Morphological and morphometric changes in the cervix uteri of the rat at term pregnancy induced by hyaluronidase

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Summary

Intracervix injection of hyaluronidase during pregnancy has been proposed to accelerate cervix ripening. We evaluated the morphological and morphometric changes of the uterine cervix of pregnant rats, caused by the action of this enzyme. Ten female rats were equally divided between an experimental group (G II) and a control group (G I). On the 20th day of pregnancy, under light microscopy, a greater thinning of the superficial muciferous epithelium, with *lamina propria* rich in blood vessels and in eosinophils was found in G II. The histometric count of G II showed a smaller number of collagen fibers (average 248 vs 552 in the control group) and a greater concentration of eosinophils (average 18.20 vs 9.20 in the control group). The Student's t-test showed a significant difference in collagen fibers ($p < 0.0001$) and in eosinophils ($p < 0.0007$). The action of this enzyme caused a predominance of flaccid connective tissue, a lower concentration of collagen fibers and an increased concentration of eosinophils, confirming its utilization in cervix ripening.

Key words: Labor; Cervix ripening; Hyaluronidase; Collagen.

Introduction

The thinning and dilation of the uterine cervix at the end of pregnancy is not merely the result of uterine contractions, but of other complex processes as well [1]; the action of hyaluronidase seems to be advantageous because the uterine cervix under normal conditions has around 8.5% of connective tissue [2].

This enzyme may contribute to a decrease in the number of C-sections especially in Brazil where the number of these surgical interventions is above the maximum desirable level of 15%, as prescribed by the World Health Organization [3]. Due to the difficulty in choosing the ideal method for the induction of labor this enzyme has been used to facilitate cervix ripening. However, there is a lack of histological results to corroborate the fact that the recommended dose really causes significant structural changes. The dose, mode and time of administration have been designed empirically.

The use of hyaluronidase in cervix ripening has been sporadically used since the 1950s at doses ranging between 200 and 1,000 units with variable results in cervix ripening and decrease of parturition time [4-10]. Doses ranging between 1,000 and 2,000 units of intracervix hyaluronidase brought about good results in cervix ripening, decreased parturition time and increased incidence of vaginal deliveries [11-14].

Spallicci, in a randomized clinical trial of term pregnancies, used a dose of 20,000 units of hyaluronidase and concluded that the time between the first dose and the beginning of labor for individuals receiving such dose was on average 44 hours (vs 60 hours for the control group) and 54 hours when a boost was given after 48 hours (vs 94 hours for the control group). Induction time was on average 14 hours less when compared to the control group [15].

In the same study, for those patients who had received hyaluronidase, the labour time was on average seven hours for nulliparous women (13 hours for the placebo group) and five hours for primiparous and multiparous women (vs 10 hours for the placebo group). The incidence of vaginal deliveries was higher in the group of women receiving hyaluronidase (83%) than in the placebo group (51%) [15].

Surita, comparing the use of hyaluronidase and Foley probe in the cervix preparation in pregnancies with indication for labour induction, concluded that both methods accelerate cervix ripening without significant changes in perinatal results, undesirable effects and complications. In the Foley probe group, labour has been triggered more rapidly with a lower induction time and oxytoxic dosage and higher occurrence of vaginal deliveries [16]. However, Kavanagh and co-workers concluded that there were no conclusive clinical trials for the use of hyaluronidase as a method of cervix preparation during pregnancy [2].

The goal of the present study was to evaluate, under light microscopy, the morphological and morphometric changes of collagen fibers and eosinophils in the uterine cervix of albino rats, caused by the local administration of hyaluronidase at the end of term pregnancy.

Material and Methods

Animals and treatment

We used albino rats (*Rattus norvegicus albinus*, Rodentia, Mammalia), at about 90 days of age, virgin and weighing

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approximately 200 g of the EPM-Wistar strain. Each male mated with three female rats, and the beginning of pregnancy was determined according to the Hamilton Wolfe technique (considered as day 0 - pregnancy day zero) [17].

Ten female rats with a positive pregnancy test were randomly distributed in two numerically equivalent groups: control group (G I) – female rats that received 1 ml of distilled water at a single dose on the 18th day of pregnancy administered to the cervix; trial group (G II) – rats that received under the same conditions as the control group a single dose of 0.02 ml of hyaluronidase diluted in 0.98 ml of distilled water (total 1 ml) on the 18th day of pregnancy through the intracervix route.

In the preparation of the enzyme given to G II, the vial with lyophilized powder was diluted in 4 ml of distilled water, with rotating movements in order to avoid frost formation and to facilitate dilution of the drug. The recommended dose for the rats was proportional to that established for human beings, and equivalent to 20,000 units [4].

Disposable syringes (1 ml) sterilized with ethylene oxide were used for the injections. The doses were given under anesthesia (ketamine + xylazine 0.1 mg/kg) with the help of a plastic guide in the form of a cone, introduced through the whole extension of the rat vagina, to guide the course of the needle (an ethylene oxide sterilized disposable needle of the "pencil tip" type). The needle was introduced into the guide and the insertion limit was defined by the local barrier sensation. Next, a light pressure was carried out with the needle on the area, traversing about 2-3 mm (uterine cervix) and 1 ml solution was injected; the dose was injected slowly (about 5 sec), keeping the needle in place for about 30 seconds in order to avoid reflux.

On the 20th day of pregnancy the rats were anesthetized again (ketamine + xylazine 0.1 mg/kg) at 9:00 a.m. The rats were immediately shaved in the abdominal region and vaginal entrance. After the abdomen had been opened, disjointing of the pubic symphysis was performed in order to facilitate the longitudinal opening of the vagina in its whole extension, thus exposing the cervix.

After careful observation of the pelvic organs, mainly the uterine horns, we proceeded with the dissection and removal of the uterine cervix. The samples were immediately placed in a 10% formaldehyde solution for fixation and left for 24 hours; next, they were dehydrated in increasing concentrations of ethyl alcohol, rendered translucent by xylol, stained and included in liquid paraffin in an oven regulated to 50°C in order to obtain histological slides containing longitudinal cuts of the uterine cervix.

Later, cuts were obtained in a microtome adjusted to 5 μm. Soon afterwards these cuts were placed on slides previously smeared with Mayer albumin and kept in an oven at 37°C for 24 hours to dry and adhere. The slides were stained with hematoxylin-eosin for morphological description and by means of Masson trichromium to indicate collagen fibers and eosinophils and to allow morphometric studies to be carried out.

For the histological study we used a microscope with 10 x ocular magnification and objectives with magnifications ranging from 4 to 100 times. To count the collagen fibers in the two groups a morphometric study was carried out based on the principles of stereology, which through quantitative analysis yields the amounts in percentages of the analyzed components in the two-dimensional plane, thus giving us an idea of what happens in the three-dimensional plane; an integrating ocular, containing 25 points distributed in geometrical fashion was employed. The lattice of the integrating ocular, projected over the histological cuts, allowed us to count the hits, i.e., the points of the ocular that fell on collagen fibers and other structures (residue). For such purpose, the integrating ocular was coupled to a light microscope with a 100 x objective, and the final magnification was 1000 x.

In order to avoid counting faults on common fields in the same slide, we used a schedule on which the cuts of the uterine cervix were divided into four quadrants, enabling the readings in a certain direction. In each rat, we counted 40 fields, totaling 1,000 hits and a grand total of 5,000 per group.

To analyze the numeric results (counting of collagen fibers), a parametric test was employed, taking into account the nature of the distribution or the variability of the measurements carried out, applying the Student's t-test. The level of rejection for the hypothesis of nullity was established in 0.05 or 5%. When the analysis of the data showed significance, we used a symbol (.) to characterize it. The averages and SEM were calculated and presented for information purposes.

The study was evaluated and approved by the Ethics Committee for Research of the Federal University of São Paulo, Paulista School of Medicine.

Results and Discussion

In the figures below the microscopic findings of the uterine cervix of rats (optic microscopy) are shown. In the control group (G I), we noted that the ectocervix was externally lined by a stratified epithelium. The lamina propria was made up of dense connective tissue, rich in collagen fibers and fibroblasts. The endocervix was lined by a cylindrical epithelium and the lamina propria was stroma-rich in collagen fibers. Below the lamina propria, both in the ectocervix as well as in the endocervix smooth muscle fibers were seen (Figures 1A and 1B).

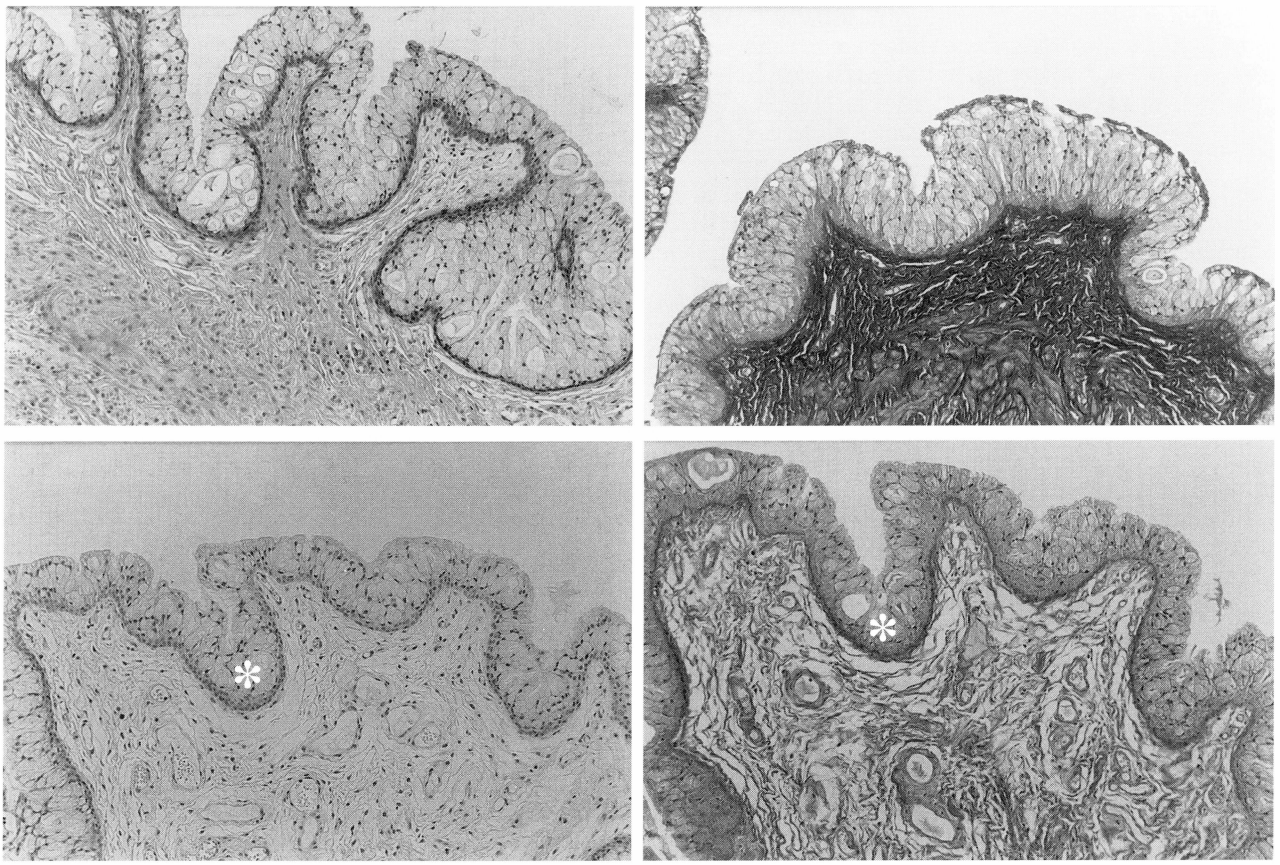
In the trial group (G II), the regions of the rat uterine cervix were similar to those of the control group, i.e., they were made up of ecto and endocervix. However, in the ectocervix where the drug had been applied, a thin superficial epithelium and lamina propria of flaccid connective tissue were noted. At this site the presence of eosinophils and well dilated blood vessels were also identified (Figures 2 A and 2B).

As for the histometric counting of collagen fibers seen in Table 1, the trial group (G II) showed a number of collagen fibers that was significantly lower in statistical terms than that found in the control group (G I). The values achieved ranged from 200 to 330 in the trial group, with an average of 248 and a standard error of 49.7. There was a significant difference between the average

Table 1. — *Values of collagen fiber counting in uterine cervices of female rats on the 20th day of pregnancy in the control group (distilled water) and in the trial group (hyaluronidase).*

	Groups	
Rats (n)	Control	Trial
1	560	330
2	600	200
3	550	220
4	520	250
5	530	240
Average	552	248.
Standard error	31.1	49.7

Paired "t" test. $p < 0.0001$.



1A 1B

2A 2B

Figure 1. — A) Photomicrography showing part of the ectocervix of a rat from the control group. Note muciferous superficial epithelium and *lamina propria* (LP) made up of connective tissue. Staining H&E, magnification 200 X. - B) Photomicrography showing part of the ectocervix of a rat from the control group. Note *lamina propria* (LP) with a high concentration of collagen fibers. Staining Masson trichromium, magnification 200 X.

Figure 2. — A) Photomicrography showing part of the ectocervix of a rat from the trial group. Note thin muciferous superficial epithelium (*), *lamina propria* rich in blood vessels and eosinophils (arrows). Staining H&E, magnification 200 X. - B) Photomicrography showing part of the ectocervix of a rat from the trial group. Note thin muciferous superficial epithelium (*), *lamina propria* rich in blood vessels and eosinophils (arrows). Staining Masson trichromium, magnification 200 X.

Table 2. — *Values of the eosinophiles counting in uterine cervices of female rats on the 20th day of pregnancy in the control group (distilled water) and in the trial group (hyaluronidase).*

	Groups	
Rats (n)	Control	Trial
1	10	15
2	12	16
3	7	17
4	8	20
5	9	23
Average	9.20	18.20
Standard error	1.92	3.27

Paired "t" test. $p < 0.0007$.

values in the control and experimental groups (552 and 248, respectively).

Regarding eosinophils, G II showed a predominance of this leukocyte (Table 1), with values ranging from 15 to 23, average 18.20 and a standard error of 3.27, when compared to G I (average 9.20 and a standard error of 1.92).

The alternatives to reduce the number of cesarean sections (C-sections) are justified due to the increased morbidity and mortality associated to this type of surgery all over the world [18].

The induction of labor is one of the main advances in present obstetrics, with the aim of avoiding maternal and fetal risks and to decrease the numbers of C-sections [19]. In the past decade, the worldwide incidence of induction varied from 15 to 25% [20]. However, in order to have this practice work effectively in the fight against elective C-sections, we should search for conditions to make it more accessible, efficient and safe. The different methods for labor induction are being chosen mainly according to the stage of cervix ripening. Physicians decide on those cases that stimulate contractions when the cervix is more ripe and reserve the use of agents that facilitate cervix maturation for the cases where the uterine cervix is not well prepared.

In practice, the most widely used method to evaluate the stage of cervix ripening is the Bishop's index [21], which consists of a score of five parameters (consistence, thinning, dilation and position of the uterine cervix,

added to presentation height). Values below five are indicative for the use of methods that act mainly on cervix ripening, without determining the early onset of uterine contractions.

The literature is pointing to changes in the concentration of collagen during pregnancy, stressing that its degradation and reorganization probably contributes to the dilation process [22, 23].

Product advantages of hyaluronidase that seem relevant to us are: its use in an outpatient setting, the low cost of an applied unit, no biological risks for mother or fetus, simple to control, well accepted by the patient as well as by health professionals and easy to use. Also it does not interfere with uterine activity, acting only in cervix cells, i.e., causing physiological cervix changes without causing uterine contraction or hyper stimulation. In the doses clinically used, the drug does not infiltrate the region of the inferior segment, allowing its use in patients who have undergone previous C-sections.

The results of our experimental trial allow us to conclude that the intracervix injection of hyaluronidase promotes evident histological changes, characteristic of cervix ripening (*lamina propria* with ectocervix made up by flaccid connective tissue and lower concentrations of collagen fibers), allowing the clinical conclusion that confirms the use of hyaluronidase in humans for this purpose.

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