Prostasomes—their effects on human male reproduction and fertility

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The prostate is a glandular male accessory sex organ vital for normal fertility. It provides the prostatic component of seminal plasma which nourishes and protects sperm following ejaculation. Prostasomes are small (40–500 nm) membrane-bound vesicles produced by epithelial cells lining the prostate acini and are a component of prostatic secretions. Although the existence of these particles has been known for many years, their full function and relevance to reproductive health are largely unknown. Proteomic studies have shown a wide range of proteins (enzymes, structural proteins and novel, unannotated proteins) present in or on the surface of prostasomes providing them with a diverse nature. Interestingly prostasomes are able to fuse with sperm, this event and the associated transfer of proteins lies at the heart of many of their proposed functions. Sperm motility is increased by the presence of prostasomes and their fusion prevents premature acrosome reactions. Prostasomes have been shown to aid protection of sperm within the female reproductive tract because of immunosuppressive, antioxidant and antibacterial properties. Clinically these functions imply a role for prostasomes in male factor infertility. However, the very functions that promote fertility may have negative connotations in later life; recent work has suggested that prostasomes are involved in prostate cancer. Clearly more work is needed to clarify the role of these novel particles and their impact on men’s health.

Key words: male fertility/prostasomes/prostate

The prostate gland

The prostate is a glandular organ whose contents are secreted during ejaculation. In humans it is the largest of the male accessory sexual glands (others being the seminal vesicles, bulbourethral and peri-urethral glands) and is situated below the base of the bladder where it completely surrounds the urethra. In a non-pathological state the prostate is the size of a walnut, weighing 20 ± 6 g. The prostate is divided anatomically into four lobes (Coffey, 2003), the anterior lobe lying anterior to the urethra at the bladder neck, the lateral lobes lying on either side of the urethra and the median lobe lying adjacent to the bladder neck. Each lobe is sub-divided into four lobules, each of which contains a prostatic duct. The prostatic ducts open into prostatic sinuses located either side of the verumontanum (Figure 1) on the posterior wall of the prostatic urethra.

The prostate can be further divided macroscopically into three zones (Figure 1). The transitional zone comprising 25% of prostatic tissue surrounds the urethra, the central zone (5% of prostatic tissue, mainly located in the median lobe) and the peripheral zone. The peripheral zone makes up 70% of the gland in a normal prostate and almost all prostate cancer occurs in this zone.
membrane and located between adjacent columnar cells. A sub-population of basal cells is believed to contain the prostatic stem cell population that gives rise to all prostatic epithelial cell lineages (Bonkhoff and Remberger, 1996). Basal cells are generally assumed to be absent in prostatic cancer; thus their presence is used to differentiate benign conditions from carcinomas (Guinan et al., 1989; Shah et al., 1991). Despite this, several studies have reported that prostatic carcinomas do stain for basal cell markers to a variable degree (Turhan et al., 1998; Yang et al., 1999).

Neuroendocrine cells account for 0.4% of the cells in the prostatic epithelial compartment (Humphrey, 2003). They are irregularly distributed throughout the epithelium and are located between basal and luminal cells. They contain several different hormones and enzymes including serotonin [5-hydroxytryptamine (5-HT)], thyroid-stimulating hormone, calcitonin and somatostatin. These cells are of neuronal origin, but very little is known of their function.

For human fertility the prostate is important as prostatic secretions contribute up to one sixth of seminal fluid volume (Coffey, 2003). The constituents (Table I) of prostatic secretions provide sperm with an optimal environment following ejaculation into the hostile milieu of the vagina. Human prostatic secretion is remarkably rich in citric acid (Kavanagh, 1994) and zinc. Citric acid provides one of the major anions in seminal plasma (Coffey, 2003) and is a potent chelator of metal ions. The prostate has the highest zinc concentration in the body. Zinc binds enzymes within the seminal plasma and acts as an antibacterial agent (Fair et al., 1973; Fair and Parrish, 1981).

Prostate-specific antigen (PSA) is a serine protease involved in liquefaction of the ejaculate. The ejaculate coagulum prevents loss of semen out of the vagina just after ejaculation. However, liquefaction must occur to enable sperm to become motile and progress into the upper regions of the female reproductive tract.

Table I. Prostatic components of seminal fluid

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
<th>Function</th>
<th>Clinical relevance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric acid</td>
<td>97–178 mM</td>
<td>Major anion of seminal plasma, potent binder of metal ions</td>
<td>Unknown</td>
</tr>
<tr>
<td>(Averna et al., 2005)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spermine</td>
<td>50–350 mg/dl</td>
<td>Unknown, linked to sperm count and motility</td>
<td>Unknown</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.1–5.4 mmol/l</td>
<td>Binds enzymes within the seminal plasma and acts as an antibacterial agent</td>
<td>Low levels associated with prostate cancer</td>
</tr>
<tr>
<td>(Elzanaty et al., 2002)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prostate specific antigen</td>
<td>110–2211 mg/l</td>
<td>Liquefaction of the ejaculate</td>
<td>High levels associated with prostate cancer</td>
</tr>
<tr>
<td>Prostatic acid phosphatase</td>
<td>Unknown</td>
<td>Hydrolysis</td>
<td>unknown</td>
</tr>
</tbody>
</table>

Prostasomes

Prostasomes were first identified by Ronquist et al. in 1978 (a,b). For over two decades, they have been investigated by both the urological and gynaecological research communities, and yet remain relatively unknown clinically. They are sub-micron, membrane-bound secretory granules produced by the luminal prostatic epithelial cells and expelled with prostatic secretions at ejaculation. Prostasomes have multiple functions, many of which are related to the process of fertilization. In addition, more recent studies have suggested prostasomes may be involved in the development of prostate cancer.
Prostasomes and male reproduction

Structure

Prostasomes range in size from 40 to 500 nm, (Figure 2) with a mean diameter of 150 nm (Ronquist et al., 1978 a,b). Two distinct morphological types have been identified—a larger, light prostasome and one which is smaller, dark and more electron-dense (Ronquist et al., 1978 a,b). Prostasomes have multilamellar lipoprotein membranes which are unique in that they contain an inherently high concentration of cholesterol, approximately 45% together with 15% phospholipids (Arvidson et al., 1989; Arienti et al., 1998). Unlike plasma membranes, which have a 1:1 ratio of cholesterol to phospholipids, the prostasome membrane has a ratio which is 2:1 (Arienti et al., 1996b; Carlsson et al., 2003). The most abundant phospholipid in prostasome membranes, constituting about 50%, is sphingomyelin (Arienti et al., 1999b). It is this unusual membrane composition that confers the prostasome with a uniqueness that, together with its ability to fuse with other cells, undoubtedly leads to its distinctive functions and behaviour.

Formation

Prostasomes are produced in the apical region of prostatic luminal epithelial cells (Figure 3). This region, near the upper pole of the nucleus, is the area in which the Golgi apparatus is most abundant (Ronquist and Brody, 1985; Nilsson et al., 1996; Stewart et al., 2004). Sahlen et al. (2002) examined benign and neoplastic areas of core prostatic biopsies by electron microscopy. Cells in both areas showed comparable secretory machinery and the content, structure and distribution of the prostasomal vesicles appeared similar. Small vesicles were visualized budding from the Golgi membrane system and this is hypothesized to represent the initial developmental stages of the prostasome (Sahlen et al., 2002). Two theories exist to explain how these membrane-bound storage vesicles are released from the epithelial cells into the lumen of the prostate duct—exocytosis and diacytosis. Exocytosis involves fusion of adjacent membranes belonging to the storage vesicle and the epithelial cell; the prostasome is then delivered into the glandular lumen (Ronquist and Brody, 1985). Diacytosis involves the storage vesicle being translocated from the cell cytoplasm through the plasma membrane intact. Both phenomena seem to occur with approximately equal frequency in the prostate gland (Ronquist and Brody, 1985).

Protein composition

Prostasomes have been found to contain multiple proteins on their surfaces. The first membrane-bound enzyme to be identified in prostasomes was ATPase whose activity is Mg\(^{2+}\), Ca\(^{2+}\) and Zn\(^{2+}\) dependent (Ronquist et al., 1978a,b). Since this initial work, many further proteins have been identified on the prostasomal membrane. A comprehensive proteomic study by Utleg et al. (2003) aimed at determining the protein composition of prostasomes identified over 139 proteins. These were classified into six categories; enzymes (35%), transport and structural proteins (19%), GTP proteins (14%), chaperone proteins (6%), signal transduction proteins (17%) and unannotated proteins (9%) (Table II).
IV, V, VII and XI) have also been identified in prostasomes. The family was identified on prostasomes by Utleg et al. (Bogenrieder et al., 2003). Aminopeptidase N (CD13), a cell surface metalloenzyme involved in the down-regulation of regulatory peptide signals through the removal of the N-terminus (Carlsson et al., 2003). Other studies had previously detected this enzyme and it has been used as a marker of prostasomes (Ronquist et al., 1988; Arienti et al., 1997b). In a study by Carlsson et al. (2003), prostasomes were analysed from three sources—seminal plasma, normal prostate and prostate cancer bone metastases. Aminopeptidase N, together with the membrane proteins CD26, CD10 and CD46 were found to be present in prostasomes from all three sources (Carlsson et al., 1994). It is difficult, however, to compare these results to free-zone electrophoresis. The samples were found to dissociate. This was then confirmed by electron microscopy. Arienti et al. (1990), Prostasomes have also been found to contain granuloplysin, a 32–37 kDa enzyme (Skibinski et al., 1994).

Significant amounts of prostasomal proteins (35%) were found to be enzymes, and these consisted mainly of hydrolases. One such hydrolase is aminopeptidase N (CD13), a cell surface metalloenzyme involved in the down-regulation of regulatory peptide signals through the removal of the N-terminus (Carlsson et al., 2003). Other studies had previously detected this enzyme and it has been used as a marker of prostasomes (Ronquist et al., 1988; Arienti et al., 1997b). In a study by Carlsson et al. (2003), prostasomes were analysed from three sources—seminal plasma, normal prostate and prostate cancer bone metastases. Aminopeptidase N, together with the membrane proteins CD26, CD10 and CD46 were found to be present in prostasomes from all three sources (Carlsson et al., 2003). However, other studies have suggested that CD13 (aminopeptidase N) and CD26 (dipeptidyl peptidase IV) may be absent from prostasomes originating from metastatic prostate cancer (Bogenrieder et al., 1997). It is difficult, however, to compare these two papers, as Carlsson uses the more robust modality of flow cytometry whilst Bogenrieder uses light microscopy at a maximum magnification of ×40, which may be too low to accurately characterize prostasomes. CD26 is a serine protease that cleaves N-terminal dipeptides from peptides with proline or alanine at their penultimate position. Neprilysin (CD10) is thought to be associated with androgen-independent progression of prostate cancer (Papandreou et al., 1998). Prostasome membranes have also been found to contain granuloplysin, a 32–37 kDa enzyme (Skibinski et al., 1994).

Transport and structural proteins are the second most abundant group (19%) of prostasomal proteins. Six members of the annexin family were identified on prostasomes by Utleg et al. (2003)—annexin I–III, V, VI and XII. Members of the annexin family (I, II, IV, V, VII and XI) have also been identified in prostasomes (Haigler and Christmas, 1990; Xin et al., 2003; Smitherman et al., 2004; Lehnig et al., 2005). Annexins are a family of proteins involved in calcium and phospholipid binding. They are characterized by a central hydrophilic pore that functions as a calcium channel (Utleg et al., 2003). This property is believed to have relevance to fertility because of the critical dependence of sperm motility on calcium. It is described later in this review how prostasomes fuse with sperm and transfer calcium to them; it is likely that annexins play a role in this transmission.

Prostasomes are believed to play a role in prostate cancer, and prostate cancer tissue has been shown to have reduced levels of annexins I and II (Smitherman et al., 2004; Lehnig et al., 2005). Smitherman et al. (2004) compared benign prostatic epithelium with androgen-stimulated prostate cancer and found annexins I and II to be decreased in prostate cancer. Additionally, they compared these to recurrent prostate cancer and, interestingly, found the levels to be further decreased in this group of patients. Similar findings were reported by Lehnig et al. (2005) when they examined whole radical prostatectomy specimens. The clinical relevance of this to the process of carcinogenesis and prostate cancer progression remains to be determined.

Prostasomal proteins identified by Utleg et al. (2003) also incorporated GTP-binding proteins (14% of the total proteins located on prostasomes) including Rho, Ras and Rab, chaperone proteins (6%) including the heat shock proteins calmodulin, ubiquitin and clusterin and some 9% of proteins which were unannotated.

It has been speculated that prostasomal protein content may change with disease state; including benign prostatic hyperplasia (BPH), prostate cancer, prostatitis and infertility. There are many important and diverse proteins present on prostasomes whose functions in relation to prostatic disease are not completely elucidated.

**Prostasome function**

Since their discovery in 1978, a variety of roles have been ascribed to prostasomes. Perhaps the most striking of these is the ability of prostasomes to undergo hydrophobic fusion with spermatozoa, thereby allowing proteins present on prostasomes to be directly transferred to sperm. This property appears to be important in aiding the passage of sperm through, and the survival in, the lower and upper female genital tract to penetrate the zona pellucida and to reach the oocyte for fertilization (Ronquist and Nilsson, 2004). The numerous enzyme systems, small signalling molecules and neuroendocrine markers associated with prostasomes suggest that these vesicles play a complex role in regulating sperm viability and function (Kravets et al., 2000). There is now a widespread consensus that their major function is to interact with and protect spermatozoa after ejaculation in order for them to retain full functional capacity in preparation for their encounter with the oocyte (Saez et al., 2003).

Prostasomes were first observed to fuse with sperm by Ronquist et al. (1990). They subjected prostasomes and sperm as separate samples to free-zone electrophoresis. The samples were found to approach each other and to fuse into a single peak that could not be dissociated. This was then confirmed by electron microscopy. Arienti et al. (1997a) confirmed this fusion in several experiments using fluorescent self-quenching microscopy and flow cytometry. Both prostasomes and spermatozoa are negatively charged and their bonding appears to be hydrophobic in character (Ronquist et al., 1990).
The interaction of spermatozoa and prostasomes is most likely limited to the environment of the vagina, because prostasomes are immobile and cannot follow spermatozoa into the upper female reproductive tract (Arienti et al., 2004). Environmental factors must be favourable for fusion to take place. Thus fusion of prostasomes with the sperm plasma membrane is pH-dependent and is favoured by an acidic pH (Arienti et al., 1997a; Carlini et al., 1997; Frenette et al., 2002). The vaginal environment is acidic whilst prostatic fluid and cervical mucus is alkaline. This may explain why prostasome/sperm fusion does not occur prematurely. Thus, the fusion of sperm with prostasomes may represent an important physiological mechanism to assist sperm in resisting the acidic milieu of the vagina (Arienti et al., 1997a, 1999a). Another factor which also seems to be important for prostasome fusion is zinc, whose optimal level is 0.1–1.5 mmol.l⁻¹ (Frenette et al., 2002).

Prostasome and sperm fusion is of high physiological importance. The transfer of lipids and biologically active proteins underlies many of the functions that prostasomes confer on sperm. Membrane fusion is required for many biological events (Kravets et al., 2000). Transfer of enzymatic activity from prostasomes to spermatozoa represents a means of modifying the composition and biological properties of the sperm membrane (Kravets et al., 2002). Prostasome function has been shown to either directly or indirectly influence sperm function. These properties will be described below.

**Prostasomes directly influencing sperm function**

**Sperm motility**
Sperm motility is vital for natural fertility in the human male and if abnormal contributes significantly to infertility. Sperm motility and movement quality are important factors in the migration of sperm through the female genital tract, especially moving through cervical mucus and penetrating the zona pellucida (Saez et al., 2003). Prostasomes have been shown to improve the ‘swim-up’ ability of sperm (Fabiani et al., 1994) in a pH-dependent manner. Spermatozoa were exposed to an acidic medium in the presence or absence of prostasomes and motility was assessed. Whilst, normally, sperm are less motile in an acidic milieu, prostasomes appear to have a protective effect and increase the percentage of motile cells (Arienti et al., 1999a). Such a system may therefore be invaluable for counteracting the effects of an acidic environment in the vagina after coitus.

Calcium is implicated as the major cation involved in sperm motility (Suarez and Dai, 1995). Palmerini (1999) measured sperm [Ca²⁺], and found that it increased after mixing prostasomes and sperm at pH values necessary for fusion. Moreover, they found proportionality between the extent of fusion and the concentration of cytosolic calcium. A rise in [Ca²⁺], was seen after 2 min but maximal values were reached after 20 min, with the [Ca²⁺], rising from 135 to 364 nmol/l. As previously discussed, prostasomes contain a calcium-dependent ATPase on their surface and by virtue of their expression of annexins also have calcium channels (Uleg et al., 2003). It has been demonstrated that there is transfer of a cytoplasmic calcium ‘burst’ to the sperm after fusion with prostasomes. The manner in which the rise of intracellular calcium occurs is unknown. It may be secondary to delivery from the prostasome or may be linked to annexins within prostasomes acting as calcium channels after fusion. This has not yet been shown to be linked to annexins, although they remain likely candidates.

In contrast, zinc has been proposed to exert a negative effect on sperm motility. Prostasome-free semen samples have reduced levels of zinc (Vivacqua et al., 2004), suggesting that the high zinc levels unique to prostatic fluid, are due to prostasomes (Stegmayer et al., 1982). Several studies have shown an inhibitory effect of zinc upon sperm motility (Riffo et al., 1992; Fuse et al., 1999). The inference is therefore that prostasomes enhance sperm motility mainly by the transfer of calcium whilst the effect of zinc upon sperm motility is inhibitory.

**Capacitation and acrosome reaction**
Both capacitation and the acrosome reaction are crucial steps that sperm must undergo before fertilization. Capacitation involves the removal of the glycoprotein coat and seminal plasma proteins overlying the acrosomal region of the sperm. Only once capacitation is complete can the acrosome reaction take place (Breitbart, 2003). The acrosome reaction allows sperm to fuse with the female egg. As previously described, prostasomes are very rich in cholesterol and approximately 45% of the total semen cholesterol is bound to prostasomes (Arienti et al., 1999b). The cholesterol: phospholipid ratio for human sperm is 0.83 (Mack et al., 1986). Cross and Mahasreshti (1997) proposed that cholesterol is transferred from the prostasomal to the sperm membrane. This has been confirmed by Kravets et al. (2000) who have shown that the sperm membrane becomes enriched for cholesterol, sphingomyelin and saturated glycosphospholipid following fusion with prostasomes. Carlini et al. (1997) measured the fluidity of the sperm membrane pre- and post-prostasome fusion and found that lipid transfer caused the sperm membrane fluidity to decrease, thereby delaying the acrosome reaction (Arienti et al., 1998). The work of Visconti et al. (1999) and Osheroff et al. (1999) showed that the converse is true, cholesterol efflux from the sperm plasma membrane was postulated (in vitro) to lead to a successful capacitation reaction therefore, supporting the conclusion that cholesterol is inhibitory to acrosomal responsiveness (Cross, 1996). Prostasome-derived cholesterol will potentially, therefore, stabilize the acrosomal cap and in doing so prevent premature activation (Cross and Mahasreshti, 1997; Arienti et al., 2004).

The fusion of prostasomes with spermatozoa has also been shown to render the spermatozoa more sensitive to the effect of progesterone on acrosome reaction induction (Arienti et al., 2002; Palmerini et al., 2003). Progesterone is thought to be one of the stimulators of the acrosome reaction, by also increasing cytosolic calcium.

An additional method by which prostasomes influence the acrosomal reaction appears to involve hydrolases, which as discussed earlier were found to be present in abundance on the prostasomal surface. Hydrolytic enzymes, such as ecto-diadenosine polyphosphate hydrolase, present on sperm acrosomal membranes are indeed essential for the acrosome reaction to occur (Breitbart et al., 2005). Human sperm have been shown to only possess this enzyme after fusion with prostasomes (Minelli et al., 2002). This suggests that the ability of sperm to undergo the acrosome reaction is dependent upon the presence of prostasomes.

**Prostasomes indirectly influencing sperm function**

**Immunological relevance**
The female genital tract is a potentially hostile environment for spermatozoa. To survive, it is essential that sperm evade potent...
female immune effectors. The male appears to have developed complex mechanisms to immunomodulate the local environment within the female reproductive tract following ejaculation, preventing immune-mediated sperm destruction and prolonging their life as they approach fertilization. Acquired and innate responses in the female reproductive tract have been shown to be curtailed temporarily following intercourse (Kelly, 1995). As a result of this, the chances of conception are enhanced. Immunosuppressive effects of human seminal plasma are mediated by several factors. There is agreement that some of the immunosuppressive activity in human seminal plasma is accounted for by high levels of the E series prostaglandins (Quayle et al., 1989). When present at high concentrations prostaglandins are responsible for a wide range of suppressive effects, including inhibition of lymphocyte proliferation, modulation of natural killer cell-mediated cytotoxicity and modification of cytokine release from antigen presenting cells (Kelly, 1995). However, seminal plasma from which sperm have been removed retains the ability to inhibit the proliferation of lymphocytes. Kelly et al. (1991) found that purified preparations of prostasomes inhibited lymphoproliferation in a dose-dependent manner. In addition, the ability of neutrophils and monocytes to phagocytose latex particles was also found to be inhibited following their interaction with prostasomes; the phagocytosing cells apparently becoming inactivated following their ingestion of prostasomes (Skibinski et al., 1992). Hence, prostasomes seem to play a complementary role to other immunosuppressive factors present in semen, neutralizing immune defences in the female reproductive tract.

Sperm are at risk of complement-mediated damage and destruction as they travel through the female reproductive tract. Functionally active complement is present at multiple sites, for example, within cervical mucus and ovarian follicular fluid (Simpson and Holmes, 1992). Spermatozoal membranes are known to contain three important and functionally active membrane proteins which regulate the activity of complement: CD55 (decay accelerating factor—DAF), CD46 (membrane cofactor protein—MCP) and CD59 (protectin). In other systems, it has been established that these proteins normally act together to effectively control the potentially damaging effects of complement activation on cell surfaces.

The complement cascade can be activated by three distinct enzyme activation pathways known as the classical, alternative and lectin pathways. These three pathways converge at the level of the third complement component, C3. C3 lies at the heart of the complement system, acting as a pivot which links the recognition and effector arms of the complement cascade. C3 possesses a labile internal thioester bond which undergoes low level spontaneous hydrolysis to form activated C3, a process known as C3 ‘tick-over’ (Holmes and Simpson, 1992). Activated C3 binds to cell or pathogen surfaces and allows the terminal components of the complement cascade to self-assemble, generating the membrane attack complex (MAC). The MAC inserts itself into the membrane creating a transmembrane pore and causing lysis. The regulators CD55 and CD46 act early in the complement cascade to control C3 activation, whereas CD59 acts at the later stages to control the MAC.

In contrast with other cells, the complement regulatory proteins display a striking regional distribution on human spermatozoa. Thus, CD59 is known to be strongly expressed on the surface of acrosome intact sperm. By contrast, CD46 is entirely restricted to the inner acrosomal membrane; this regulator is therefore exposed directly to the female environment only after the acrosome reaction has taken place. CD55 is present at relatively low levels on sperm by comparison with the other two regulators. On this basis, Simpson and Holmes (1994a) have suggested that CD59 is especially important for protecting sperm from complement in the lower female genital tract. On the other hand, CD46 appears to exert its regulatory role in the upper female genital tract, proximal to the site of fertilization, when the acrosome reaction has taken place.

On the basis of studies involving sequential ultracentrifugation and phase separation in the detergent Triton X-114, Simpson and Holmes (1994b) showed that CD46 was present in a membrane-associated form in the particulate fraction of seminal plasma following the removal of sperm. These observations clearly suggested that CD46 was present on prostasomes. In addition, Rooney et al. (1993) found that CD59 was also present on prostasomes and, moreover, showed that functional CD59 could be transferred from prostasomes to red blood cells. Babiker et al. (2005) have since confirmed this, showing that CD59 could be transferred from prostasomes to red blood cells and malignant prostate cells deficient in CD59. This resulted in protection of these cells against complement-mediated lysis, thus showing that a fully functional protein had been transferred. The authors suggested that this could be a mechanism by which prostasomes protect malignant prostatic cells from complement attack. For fertility, the transfer of complement inhibitory molecules, especially CD59, to sperm following prostasome fusion could provide an important mechanism to ensure that sperm are protected against functional complement whilst in the female reproductive tract.

**Antioxidant attributes**

Due to the high content of unsaturated fatty acids within their membranes, human sperm are very sensitive to oxidative stress which results in peroxidative damage (MacLeod, 1943). Free radicals are also purported to be involved in inflammation and the process of carcinogenesis. They are thought to impair male fertility (Male Infertility Best Practice Policy Committee of the American Urological Association; The Practice Committee of the American Society for Reproductive Medicine, 2004). Free radicals are known to be produced by polymorphonuclear neutrophils (PMNs) and this could be one mechanism by which sperm are damaged by the female immune system. Prostasomes have been shown to have antioxidant properties enabling them to inhibit superoxide generation by leucocytes (Skibinski et al., 1992; Saez et al., 1998). This antioxidant activity occurs through the inhibition of NAPDH oxidase activity, which is present in PMNs. This arises by rigidification of the PMN plasma membrane through lipid transfer following fusion with the prostasomes (Saez et al., 1998, 2000).

**Antibacterial properties**

Several studies have suggested the presence of an antibacterial agent associated with prostasomes. Carlsson et al. (2000b) observed a dose-dependent growth inhibition when prostasomes were incubated in a medium containing Bacillus megaterium. More recently there have been suggestions as to what may provide this antibacterial activity. Andersson et al. (2002) investigated the human cationic antimicrobial protein hCAP-18. This is one of several antimicrobial peptides discovered to act as effector molecules in innate immunity. hCAP-18 is the only known human cathelicidin and releases the antimicrobial peptide LL-37 which has a broad spectrum of activity against gram positive and negative
bacteria (Bals et al., 1998). Andersson et al. (2002) have shown using western blotting and flow cytometry that 70% of the hCAP-18 in seminal plasma is localized to the prostasomal component. They hypothesize that hCAP-18 may play an important role in antimicrobial defence during human reproduction.

Coagulant activity

Semen is a potent coagulator of blood, and dilutions of seminal plasma decrease the clotting time of normal plasma from 273 to 42 s (Fernandez et al., 1997). This confers positive advantages to the female and male during intercourse as any abrasions and bleeding will be rapidly healed. Hence, sperm and seminal products are less likely to enter the female’s bloodstream, thereby decreasing the risk of forming anti-sperm antibodies. Additionally, there will be less chance of transmitting blood-borne infections, such as HIV.

Seminal coagulant activity is likely to be due to the presence of tissue factor (CD142) on the surface of prostasomal membranes (Fernandez et al., 1997; Carson and De Jonge, 1998). Tissue factor is a cell membrane-associated receptor and an essential clotting cofactor for factor VII (Fernandez et al., 1997). Activated factor VII (VIIa) is an initiator of the clotting cascade and activates factor X and IX, leading to the activation of thrombin. As such, tissue factor has critical functions in haemostasis and thrombogenesis and is also implicated in the cellular immune response.

Semen liquefaction

Ejaculation occurs into the vagina where semen forms a coagulum for approximately 20 min before liquefying. This ensures the semen remains in the vagina following intercourse. The enzyme PSA plays an important role in this process by controlling the liquefaction of semen. Prostasomes are likely to contribute to this process as they contain PSA (Utleg et al., 2003). There is also evidence that tissue factor (CD142) associated with prostasomes is involved in sperm coagulation and liquefaction (Lwaleed et al., 2004). In a series of experiments Lwaleed et al. (2005) showed the presence of both tissue factor and tissue factor pathway inhibitor in semen. Tissue factor was associated with prostasomes and acts as an initiator of the haemostatic system, which is believed to be responsible for semen coagulation. Tissue factor pathway inhibitor, which is found in the seminal plasma and also possibly associated with prostasomes, was found to be positively associated with seminal liquefaction time.

Clinical implications of prostasomes

Infertility

It is clear from the evidence presented in the previous section that prostasomes contribute to the normal fertilization process. From the moment of ejaculation, they help to protect sperm because of their immunosuppressive, antibiotic and antioxidant properties. By enhancing motility and stabilizing the sperm membranes to prevent untimely capacitation, the prostasome helps to ensure that sperm reach the oocyte and are then able to acrosome react and penetrate the zona pellucida for fertilization. Disruptions to prostasome function are therefore likely to contribute to impairment of fertility.

Antisperm antibodies (ASA) are present in 5% of men with infertility (Impey, 1999), but the corresponding antigens are poorly characterized. A high percentage of infertile patients have been shown to have anti-prostasome antibodies (Allegrucci et al., 2001). Carlsson et al. (2004a,b) showed that the prostasomal antigens recognized by high titre-antisera of infertile men were generally different from the sperm antigens recognized by the same sera. Further studies on the prevalence of anti-prostasome antibodies showed that 97% of immunoinfertile patients’ sera contained IgG antibodies against seminal prostasomes. Characterization revealed that the two dominant immunogens were prolactin-inducible protein and clusterin (Carlsson et al., 2004c).

Prostasomes are known to improve sperm motility (Fabiani et al., 1994; Arienti et al., 1999a). Asthenozoospermia is a common finding in male factor infertility. Kravets et al. (2000) have suggested the use of a medium containing prostasomes could be instrumental in assisting reproductive technologies associated with low sperm motility.

Prostate disease

Prostate cancer arises as a result of malignant growth, and in the United Kingdom (UK) this disease is diagnosed in approximately 30,000 men each year (Cancer Research, UK). The natural history of prostate cancer remains relatively unknown. However, it is well recognized that the incidence of prostate cancer increases with age, with the largest number of cases being diagnosed in the 70- to 79-year-old age group (Cancer Research, UK). Ronquist and Nilsson (2004) have recently suggested that the functions of prostasomes change with the ageing process. They conject that prostasomes are protective of fertility during early- to mid-adulthood, but after the age of 50, they turn against the host cell and are conducive to the transition of a normal prostate epithelial cell into a neoplastic one. They hypothesize that this exploitation of the host’s physiological systems lays the foundation for the very high prevalence of prostate cancer after the age of 50 (Ronquist and Nilsson, 2004). However, Nilsson et al. (2001) showed higher levels of serum antibodies to prostasomes in patients with prostate cancer as compared to healthy controls, and Carlsson et al. (2000a) demonstrated that prostasomes could inhibit the growth of prostate cancer cell lines in culture. The cause of this inhibition is unknown, although it is possible that this is due to the high concentrations of zinc which are known to be present in prostasomes. Zinc has been shown to induce apoptosis in prostatic cells (Carson and De Jonge, 1998) and to reduce invasiveness in prostate cancer cell lines (Cross and Mahasreshti, 1997). These observations suggest that the high levels of zinc observed in the prostate may have a protective effect in terms of the development of malignancy. Several studies have shown that zinc levels are reduced in prostate cancer (Zaichick et al., 1997; Bataineh et al., 2002), and it is believed that the prostate looses the ability to concentrate zinc with the development of malignancy (Saez et al., 1998). The evidence does not entirely support Ronquists’ theory, but it is clear that prostasomes have the potential to impact upon the formation of prostate cancer.

As discussed previously, prostasomes have properties that promote sperm function. Ironically, these are properties that could aid the survival and progression of prostate cancer cells. Proteins mediating these effects include phosphorylation proteins, tissue factor, CD59 and angiotension converting enzyme (ACE). Phosphorylation/dephosphorylation of proteins plays a critical role in regulating cell proliferation and malignant transformation. Prostasomes contain phosphorylation enzymes on their surface and it has been hypothesized by Mearini (2004) that prostasomes...
by virtue of these enzymes are involved in neoplastic prostate transformation. Mearini (2004) examined prostasomes from prostate cancer patients and showed that enzyme activities and immunodetection of prostate proteins were different from prostasomes obtained from healthy donors. However, they did detect differences in patterns of serine-phosphorylated proteins present in normal and neoplastic prostate tissue suggesting that this may be a tool for prostate cancer diagnosis.

Angiogenesis is an important step in carcinogenesis. There is, as yet, no consensus upon whether prostasomes inhibit or stimulate angiogenesis. Delves et al. (2005a,b) studied the effect of prostasomes on human umbilical vein endothelial cells, finding that they were inhibitory to angiogenesis in this experimental model. However, Hicks et al. (2005) suggested that prostasomes might instigate angiogenesis in prostate cancer. They have related angiogenesis to the prostasomal protein tissue factor (CD142). Tissue factor is also known to have critical functions in haemostasis and thrombogenesis. There is an increased risk of thromboembolic disease in patients with prostate cancer, and this may be in part related to prostasomal CD142. Another cluster of differentiation (CD) factor commonly associated with prostasomes is CD13 (aminopeptidase N)—a zinc metalloenzyme. Metalloenzymes are known to activate growth factors and can cause angiogenesis and thus may support a role for the prostasome in early malignant transformation (Ronquist and Nilsson, 2004).

Two other less well-characterized prostasomal enzymes, ACE and chromogranin A, have also been implicated in the development of prostate cancer. ACE sustains a high concentration of angiotensin II, which favours cell proliferation and thus may contribute to the growth of prostate cancer (Ronquist and Nilsson, 2004). Chromogranin A, which is often associated with neuroendocrine cells, is present at higher concentrations (10-fold) on prostasomes derived from prostate cancer bone metastases as compared to those from seminal fluid or native prostate (Carlsson et al., 2003). Further research would be valuable in determining whether this information can be exploited for diagnostic purposes.

Certainly much is still to be established regarding the prostasome and its relationship to prostate cancer development and progression. As the evidence above shows, there is an urgent need to pursue work in this field especially in light of the high incidence of this malignancy, and the financial burden it places on the health service.

Finally, further elucidation of the prostasome’s role in promoting fertility may highlight potential avenues for new fertility treatments.

References


Prostasomes and male reproduction


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