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Research Article

EVALUATION OF PATHOGENIC POTENTIAL OF MYCOPLASMA MYCOIDES SS MYCOIDES (LC VARIANT) ASSOCIATED WITH KID-ARTHRITIS IN LABORATORY MODELS

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ABSTRACT

A strain of Mycoplasma mycoides ss mycoides (LC variant) isolated from synovial fluid of swollen knee-joint of a kid was tested for its pathogenic potential in organ cultures (hamster tracheal rings, rabbit fallopian tube), rat mammary gland and genito-urinary challenge in female hamsters. The organisms grew well and multiplied in both the organ cultures, stopped their ciliary activity and caused histopathological lesions. At the end of the experiments, *M. mycodes* ss *mycoides* (LC) could be recovered from infected tracheal rings, fallopian tube explants, mammary gland of rat and genital organs (ovary, uterus, vagina) of female hamsters. Significant histopathological lesions included: denudation of epithelium lining and loss of cilia along with infiltration of lymphocytes with oedema in lamina propria of tracheal rings and fallopian tube explants, lymphocytic and macrophagic infiltration in interaccinar and interlobular tissues followed by desquamation and distortion of accinar cell lining, fibroblastic cell proliferation in interstitium and hyperplasia of accinar ducts of mammary gland of rats, degeneration and necrosis of ovarian tissue and infiltration of hymphocytes, macrophages along with fibroblastic cell hyperplasia in ovary, uterine and vaginal tissues of female hamsters. The results of these experimental study revealed that *M. mycoides* ss *mycoides* (LC) was pathogenic strain.

Key words: *M. mycoides* ss *mycoides* (LC), kid arthritis, mycoplasma pathogenicity

Mycoplasma mycoides ss *mycoids* (LC variant) has been isolated from goats, sheep and cattle suffering from fibrinous peritonitis (Laws, 1956), arthritis, mastitis (Perreau, 1979), pneumonia (Ojo, 1976, Littlejohns and Cottew, 1977) and ocular infections (Jones and Barber, 1969). Only a few reports (Mac Owan, 1984, Kapoor, 1993) are available in literature regarding its experimental pathogenicity in ruminants. However, i*n-vitro* organ cultures including hamster tracheal organ culture (Garg *et al.*, 1991, Kapoor *et al.*, 1993) and rabbit fallopian tube organ culture (Singh *et al.*, 1991,) have been used to study the pathogenicity of mollicutes of bovine and equine origin. Rat mammary gland and genitourinary challenge in female hamster have also been exploited to study the pathogenic potential of various mollecutes of bovine and equine genital tract (Garg *et al.*, 1991, Kumar *et al.*, 1994, Singh *et al.*, 1997). This paper describes the pathogenic status of a *M. mycoides ss mycoides* (LC) isolated from arthritic–kid in organ cultures (hamster tracheal and rabbit fallopian tube), rat mammary gland and genito-urinary challenge in female hamsters.

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MATERIALS AND METHODS

Mycoplasma culture: *Mycoplasma mycoides* ss *mycoides,* LC variant (P113/87) used in this study was isolated from synovial fluid of an arthritic kid at Mathura (U.P.). The *Mycoplasma* strain was maintained in PPLO broth medium devoid of thallium acetate (Garg *et al.*, 1988). The PPLO broth medium having ammonium reineckate (50 µg/ml) was used to recover mycoplasma from rat mammary gland tissues and genital as well as visceral organs (lungs, liver, heart, spleen) of hamsters.

Experimental animals: Adult golden hamsters weighing 80-100 g, female rabbits of about 6-8 months age and 1 kg body weight, and Norway albino female rats at 7-10 days lactation were procured from the disease free small animal

house of the university.

Organ cultures: Hamster tracheal ring (HTR) and rabbit fallopian tube (RFT) explants were prepared and maintained in Eagle's BSE-Basal medium supplemented with Hank's salt and L-glutamine without sodium bicarbonate (Hi-media) as described earlier (Singh *et al.*, 1991, Kapoor *et al.*, 1993). The ciliary action of epithelial cells at inner surface of the hamster tracheal rings and outer fringes of RFT explants was observed using an inverted microscope (x300).

Inoculation of organ explants: Six to eight selected tracheal rings and RFT explants showing vigorous ciliary activity in 1.5 ml Eagle's medium were infected with 0.5 ml *M. Mycoides* ss *mycoides* (LC) inoculum having $2x10^6$ CFU. Three to four tracheal rings and RFT explants were kept as uninfected control having equal volume of sterile PPLO broth. Infected and control tracheal rings were observed on alternate day for their ciliary activity score (Chandler and Barile, 1980). Similarly, RFT explants were examined daily for ciliary activity till the day on which infected explaints lost their ciliary activity completely. The recovery of *M. mycoides* ss *mycoides* (LC) was made on alternate days from both the organ cultures. At the termination of RFT experiments, the number of *M. mycoides* ss *mycoides* (LC) as CFU/ml was determined separately in medium and RFT explants. The tracheal rings and RFT explants were also preserved in neutral buffered formalin and processed for histopathological examination.

Infection of rat mammary glands: The technique described previously (Kumar *et al.*, 1994) was used to inoculate the rat with *M. mycoides* ss *mycoides* (LC) via intramammary route. The third, fourth and fifth mammary glands from the front on left side (L_3, L_4, L_5) of the five lactating rats were infected with 0.1 ml (10^6 CFU/gland) via intramammary route using 30 G x ½" needle (Thomas, USA). The right third and fourth glands (R-3, R-4) of the same rat served as uninoculated controls while right fifth (R-5) gland was inoculated with 0.1 ml sterile PPLO-broth as control. One rat each was examined and sacrificed at 1,2,3,4 and 6 day post infection (day PI). At necropsy, the glands were cut into two halves, the one half was grounded with sterile sand in 1.8 ml PPLO broth having 50 µg/ml ammonium reineckate in order to recover the inoculated organisms and the other half was fixed in neutral buffered formaline for histopathlogy.

Genito-urinary challenge of female hamsters: The technique described previously (Taylor- Robinson, 1983) was used to challenge female hamster through genito-urinary tract. In a group of six healthy hamsters devoid of any bacterial infection and cytological vaginal response, three were infected with 0.5 ml *M. mycoides* ss *mycoides* (LC) culture in log phase having 10^6 organisms inserting the nozzle of a syringe into the vagina whereas remaining three were kept as sterile PPLO-broth inoculated controls. Vaginal swabs were taken daily up to 3-day PI and thereafter on alternate day up to 15-day PI for preparation of smears to determine cytological response and for recovery of *M. mycoides* organisms. One hamster each from infected and control groups was sacrificed at 3, 7 and 15-days PI and tissues from genital (ovary, FT, uterus, vagina) as well as visceral organs (liver, lungs, heart, spleen, kidneys) were collected and examined for *M. mycoides* ss *mycoides* (LC) and histopathological changes.

RESULTS AND DISCUSSION

The cilia stopping effect (CSE) in hamster tracheal rings decreased from an average ciliary activity score of 265 at 0-day to 5 at 6-day PI in comparison to uninoculated control rings in which score decreased from an average of 275 at 0 day to 255 at 6-day PI (Fig 1). The results obtained with HTR organ culture revealed that the reduction in per cent ciliary activity in tracheal rings infected with *M. mycoides* ss *mycoides*, LC type (P113/87) was 98.1% at 6-day PI in comparison to 7.27% in uninfected control rings, which is indicative of its pathogenicity. Complete ciliostasis was observed in RFT explants infected with *M. mycoides* ss *mycoides* (LC) at 8-day PI in comparison to vigorous ciliary activity of uninfected explants up to 18 days, which also suggested the pathogenic nature of tested *M. mycoides* ss (LC) isolate. These observations were substantiated by recovery of *M. mycoides*

ss *mycoides* (LC) from infected but not from uninfected HTR and RFT explants along with significant histopathological lesions viz. denudation of lining epithelium, loss of cilia and mild infiltration of lymphocytes in lamina propria. Hamster tracheal ring and RFT explant organ cultures have been used previously to evaluate the pathogenic potential of *M. mycoides* ss *mycoides* (LC) of bovine origin (Kapoor *et al.*, 1993, Singh *et al.*, 1991, 1997), *M. canadense* from bovine mastitis (Garg *et al.*, 2004) and *M. equirhinis* from equine metritis (Garg *et al.*, 1991) describing similar CSE and histopathological changes. These observations also suggest that both HTR and RFT organ cultures seems equally sensitive to pathogenic determinants of *M. mycoides* ss *mycoides* (LC type).

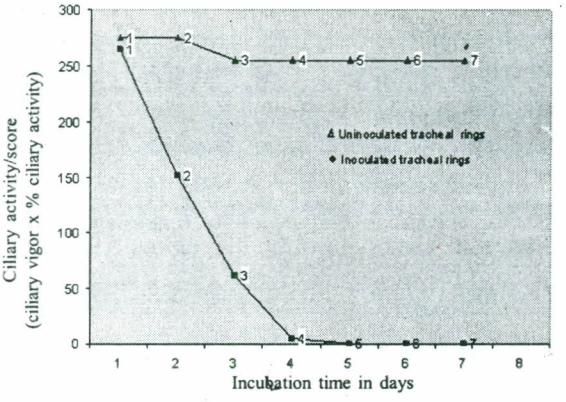

Fig 1. Cilia stopping effect (CSE) of *M. mycoides* ss *mycoides*, LC type (P-113/87) in hamster tracheal organ culture.

In rat mammary gland model, recovery of *M. mycoides* ss *mycoides* (LC) isolate and gross changes like mild to dark red area around the mammary gland on reflected skin, were observed up to 4-day PI in infected glands (L_3, L_4, L_5) but not in control glands (R_3, R_4, R_5). The main histopathological features included mild neutrophilic infiltration in alveolar lumen and infiltration of lymphocytes and macrophages in interalveolar tissues along with desquamation of alveolar cell lining and distortion of alveoli leading to narrowing of lumen due to fibroblastic cell proliferation in interstitium and hyperplasia of lining cells of alveolar duct which were noticed at 3 to 6-day PI. Similar histopathological changes in rat mammary gland have been reported earlier with *Mycoplasma* F-38 (*M. capripneumoniae*) and *M. mycoides* ss *capri* (Kumar, 1986) and *M. mycoides* ss *mycoides*, LC variant (Singh *et al.*, 1991, Kapoor *et al.*, 1997).

Genito-urinary challenge of female hamsters, resulted moderate neutrophillic response in vaginal smears up to 7-day PI along with recovery of *M. mycoides* ss *mycoides* (LC) from vagina up to 15-day PI in pure culture but not from the vagina of control group. The infected organisms could also be recovered from uterus, fallopian tube, ovary, heart- blood and pooled visceral organs. Histopathological changes encountered included degeneration and necrosis of ovarian tissues, mild lymphocytic and macrophagic infiltration along with mild fibroblastic hyperplasia in uterine and vaginal tissues. In addition, the hyperplasia of mesenchymal, Von-Kupffer's and reticular cells was observed in lungs, liver and spleen respectively. Studies pertaining to genito-urinary challenge of hamsters are scanty. However, the observations on recovery of infected organisms, cytological response in vaginal smear and histopathological changes are in agreement with earlier findings (Garg *et al.*, 1991).

The observation of *in-vitro* experimental infection of hamster tracheal rings and rabbit FT organ cultures as well as *in-vivo* inoculation in rat mammary gland and genitor-urinary challenge of female hamsters with *M. mycoides* ss *mycoides* (LC) isolated from synovial fluid of knee joint of a kid was strongly suggestive of its pathogenic role in producing kid arthritis.

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