in this study. All animals received humane care, and their general health conditions were checked and randomly classified in to two groups. Their fasting blood glucose (FBS) was tested using the glucose oxidase method [24].

**Induction of diabetes mellitus**

Diabetes mellitus was induced in 3 dogs by an intravenous (IV) injection of alloxan tetrahydrate (Sigma, St. Louis, MO, USA) (100 mg/kg). The animals were fasted 12 h before and after alloxan injection. A blood glucose level of above 200 mg/dl and polydipsia and polyuria for at least 1 week were considered to be signs of DM and selection for experiment. Four weeks following induction of DM, the animals were anesthetized and sacrificed by IV injection of 1 g sodium thiopental. Whole reproductive systems including the testes, urethra, prostate, and penis, were dissected and stripped from fat and extraneous materials.

**Measurement of total soluble protein and arginase activity**

The prostrate, epididymis, penile urethra, testes, vas deferens, corpus cavernosum, and corpus spongiosum tissues were separated, washed several times with normal saline, and blotted. Tissue extracts were prepared by freezing 0.5 g of the samples in liquid nitrogen, homogenizing with a hand-homogenizer, and suspending the homogenate in 4 ml of 0.025 M sodium phosphate buffer (pH 7.2). The suspensions were centrifuged
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for 15 min at 4,000 g in an MSE high speed refrigerated centrifuge. The supernatants were collected and the activity of arginase was measured by the urea method [25]. The arginase specific activity (ASA) was calculated by dividing the amount of arginase activity by the amount of total soluble protein (TSP). The protein concentration in the crude extracts of different tissues was measured by the Lowry method [26].

Serum testosterone measurement

Blood was collected from the saphenous vein, and testosterone was measured in the serum using the radioimmunoassay (RIA) method.

Statistical analysis

Statistical analysis of the data was conducted using analysis of variance (ANOVA). Differences between means were estimated using Duncan’s test. All values were expressed as means ± SD, and differences of P<0.05 were considered significant.

Results

The values of TSP (mg/g of tissue) and ASA (mIU/g of tissue) in the livers and different parts of the reproductive systems of the normal (untreated) and diabetic dogs are presented in Table 1. It is obvious from the result that ASA in liver is about 30 times higher than that in the reproductive system (947 vs. 27.5). There was variation in the TSP of the examined tissues, with maximum level found in the liver and the minimum level found in the corpus cavernosum in both groups. There was no significant difference in ASA among reproductive tissues of the normal dogs; however, there was an increase in the ASA of some parts of the reproductive system in the diabetic group, and the differences were significant for the prostate, corpus spongiosum, epididymis and corpus cavernosum (P<0.05). In the diabetic group, ASA was significantly increased in the liver (data not shown), epididymis, prostate, corpus cavernosum, and corpus spongiosum (P<0.05). The serum testosterone concentration was not reduced in the diabetic dog (2.83 ± 0.18 and 2.18 ± 0.29 for the normal and diabetic groups, respectively).

Discussion

In mammals, the full urea cycle is functional in the liver [27]. The highest rates of arginine synthesis occur within the hepatic urea cycle, which is localized within periportal hepatocytes. Net arginine synthesis by the liver is only possible if the urea cycle is replenished by necessary intermediates, such as ornithine. In the livers of the diabetic dogs, ASA increased significantly in comparison with the normal animals. Increased activity of liver arginase has been demonstrated in diabetic rats [28–30]. Insulin-dependent diabetes is accompanied by increased food consumption, amino acid metabolism, and ratio of blood glucagon to insulin, all of which would tend to increase urea cycle and arginase activity [31].

Low fertility has recently received attention as a reproductive complication in diabetics and seems to be related to abnormal spermatogenesis [4] or

Table 1. Comparison of mean (±SD) of total soluble proteins (TSP), arginase activity (AA), and arginase specific activity (ASA) in the livers and reproductive systems of diabetic and non-diabetic male dogs (n=3)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Non-Diabetic</th>
<th>Diabetic</th>
</tr>
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<tbody>
<tr>
<td>Tissues TSP</td>
<td>AA (IU/g tissue x 10^2)</td>
<td>ASA (IU/mg protein x 10^-3)</td>
</tr>
<tr>
<td>Liver*</td>
<td>105.12 ± 5.96</td>
<td>9999.60 ± 11.26</td>
</tr>
<tr>
<td>Corpus cavernosum*</td>
<td>10.25 ± 3.45</td>
<td>37.30 ± 0.12</td>
</tr>
<tr>
<td>Corpus spongiosum*</td>
<td>11.53 ± 7.79</td>
<td>28.30 ± 0.14</td>
</tr>
<tr>
<td>Prostate*</td>
<td>26.91 ± 3.9</td>
<td>59.00 ± 0.24</td>
</tr>
<tr>
<td>Epididymis*</td>
<td>17.94 ± 9.85</td>
<td>37.30 ± 0.12</td>
</tr>
<tr>
<td>Vas deferens</td>
<td>20.51 ± 2.25</td>
<td>82.00 ± 0.52</td>
</tr>
<tr>
<td>Urethra</td>
<td>28.20 ± 5.97</td>
<td>66.00 ± 0.21</td>
</tr>
<tr>
<td>Testes</td>
<td>44.86 ± 2.25</td>
<td>119.60 ± 0.87</td>
</tr>
</tbody>
</table>

* Significant difference in AA and ASA between the two groups (P<0.05).
abnormal circulation to erectile tissues [12]. Our data shows that arginase is present in all parts of the normal dog reproductive system with no significant differences. However, significant variation has been reported for ASA in different parts of the reproductive system of the ram [17]. In the diabetic dogs in this study, ASA increased significantly in the corpus cavernosum, corpus spongiosum, prostate, and epididymis. Increased arginase specific activity in some parts of the reproductive system might be due to high levels of cell division and high differentiation rates [15] or less participation in sexual arousal [11]. The presence of arginase in extrahepatic tissues might indicate that these tissues use arginase for purposes other than urea synthesis.

Arginase shares arginine as a common substrate with nitric oxide synthase (NOS), the enzyme that synthesizes NO, and NO is the principal mediator of penile erection. Arginase may downregulate NO production in the penile corpus cavernosum and cause alteration of normal penile homeostasis and erectile dysfunction in diabetic patients [12]. The corpora cavernosa and corpus spongiosum consist of sinusoid system lines composed of endothelial cells. Erection depends on increased arterial blood flow into the corpora cavernosa of penis. Erection is induced by a NO mechanism. Induction of arginase synthesis may be due to secretion of cytokines by T lymphocytes [32], inhibition of NOS, or increase of NO degradation. In diabetics, the concentration of NO decreases due to increases of superoxides; this enhances NO degradation. NO is essential for smooth muscle relaxation, and a decrease in NO disturbs vasodilatation and erection [11].

The arginase activity in the prostate of the diabetic dogs increased significantly; however, this value was previously reported as unchanged in diabetic rats [5]. The prostate secretes high amounts of inorganic ions [33]) and a fluid rich in proteins such as acid phosphatase and prostate-specific antigen, a kallikrein-like protease [34]. It has a small contribution on the fluid volume of semen in most species; however, in the dog, the prostate is the only accessory sex gland and source of seminal plasma [35]. Greatly increased seminal plasma abnormalities, as reported in diabetics [4], could be due to alteration of this enzyme.

Polyamine synthesis is stimulated in the prostate and seminal vesicle by testosterone, and these compounds in turn increase RNA synthesis. Androgens also stimulate remarkable growth of these accessory organs of reproduction [36]. Non-insulin diabetic patients has been reported to have disordered androgen synthesis [37]. Alterations of androgens could be responsible for the increase in ASA in prostate; however, we did not see a significant reduction in testosterone in the diabetic group, which does not seem be explainable.

The results of this study showed that arginase is present, but probably does not play a significant role in ammonia detoxification, in different parts of the dog reproductive system, although it might be important polyamine biosynthesis. Diabetes mellitus causes an increase in arginase activity in the epididymis, corpus cavernosum, corpus spongiosum and prostate and may possibly contribute to consequent erectile dysfunction and low fertility in these patients.

References