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Ageing of the human oviduct: lectin histochemistry

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Summary. The aim of the present research was to investigate the changes of the sugar residues in the oviduct in the course of ageing in postmenopausal women vs normally menstruating women, by means of lectin histochemistry.

Twenty asymptomatic postmenopausal women (48-83 years old) were recruited among patients who underwent a vaginal hysterectomy. Eight normally menstruating women were recruited as controls. Fragments of Fallopian tubes (pars ampullaris) were fixed in 10% formalin and routinely processed. The sections were labelled with HRP-lectins (PNA, SBA, DBA, WGA, Con A, LTA, UEAI). Some sections were pre-treated with neuraminidase prior to staining with HRP-lectins.

Among the postmenopausal patients, our histochemical data showed that there was no difference in the localization and distribution of sugar residues of glycoconjugates as detected by various HRP-lectins. Moreover, our results demonstrated that the oviductal epithelium is characterized by apical reactivity in both ciliated and non-ciliated cells. In the course of ageing, the ciliated cells changed their morphology from bathyprismatic to large and rounded shape. ConA lectin reacted intensely with such highly degenerating ciliated cells and could be considered a marker of these cells. The degenerating ciliated cells are also characterized by the absence of sialic acid. In comparison with the sugar residues present in the control group, the oviductal epithelium of postmenopausal women is characterized by the loss of reactivity with DBA, WGA and ConA. Moreover, PNA reactive material was present at the free border of the ciliated and non-ciliated cells. The latter findings were statistically confirmed and could be considered strictly related to the ageing process.

Key words: Sugar residues, Lectins, Fallopian tubes, Postmenopause

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Introduction

The oviduct of many mammalian species (Jansen and Bajpai, 1982, 1983; Odor et al., 1983; Oliphant, 1984; Abe, 1996; Shirley and Reeder, 1996; Reeder and Shirley, 1999) including the humans (Amso et al., 1994; Donnez et al., 1985; Crow et al., 1994; Clyman, 1996) has been widely investigated from the morphological point of view. It is well known that in women the epithelial lining of the oviduct, which consists of four types of cells, ciliated, non-ciliated, peg and basal cells, is characterised by changes in response to oestrogen and progesterone fluctuations during the menstrual cycle. Changes in the size of ciliated cells and in the different secretory activity of the non ciliated cells were reported (Hafez, 1982). Sugar residues of glycoconjugates in the epithelial lining of the Fallopian tubes during the estrogenic and progesteronic phases of normally menstruating women have been studied by Schulte et al. (1985), Wu et al. (1993) and Kiss et al. (1998).

In the course of the postmenopause, the oviductal epithelium shows a decrease in the secretory function and a gradual atrophy of all cell types (Hafez, 1982). The oligosaccharide component of the glycoconjugates in the oviductal epithelium of one post-menopausal woman was investigated by Schulte et al. (1985).To date, no other data are available on the oligosaccharide content of glycoconjugates in post-menopausal women. The aim of the present study was to investigate changes of the sugar residues during ageing of the oviduct in post-menopausal women vs. normally menstruating women, by mean of lectin histochemistry.

Materials and methods

The study was carried out on 20 asymptomatic postmenopausal women (48-83 years old) who had a diagnosis of prolapsus uteri. These patients were admitted between December 1997 and July 1998 to the Department of Gynaecology and Obstetrics of the University of Florence for vaginal hysterectomy (Table 1). The indication for surgery was urinary incontinence or perineal discomfort. None of the patients had hormonal therapy for at least 5 years. Furthermore, none

was affected by diabetes or hypertension. Table 1 shows clinical profiles of the patients.

In addition, 8 normally menstruating women subjected to hysterectomy for benignant pathology were included in the present study. Based on menstrual records and endometrial histology 6 of these patients were in the follicular phase and 2 in the luteal phase at the time of study (Table 1).

Fragments were collected from the endometrium and tubes (pars ampullaris) immediately after removal of the uterus. The oviductal specimens were fixed in 10% formalin for at least 24 hours and embedded in paraffin.

This study was carried out with the approval of the Local Ethical Committee and consent to use the oviductal specimens for lectin histochemistry was obtained from all patients recruited.

Lectins histochemistry

5 μm thick paraffin sections were prepared for lectin histochemistry. After hydration, sections were treated with 0.3% hydrogen peroxide for 10 min (to inhibit the endogenous peroxidase), rinsed in distilled water and washed with 1% bovine serum albumin (BSA) (Murata et al., 1983) in 0.1M phosphate buffered saline (PBS) pH 7.2. The sections were then incubated for 30 min at room temperature in horseradish peroxidase-conjugated lectins (HRP-lectin conjugated) dissolved in phosphate buffered saline (0.1M PBS pH 7.2, 0.1M each of NaCl, 0.1 mM $CaCl_2$, $MgCl_2$ and $MnCl_2$) and then rinsed three times in PBS. The optimal concentration for each lectin (Sigma Chemical Co., St. Louis, MO) which allowed maximum staining with minimum background was as follows: PNA (*Arachis hypogaea*, binding specificity D-Gal (ß1→3)-D-GalNAc) 25 μg/ml, SBA (*Glycine max* binding specificity α/ß-D-GalNAcD-Gal) 20 μg/ml, DBA (*Dolichos biflorus*, binding specificity α-D-GalNAc) 25 μg/ml, WGA (*Triticum vulgare* binding specificity $(\alpha\text{-D-GlcNAc})_n$ and sialic acid) 20 μg/ml, ConA (*Canavalia ensiformis* binding specificity α-D-Man) 50 μg/ml, LTA (*Lotus tetragonolobus* binding specificity α-L-fucose) 25 μg/ml and UEA I (*Ulex europaeus* binding specificity α-L-fucose) 25 μg/ml. Staining of the sites D-Gal (ß1→3)-D-GalNAc containing bound lectin-HRP was obtained by incubating the slides with PBS (pH 7.0), containing 3,3'diaminobenzidine (DAB) (25 mg/100 ml) and 0.003% hydrogen peroxide, for 10 min at room temperature. Specimens were rinsed in distilled water; dehydrated using graded ethanol solutions, cleared in xylene and mounted in Permount. Controls for lectin staining included: 1) substitution of unconjugated lectins for lectin-HRP conjugates; 2) exposure to HRP and substrate medium without lectin; 3) oxidation with 1% periodic acid for 10 min prior to lectin staining; 4) exposure of sections to 10-20 μg/ml of each lectin-HRP

Table 1. Clinical profiles of the patients.

BIOPSY No.	AGE	BLOOD GROUP	NUMBER OF PREGNANCIES	ENDOMETRIAL DATING
Fertile women				
97/17830	50	B+	2	Early proliferative
97/20029	47	0-	2	Early proliferative
97/17266	49	0+	1	Late secretive
97/15067	55	0+	1	Late secretive
97/18856	44	B+	2	Late secretive
97/19913	52	0-	-	Middle secretive
97/19912	47	A+	1	Late secretive
97/21298	49	0+	1	Late secretive
Women in postmenopause				
97/12931	69	0+	4	21
97/13163	66	A+	2	13
97/16355	63	0+	2	23
97/16782	66	A+	3	26
97/12939	58	0-	2	2
97/15069	74	A+	3	25
97/15490	59	0+	2	6
97/16678	72	A-	2	20
97/16677	48	0+	5	1
97/21299	62	A-	2	9
97/21661	57	0+	3	5
97/21945	52	0+	2	1
97/16866	63	A-	2	14
97/19216	57	B+	3	4 months
97/17091	68	0-	2	24
97/16060	59	0+	2	5
97/18627	61	A+	1	13
97/20187	83	A+	2	28
97/13074	75	A+	2	23
97/25595	49	A-	-	6 months

conjugate containing 0.1M D-galactose, D-glucose, D-mannose, L-fucose, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine and methyl-α -manno-pyranoside.

Sialidase digestion

In some experiments sialic acid was removed by pre-treating the sections for 18 hr at 37 °C in a solution of sodium acetate buffer 0.25M, pH 5.5, containing 0.1 unit/ml sialidase (neuraminidase Type X from *Clostridium perfrigens* (Sigma Chemical. Co., St. Louis, MO)), 5.0 mM $CaCl_2$ and 154 mM NaCl, prior to staining with lectin-HRP conjugates. Controls containing the sialidase buffer without the enzyme were also prepared.

Statistical evaluation

The statistical significance of the differences between the number of subjects reactive with the lectins in the two groups of women (normally menstruating vs postmenopausal) was evaluated using the Fisher's exact test. Differences between the two groups were considered if $p<0.05$.

Results

The epithelium of the pars ampullaris of the oviduct in the postmenopausal women showed a decrease in number of ciliated cells, which often appeared in clusters. No secretory activity was observed in postmenopausal oviductal epithelium. Especially in late postmenopause, some clusters of ciliated giant cells were observed.

Control group (Table 2)

Lectin histochemistry showed in general no differences in glycoconjugate content of the epithelial cells during the estrogenic phase in comparison to the progesteronic one. For this reason the following results are representative of the lectin staining of the oviducts in 8 fertile women, irrespective of the menstrual phase.

DBA

This lectin reacted with the apical portion of the ciliated and non-ciliated cells (1 subject). Only 2 patients showed lectin binding at the cilia of the ciliated cells. One subject belonging to the blood group A showed

Table 2. Fertile subjects (8 women).

	DBA		SBA		WGA		ConA		PNA		LTA		UEA-I	
	Cil	Ncil	Cil	Ncil	Cil	Ncil	Cil	Ncil	Cil	Ncil	Cil	Ncil	Cil	Ncil
Apical portion	1		6	8	8	8	**8**			2	5	4	6	6
Cilia	2		7		8$^-$		7		7$^+$		2		5	
Supranuclear cytoplasm			1	1	1	1	7	7			1	3	4	5
Cytoplasm	**5**	**5**												
Golgi Region					**7**	**7**	1				2	1		1

Cil: ciliated cells; Ncil: non-ciliated cells; $^+$: increase of reactivity after neuraminidase digestion; $^-$: decrease of reactivity after neuraminidase digestion. In bold and underlined: Fisher's exact Test revealed a significative difference ($p< 0.05$) vs the post-menopausal subjects.

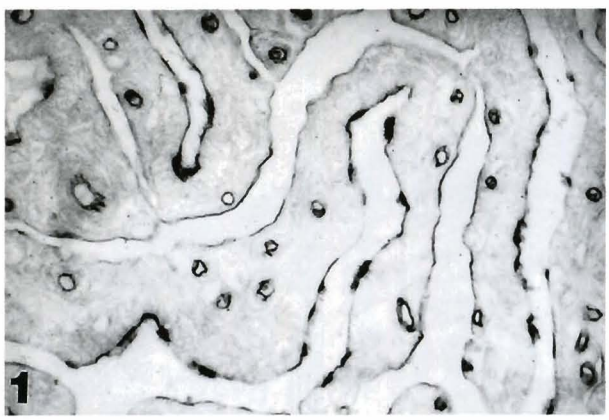

Fig. 1. DBA-HRP. 66 years old woman. Postmenopause. The apical portion of the ciliated and non ciliated cells, the cilia of the ciliated cells of the oviductal lining epithelium and the vascular endothelium reacts with DBA. x 360

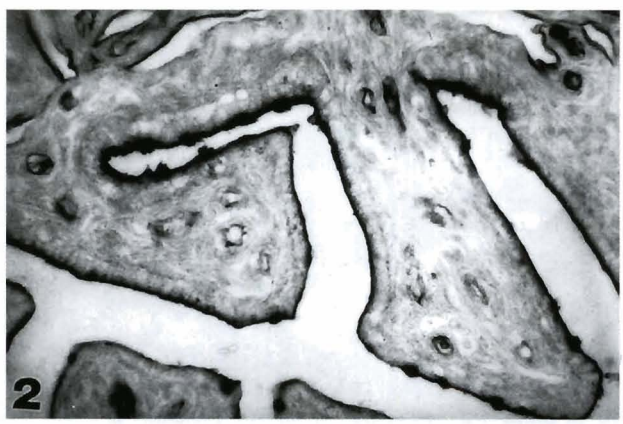

Fig. 2. SBA-HRP. 75 years old woman. Postmenopause. The apical portion of all the cells of the oviductal lining epithelium intensely reacts with SBA; the vascular endothelium is also reactive. x 360

reactivity in the vascular endothelium.

SBA

SBA reacted with the apical portion of the ciliated cells (6 patients) and of non-ciliated cells (8 patients) and with the cilia of the ciliated cells (7 patients). Only 1 patient, the only one in the middle secretory phase of the menstrual cycle, showed lectin reactivity in the supranuclear cytoplasm of ciliated and non-ciliated cells. All the patients showed reactivity at the vascular endothelium.

WGA

The apical portion and the cilia of the ciliated cells and the apical portion of the non-ciliated cells reacted with WGA in all the examined patients. Only 1 patient showed lectin reactivity at the supranuclear cytoplasm of ciliated and non-ciliated cells. In 8 patients the cilia of the ciliated cells showed reactivity with this lectin. The Golgi region of the ciliated and non-ciliated cells reacted with WGA in 7 patients. (Fig. 3) After neuraminidase digestion a decrease in reactivity in the cilia of the ciliated cells was observed.

ConA

The apical portion (8 patients) and the cilia (7 patients) of the ciliated cells and supranuclear cytoplasm of the ciliated and non ciliated cells reacted with Con A (Fig. 5). One patient revealed lectin binding at the Golgi region of the ciliated cells. The Golgi region of the ciliated cells of the other subjects did not react with ConA.

PNA

This lectin reacted with the apical portion of the

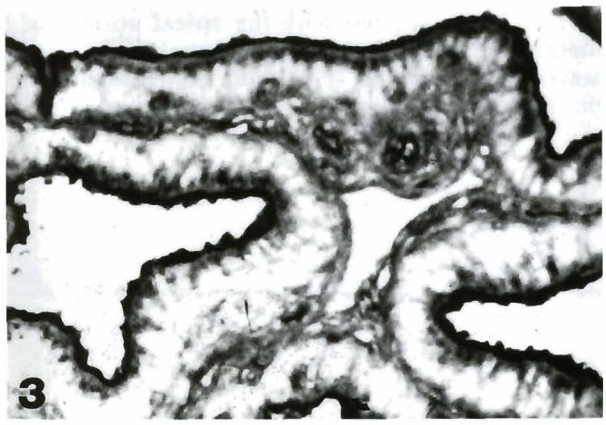

Fig. 3. WGA-HRP. 49 years old woman. Endometrial dating: late secretive. The apical portion and the Golgi region of all the oviductal epithelial cells reacts with WGA. x 900

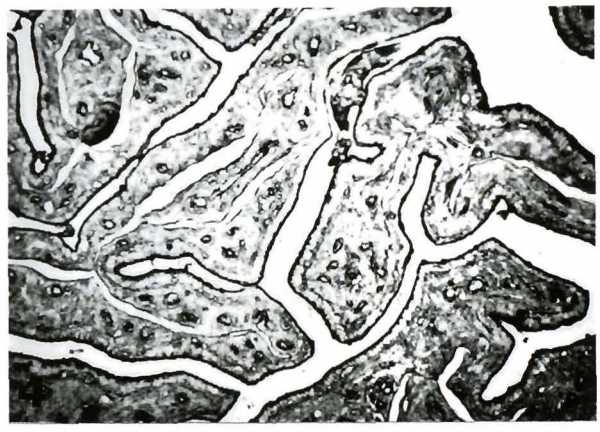

Fig. 4. WGA-HRP. 75 years old woman. Postmenopause. The apical portion of all the cells of the oviductal lining epithelium and the vascular endothelium intensely reacts with this lectin. x 180

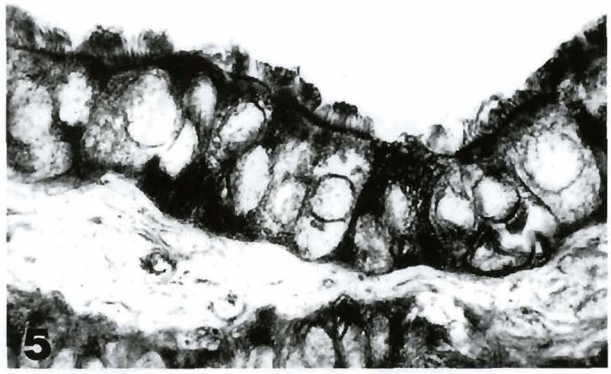

Fig. 5. ConA-HRP. 49 years old woman. Endometrial dating: late secretive. The apical portion and the cilia of the ciliated cells of the oviductal lining epithelium reacts with Con A. Within the ciliated and non ciliated cells the supranuclear cytoplasm intensely reacts with this lectin. x 900

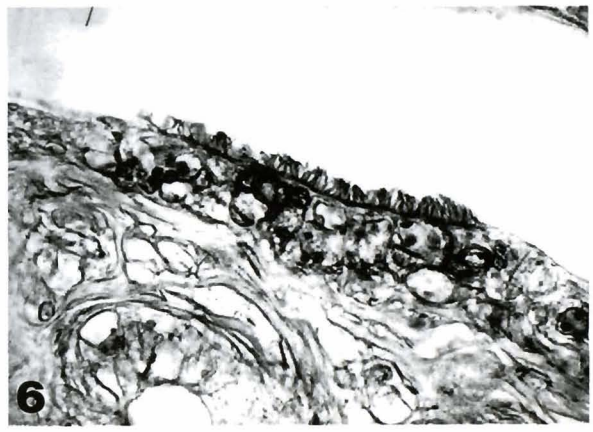

Fig. 6. ConA- HRP. 58 years old woman. Postmenopause. The apical portion, the cilia and large cytoplasmic granules of the degenerating cells of the oviductal lining epithelium intensely reacts with Con A. x 900

non-ciliated cells in 2 patients. In 7 patients the cilia of the ciliated cells reacted with PNA. 2 patients showed reactivity at the vascular endothelium. After neuraminidase digestion an increase in reactivity in the cilia of the ciliated cells was observed.

LTA

LTA reacted with the apical portion of the ciliated cells (5 patients) and of the non-ciliated cells (4 patients). In 2 patients reactivity at the cilia of the ciliated cells was observed. Supranuclear cytoplasm was seen to react at the ciliated (1 patient) and non-ciliated cells (3 patients). The Golgi region reacted with LTA at the ciliated (2 patients) and non-ciliated cells (1 patient). 5 patients showed reactivity at the vascular endothelium.

UEAI

6 patients showed lectin reactivity at the apical portion of the ciliated and non-ciliated cells. The cilia of the ciliated cells were seen to react with UEAI in 5 patients. Supranuclear cytoplasm of the ciliated (4 patients) and non-ciliated (5 patients) showed UEAI reactivity. Only in 1 subject the Golgi region of the non-ciliated cells reacted with UEAI. All the patients showed reactivity in the vascular endothelium and in the stromal cells.

Postmenopausal subjects (Table 3)

DBA

In 10 subjects (all belonging to blood group A) the apical portion of the ciliated and non-ciliated cells showed reactivity with DBA. In 2 cases the cilia of the ciliated cells reacted with the lectin (Fig.1) and in 2 subjects the cytoplasm of the ciliated and non-ciliated cells reacted with DBA. 8 patients showed reactivity in the vascular endothelium

SBA

SBA reactivity at the apical portion of the ciliated and non-ciliated cells was observed in 19 subjects (Fig. 2). The cilia of the ciliated cells reacted with SBA in 12 patients. 1 patient showed SBA reactivity in the cytoplasm of the non-ciliated cells. All the patients showed reactivity at the vascular endothelium

Table 3. Postmenopausal subjects (20 women).

	DBA		SBA		WGA		ConA		PNA		LTA		UEA-I	
	Cil	Ncil	Cil	Ncil	Cil	Ncil	Cil	Ncil	Cil	Ncil	Cil	Ncil	Cil	Ncil
Apical portion	10	10	19	19	20	20	11	14	**14±**	**14±**	9	9	16	16
Cilia	2		12		13⁻		11		9⁺		5		5	
Supranuclear cytoplasm					6	3	4	1		2	1	1		
Cytoplasm	2	2		1									1	1
Golgi Region					1	2								

Cil: ciliated cells; Ncil: non-ciliated cells; ⁺: increase of reactivity after neuraminidase digestion; ⁻: decrease of reactivity after neuraminidase digestion. In bold and underlined: Fisher's exact Test revealed a significative difference ($p < 0.05$) vs the post-menopausal subjects.

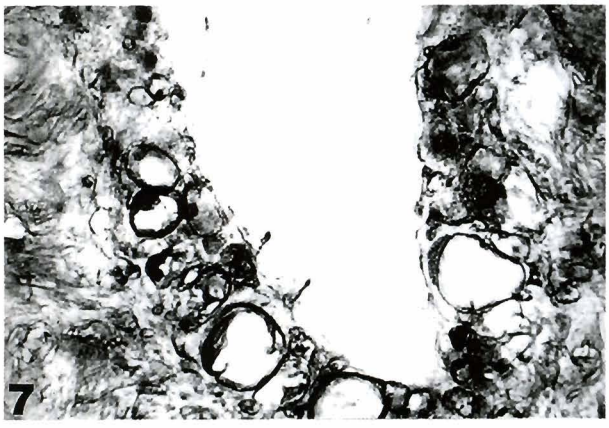

Fig. 7. Con A-HRP 61 years old woman. Postmenopause. The disappearance of the degenerating oviductal epithelial cells probably gives results in the presence of rounded empty spaces whose border is strongly reactive with ConA. x 900

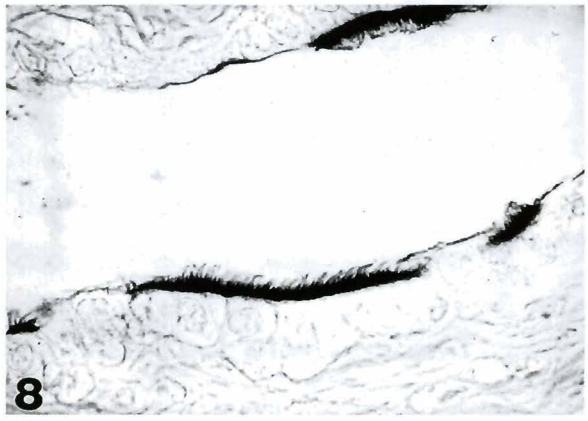

Fig. 8. PNA-HRP. 58 years old woman. Postmenopause. The apical portion of all the epithelial cells and the cilia of the ciliated cells of the oviduct reacts with PNA. x 900

WGA

All the patient showed WGA reactivity at the apical portion of the ciliated and non-ciliated cells (Fig. 4). WGA reactivity was observed at the cilia of the ciliated cells in 13 subjects and reactive supranuclear cytoplasm was seen at the ciliated cells in 6 patients and in the non-ciliated cells in 3 patients. WGA reactivity at the Golgi region was observed in the ciliated cells in 1 patient and in the non-ciliated cells in 2 subjects. After neuraminidase digestion a decrease in reactivity in the cilia of the ciliated cells was observed.

Con A

11 subjects showed Con A reactivity in the apical portion of the ciliated cells and 14 patients in the non-ciliated cells. The cilia of the ciliated cells reacted in 11 subjects. Reactive supranuclear cytoplasm was seen in the ciliated cells in 4 patients and in the non-ciliated cells in 1 patient. The ciliated cells characterized by Con A reactive granules appeared highly degenerating and arranged in clusters (Fig. 6). The detachment of the clusters of degenerating ciliated cells gave rise to large empty spaces whose borderline was strongly reactive with this lectin (Fig. 7).

PNA

PNA reactivity was observed at the apical portion of the ciliated and non-ciliated cells in 14 patients (Fig. 8). 9 patients showed reactivity over the cilia of the ciliated cells and 2 patients in the supranuclear cytoplasm of non-ciliated cells. After neuraminidase treatment, an increase of reactivity in the free border and in the cilia of the oviductal epithelial cells was seen, while a lack of reactivity in the degenerating ciliated cells was detected (Fig. 9).

LTA

LTA reactivity was seen at the apical portion of ciliated and non-ciliated cells in 9 patients. 5 subjects showed LTA reactivity over the cilia of the ciliated cells and 1 patient in the granules of ciliated and non-ciliated cells. 10 patients showed reactivity in the vascular endothelium.

UEAI

In 16 patients, the apical portion of the ciliated and non-ciliated cells showed reactivity with UEAI. In 5 patients the cilia of the ciliated cells reacted with the lectin and diffuse cytoplasmic reactivity of the ciliated and non-ciliated cells was observed in 1 subject (Fig. 10). All the patients showed reactivity in the vascular endothelium

Statistical evaluation

The statistical significance of the differences in reactivity with the lectins between the number of subjects in the 2 groups of women (normally menstruating vs. postmenopausal women) is reported in table 2 and 3 and is indicated by number in bold and underlined.

Discussion

Although a large part of the biological roles of the glycoconjugate oligosaccharides remains to be elucidated, their importance in some biological processes such as those related to the development, the functional maturation and the maintaining of the

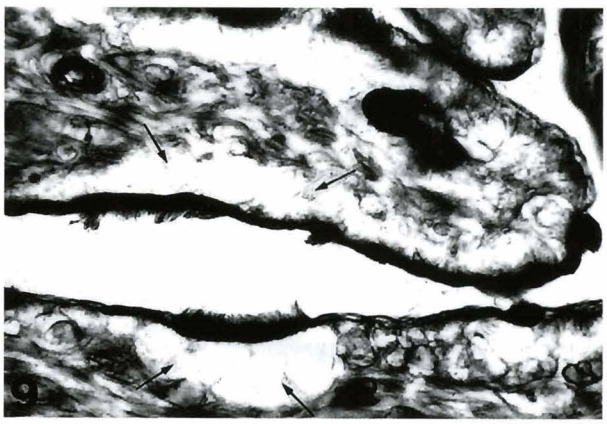

Fig. 9. Neuraminidase-PNA-HRP. 58 years old woman. Postmenopause. After neuraminidase treatment an increase in reactivity is observable at the apical portion of the oviductal epithelial cells and at the cilia of the ciliated cells. Lack of reactivity is detectable at the cytoplasm and at the baso-lateral membrane of the degenerating ciliated cells arranged in clusters (arrows) x 900

Fig. 10. UEAI-HRP. 63 years old woman. Postmenopause. The apical portion of all the oviductal epithelial cells, the cilia of the ciliated cells reacts with UEAI. In this case also the cytoplasm of the epithelial cells is reactive. x 900

integrity of some structure seem to be sufficiently ascertained (Damjanov, 1987; Varki, 1993). Surprisingly the role played by the glycoconjugate oligosaccharides during the ageing processes has been neglected.

Our lectin histochemical study has allowed the drawing of a basic picture of the oligosaccharidic distribution in postmenopausal human oviducts.

In postmenopausal subjects, a broad range in reactivity to any given lectin was observed in ciliated and non ciliated secretory cells. We have tried to correlate this individual variability with the age and with the years of menopause of the patients. This attempt gave negative results. We believe that such differences among the subjects might be due to individual characteristics, which remain to be determined.

Even in absence of hormonal stimulation, the epithelial lining of postmenopausal oviduct was characterised by a large amount of sugar residues; this abundance of oligosaccharides was also found at the epithelial lining of postmenopausal endometria (Gheri et al., 1996). From a morphological point of view, the postmenopausal oviductal epithelium showed signs of degeneration, characterized by various degrees of atrophy. In fact, the oviductal epithelium during early menopause, appears quite similar to that of the postmenstrual and premenstrual phases; afterwards a remarkable deciliation and little evidence of secretion were observed (Hafez, 1982). As far as the oligosaccharidic content of glycoconjugates and their distribution are concerned, our histochemical data demonstrated that the degree of degeneration of the oviductal epithelium is independent from the age of the patient and from the time of the last menstrual cycle. In fact, the same lectin reactivity was observed in early postmenopause and in late postmenopause. The oviductal epithelium of the postmenopausal patients also appeared characterized by clusters of ciliated cells. The extension of these clusters seemed to be independent from the age of the patients. It is to be noted that our histochemical data have shown that tracts of oviductal epithelium were characterized by apical lectin reactivity at the ciliated and non-ciliated cells. It is difficult to ascertain the role of glycoconjugate sugar residues at the apical portion of the oviductal epithelial cells. It is possible that glycoconjugates present at this site could cooperate in maintaining cellular polarity, as reported also for other organs (Damjanov, 1987; Gheri Bryk et al., 1994).

Ciliated cells, in the course of ageing, tend to modify their shape from bathyprismatic to large and rounded cells. Con A lectin intensely reacted with these highly degenerating ciliated epithelial cells, often arranged in clusters. The detachment of clusters of degenerating ciliated cells from the basal lamina probably gave rise to rounded empty spaces, whose borderline was strongly reactive with Con A. Con A lectin could be considered a marker of ciliated degenerating oviductal cells, thus revealing the presence of a large amount of glycoconjugates rich in terminal α-D-mannosyl residues. Absence of sialic acid was observed at the level of the cytoplasm and of the baso-lateral surface of these cells. The absence of sialic acid at the baso-lateral surface of the degenerating ciliated cells could explain the loss of cell to cell adhesion (Varki, 1993). The presence of sialic acid at the cilia of the degenerating ciliated cells suggests the preservation of their functional activity; as suggested by Schulte and Spicer (1985) and Ito et al. (1990) sialic acid keeps the cilia separated from one another in order to maintain the ciliary motility.

DBA reactivity detected at the ciliated and non-ciliated cells seems to be strictly related to the blood group A of the patients. This is due to the fact that the patients are secretors and express the blood group antigens also in the glycoprotein of the tissue secretions. In comparison with the sugar residues present in the control group, the oviductal epithelium of post-menopausal women were characterized by the loss of reactivity for DBA, WGA and Con A at the level of cellular compartments and/or organelles. This finding has been confirmed from a statistical point of view. The loss of terminal α-D-galactosamine at the cytoplasm, D-glucosamine at the Golgi region and α-D-mannose at the apical portion of the ciliated cells of the lining epithelium could be considered a consequence and/or a cause of the ageing process. In particular the lack of reactivity at the Golgi region, which was a constant finding in the oviductal epithelium of the control group, seems to indicate a loss or a faulty in the activity of the cytoplasmic organelle which is specialized to the assembly of the glycoproteic chains. During the ageing process, the apical portion of the ciliated and non-ciliated cells appeared characterized by reactivity with PNA, thus revealing the presence of D-galactose (ß1→3)-N-acetyl-D-galactosamine at these sites. The presence of PNA reactive material at the above mentioned site could be considered a typical feature in the ageing of the oviductal epithelium, as also demonstrated from a statistical point of view.

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