SIALIC ACID AND SIALIDASE ACTIVITY IN HUMAN ENDOMETRIAL TISSUE, UTERINE FLUID AND PLASMA UNDER DIFFERENT CONDITIONS OF UTERINE DYSFUNCTION

By

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ABSTRACT

Sialic acid content in endometrial tissue and sialidase activity in the endometrium, uterine fluid and plasma has been measured in normal and in conditions of uterine dysfunctions. It has been observed that an antagonistic effect exists between endometrial sialidase and sialic acid content, but in all the cases of uterine dysfunctions, the sialidase activity in uterine fluid and plasma decreases, whereas it increases during pregnancy.

Sialic acid is an important constituent of mucopolysaccharides and mucoproteins. In endometrial tissue, sialic acid plays an important role by acting as a hormone receptor site as well as by regulating the secretory processes and movement of sperm (Soupart & Clewe 1965; Glick & Githens 1965; Glick et al. 1966; Carlborg & Gemzell 1969). Tissue content of sialic acid is mostly regulated by the enzyme sialidase or neuroaminidase (E. C. 3.2.1.18) which regulates the hydrolytic degradation of glycoproteins, mucopolysaccharides and sialolipids. It has been reported that tissue sialidase is responsible for the turnover of both tissue and plasma glycoproteins in the body (Srivastava et al. 1970; Price & Ashwell 1971). It has also been reported that tissue sialidase activity might regulate the activity of certain glycoprotein hormones like FSH, LH, erythropoietin etc. (Mahadavan
Plasma sialidase is a good index of tissue sialidase activity. In endometrial tissue, sialidase might participate in the regulation of tissue sialic acid content and thereby may regulate the secretory activity and other steps related to implantation. In the present communication attempts have been made to correlate the sialic acid content of endometrial tissue and sialidase activity of endometrial tissue, uterine fluid and plasma obtained from normally cyclic, pregnant and lactating women, and from cases with disturbed endometrial differentiation due to the use of copper – IUD’s and contraceptive steroids.

METHODS AND MATERIALS

Twenty normal subjects, chosen for control endometrial biopsy were in the age group of 20–30 years and had normal menstrual cycles at intervals of 28 ± 2 days. Five women bearing IUD’s for at least six months, 8 women using the long-acting steroid preparation Floraconta® (dihydroprogesterone and oestradiol-17-enanthate in 100:5 ratio) supplied by I.C.M.R., New Delhi, and 6 women with lactational amenorrhoea of 4–8 months duration were studied. Early pregnancy cases were chosen (mostly within 3–8 weeks of pregnancy) from the Medical Termination of Pregnancy Unit of S.S.K.M. Hospital, Calcutta. The diagnosis of pregnancy was confirmed by the Pregnosticon Drip-dot test (Organon Inc.).

Endometrial biopsy specimens after collection were immediately put into normal saline and kept on ice. The uterine fluid was collected by washing the uterine cavity with cold sterile normal saline. Venous blood was collected from each patient and mixed with an anticoagulant mixture of 2% EDTA in normal saline.

The biopsy specimen was washed with normal saline, homogenised in saline and centrifuged at 3500 x g for 10 min in the cold. The supernatant was used for the enzyme assay. The uterine fluid was centrifuged at 2000 x g so as to make it free from contaminating blood. Anticoagulated blood was centrifuged and the plasma was collected. All these operations were made within 2 h of collection of the specimens at a temperature between 2–4°C.

For determination of sialic acid content a part of homogenate was boiled with 10% perchloric acid, centrifuged and along with the supernatant sialic acid was determined according to the method of Warren (1959). The tissue content of sialic acid was expressed as micromoles per milligram of protein. Protein was determined according to the method of Lowry et al. (1951). Sialidase activity was assayed according to the method of Mahadavan & Tappel (1967) using ovomucoid as the substrate and the activity was expressed as micromoles of sialic acid hydrolysed per gram of protein per 3 h of incubation.

RESULTS

Fig. 1 indicates the cyclical variation of sialic acid content and sialidase activity during the oestrogenic and progestogenic phase of the endometrium and in early pregnancy. It will be evident that the sialic acid content gradually increases from the early proliferative phase and reaches a maximum value during the late
secretory phase. On the contrary, in early pregnancy the sialic acid content of the endometrium is very low. With regard to the sialidase activity it is found that a gradual increase from the early proliferative phase endometrium to the late secretory endometrium takes place. A similar pattern is found in the uterine fluid which constitutes endometrial secretion. In plasma, sialidase activity follows a linear increase during the oestrogenic and progestogenic phases and reaches a maximum value during the progestogenic phase.

Fig. 2 indicates the sialic acid content and sialidase activity in different types of uterine dysfunction. Here we find the sialic acid content of the endometrium is very high in case of chronic steroidal contraceptive users, copper – IUD users and lactational amenorrhoeic subjects. In all cases with impaired endometrial function brought about by the use of contraceptive steroids and copper – IUD's or by lactational amenorrhoea, sialidase activity was found to be much lower than during the normal luteal phase of the cycle. Such a drop in activity is not only found in endometrial tissue and uterine fluid indicating diminished secretory activity, but also in plasma. On the other hand, in plasma samples obtained during early pregnancy, we find a very large increase in plasma sialidase activity as is evident in Fig. 1.

Sialic acid content and sialidase activity of human endometrium, uterine fluid and blood plasma during the early proliferative phase, ovulatory phase, late secretory phase and in early pregnancy. S = Sialic acid content of the endometrium. T = Sialidase activity of endometrial tissue, F = Sialidase activity of uterine fluid, B = Sialidase activity of blood plasma. Each bar represents the mean (± sd) values based on the determinations of 5–8 separate preparations.

Fig. 1.

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Sialic acid content and sialidase activity of human endometrium, uterine fluid and blood plasma under different conditions of uterine dysfunction as indicated in the figure. S = Sialic acid content of the endometrium, T = Sialidase activity of endometrium tissue, F = Sialidase activity of uterine fluid, B = Sialidase activity of blood plasma. Each bar represents the mean (± sd) values based on the determinations of 5–8 separate determinations.

**DISCUSSION**

The data presented in this communication seem to indicate an endocrine regulation of sialidase and sialic acid content of endometrial tissue. Sialidase is a lysosomal enzyme (Mahadavan et al. 1967). It is well known that the release of lysosomal enzymes are favoured by hydrophilic steroids which are known to cause lysosomal leakage by interacting with the lysosomal membrane (Bangham et al. 1965). From the chemical structure of oestrogen and progesterone it appears that progesterone is more hydrophilic than oestrogen. This perhaps explains the enhanced sialidase activity in the endometrial tissue as also in uterine fluid during the progestogenic phase. This idea is further supported by the fact that during early pregnancy when the endometrium is predominantly progestogenic, the sialidase activity is much higher. In lactational amenorrhoea both the steroid secretion by the ovary and the release of sialidase are low. This could explain the reduced sialidase activity in both tissue and fluid. On the basis of chemical
structure it appears that the steroidal components of Floraconta® are more hydrophobic than their natural counterparts; as a result they probably stabilize the lysosomal membrane (Bangham et al. 1965). This may explain the diminished sialidase activity in the tissue and fluid of subjects using steroidal contraceptive. Kontula et al. (1974) reported that the metal releasing IUD’s makes the endometrium infertile by preventing ovarian steroids to bind with the hormone receptor proteins. This could explain the diminished sialidase activity in tissue and fluid in copper – IUD fitted cases.

From Fig. 1 it is evident that plasma sialidase is also under the endocrine control of ovarian steroids. Although no definite explanation can be given for such a variation in plasma, it appears that the enhanced sialidase activity in the progestogenic phase is due to increased washing out of the tissue sialidase into the systemic circulation. The diminished plasma sialidase activity in the above mentioned cases of uterine dysfunction is probably due to a reduced washing out of tissue sialidase. The probable explanation of this observation is a reduced turnover of tissue and plasma glycoproteins and an altered regulatory role of sialoprotein hormones like FSH, LH, HCG, erythropoietin (Mahadavan et al. 1967; Vaitukaitis & Ross 1972) and also a decreased half life in the plasma.

The data presented in this communication indicate, in general, the existence of an antagonistic relationship between sialic acid content and sialidase activity in almost all the conditions studied, although in amenorrhoeic conditions, the comparative decrease in sialidase activity is not so marked (Fig. 2). In the normal endometrium the higher sialic acid content during the progestogenic phase of the cycle as compared to the oestrogenic phase is perhaps due to enhanced tissue sialoprotein synthesis during this phase of the cycle. The rather low sialic acid content of the early pregnancy endometrium is also due to much higher sialidase activity which splits sialoproteins and thereby reduces the tissue sialic acid content. Diminished sialidase activity in the tissue leads to accumulation of sialic acid – this probably explains the elevated sialic acid content under different conditions of uterine dysfunction. It appears from this study that the endocrine control over tissue sialic acid level is probably mediated by sialidase activity. Similar endocrine control over skin sialic acid level has been reported by Rabbiosi & Gionetti (1969).

The endocrine regulation of endometrial sialic acid level may perhaps explain the mechanism of regulation of endometrial secretion by ovarian steroids. It is also evident from Fig. 2 that elevated sialic acid level in endometrial tissue leads to diminished activity of sialidase in uterine fluid. Altered membrane polarity caused by the accumulation of sialic acid may perhaps lead to altered permeability of proteins particularly sialidase by which its secretion is depressed (Glick & Githens 1965; Glick et al. 1966; Cautrecasas & Illiano 1971). Thus sialoproteins could participate in the hormone mediated regulation of endometrial function.
ACKNOWLEDGMENTS

The authors would like to express their thanks to Dr. S. K. Banerjee, Associate Professor, Department of Obstetrics and Gynaecology, S. S. K. M. Hospital, Calcutta and Dr. Runu Mukherjee for their cooperation in collecting the clinical materials from the Out-patient Department of S. S. K. M. Hospital, Calcutta. Thanks are also due to WHO for their help.

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Received on April 17th, 1975.