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Effect of protein C inhibitor (PCI) on in vitro fertilization

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1. Introduction

Protein C inhibitor (PCI) is a non-specific serpin-type inhibitor (Suzuki et al., 1989) present in plasma and many other body fluids and secretions (Geiger, 1988; Laurell et al., 1992). PCI is synthesized throughout the male reproductive tract and high concentrations are found in seminal plasma (Laurell et al., 1992). In seminal plasma PCI has been shown to interact with prostate specific antigen (Christensson and Lilja, 1994). Additionally PCI inhibits the sperm protease acrosin and is immunocytochemically localized to disrupted acrosomal membranes of washed sperms, while intact sperms are negative for PCI-antigen (Zheng et al., 1994). Therefore, by inhibiting prematurely released and activated acrosin in the male reproductive tract PCI might protect spermatozoa, surrounding tissues and seminal plasma proteins from proteolytic damage. Under physiological conditions, however, acrosin is activated and released only in the female reproductive tract in the immediate vicinity of the ovum and is thought to be involved in the fertilization process by digesting a pathway for the sperm through the zona pellucida of the ovum. Inhibition of acrosin by PCI might therefore have an influence on the sperm/egg interaction. It was our aim to study PCI synthesis in the female reproduc-

tive tract and to analyze the effect of PCI on fertilization. For this purpose we used a mouse in vitro fertilization model.

2. Methods

For studying PCI synthesis total RNA was isolated from ovaries obtained from female Balb-C mice as described (Chomczynski and Sacchi, 1987). After electrophoresis, Northern blotting (Virca et al., 1990) was performed using a ³²P-labelled 1.3 kb fragment of mouse PCI-cDNA.

Mouse (OF1) epididymal spermatozoa were collected from the cauda epididymis and from the vas deferens into 1 ml of IVF medium supplemented with 3% BSA and incubated for 2 h at 37°C to allow sperm maturation (capacitation).

In female mice (OF1) superovulation was induced by intraperitoneal injection of 10 IU of pregnant mare's serum gonadotropin followed 48 h later by injection of 10 IU human chorionic gonadotropin. 13–14 h later mice were sacrificed and eggs were removed from the swollen ampulla of the oviduct and collected into a 200 µl drop of IVF medium containing 3% BSA covered by light mineral oil. The cumulus cells were removed by treatment with

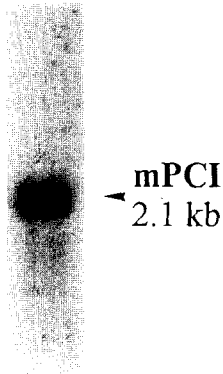


Fig. 1. PCI synthesis in the mouse ovary (Northern blot). Experimental details are given in the 'Methods' section.

hyaluronidase (5 min, 0.3 mg/ml) and washing with M16 medium. 30–40 eggs were obtained from one mouse and were cultured in 35 mm Petri dishes in

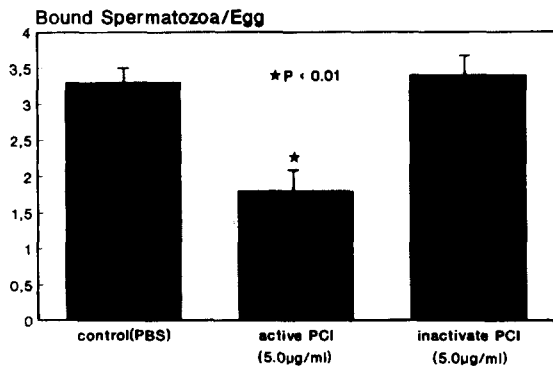
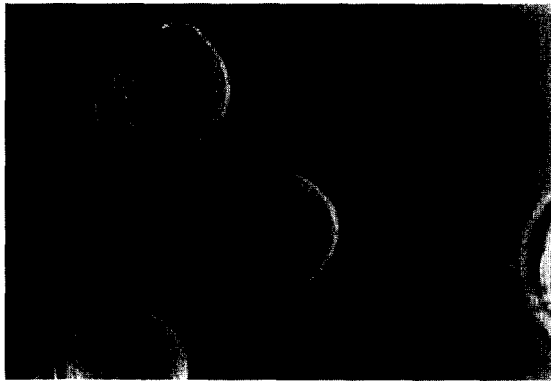


Fig. 2. Effect of PCI on mouse sperm-egg binding. Mouse eggs were incubated for 60 min with mouse spermatozoa in the absence or presence of active or heat inactivated PCI (5 µg/ml) as described in 'Methods'. Thereafter the number of zona pellucida bound spermatozoa (arrows, upper panel) was counted under a microscope.

200 µl drops of IVF medium (3% BSA) under mineral oil until further use.

The effect of PCI on sperm-egg binding and on in vitro fertilization was studied by incubating zona intact mouse eggs (8–10 in each group) with capacitated mouse epididymal spermatozoa (1×10^5 /ml) at 37°C in the absence or presence of active or heat inactivated PCI (5 µ/ml each) in 200 µl drops of IVF-media. After 60 min eggs were removed and washed by passing through 3 changes of M16 media. The number of zona pellucida bound spermatozoa was counted under a microscope. The success of fertilization (formation of 4- to 8-cell embryos) was evaluated after additional incubation for 30 h at 37°C by counting the number of fertilized eggs.

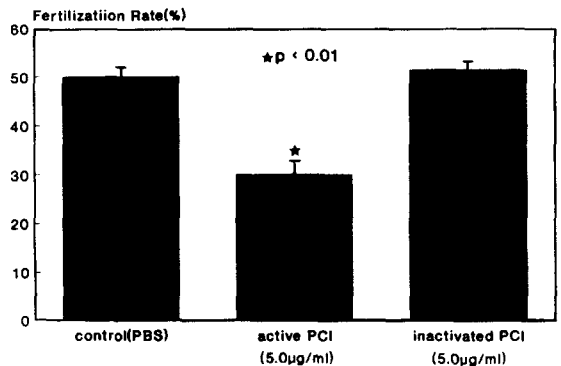


Fig. 3. Effect of PCI on in vitro fertilization. Incubation of mouse eggs with mouse spermatozoa in the absence or presence of active or heat inactivated PCI (5 µg/ml) was performed as described in the legend to Fig. 2. The success of fertilization (formation of 4- to 8-cell embryos) was evaluated after additional incubation at 37°C for 30 h by counting the number of fertilized eggs. Upper panel: Fertilized (long arrow) and unfertilized (short arrow) mature eggs.

3. Results and discussion

As can be seen from the Northern blot shown in Fig. 1 PCI mRNA (2.1kb) is present in mouse ovaries, indicating that PCI is synthesized in these organs. Since PCI is therefore present not only in the male, but also in the female reproductive tract, the interaction of PCI with sperm proteases (e.g. acrosin) may have an influence on the fertilization process. After having assured that human PCI inhibits mouse sperm proteases (mainly acrosin, not shown), we analyzed the effect of PCI on sperm-egg binding and on in vitro fertilization. In a mouse system active human PCI (5 $\mu\text{g}/\text{ml}$) inhibited sperm-egg binding by ca. 45% (Fig. 2) and in vitro fertilization by ca. 40% (Fig. 3). Heat inactivated PCI had neither an effect on sperm-egg binding nor on in vitro fertilization.

These data suggest that modulation of the fertilization process by PCI is caused by a mechanism involving the inhibition of sperm and/or oocyte proteases.

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