Colchicine-estrogen interactions[1]

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Summary. Colchicine does not block estrogen-induced recognition of uterine blood vessel surface by eosinophils, but interfers with their migration through endothelial lining and therefore blocks estrogen-induced uterine eosinophilia and edema.

2 or possibly 3 estrogen receptor systems, involved in independent mechanisms of estrogen action, exist in the rat uterus[2,3]. The cytosol-nuclear estrogen receptor system has been supposed to mediate the genomic response to estrogens, i.e., RNA and protein synthesis[4]. The eosinophil-estrogen receptor system was postulated to mediate some early parameters of estrogen stimulation in the uterus, such as edema, increase in vascular permeability and release of histamine[2,5,6].

There are no eosinophils in the immature rat uterus, but they are attracted to this organ by estrogens[7]. Any condition blocking the estrogen-induced migration of eosinophil leukocytes to the uterus, or interferring with the release of eosinophil content into this organ, should modify those parameters of estrogen stimulation mediated by the eosinophil-estrogen receptor system. In the present work, colchicine was used to inhibit eosinophil migration to uterine tissue, and thus to interact with those parameters of estrogen stimulation which are mediated by the uterine eosinophils.

Material and methods. Female 50±2 g b.wt Sprague-Dawley rats were used in the present experiments: 4 groups of animals were used: colchicine, estrogen, colchicine + estrogen-injected and control animals. The animals were sacrificed 6 h after the i.v. injection of 15 µg estradiol-17β in saline and/or 7 h after the i.p. injection of 10 µg colchicine in saline. The solution of colchicine was freshly prepared before each experiment from a stock solution kept in a light proof bottle at 4 °C. This is important to prevent the conversion of colchicine to lumicolchicine which has impaired biological properties[8]. The animals were killed by cervical dislocation, the left uterine horn wet weight was determined and the right uterine horn was fixed in neutral formalin for tissue eosinophil quantification[9] and determination of eosinophil location within or outside the vascular space.

Results. Colchicine induces an important decrease in the number of uterine eosinophils after estrogen administration (table 1). In estrogen-treated rats, most uterine eosinophils are located in the extravascular space of uterine stroma; instead, in colchicine + estrogen-treated rats, most eosinophils are located in the intravascular space, most of them attached to the endothelial lining of small venules, and only a few are located in the extravascular space (table 2). In

Table 1. Effect of colchicine on estrogen induced uterine eosinophilia and increase in uterine wet weight, 6 h after estrogen administration

	Estrogen-treated rats	Colchicine-treated rats	Estrogen- and colchicine-treated rats	Control rats
Count of eosinophils per uterine cross section	8.06±0.99	0.014	0.095	0.013
Uterine wet weight (mg)	82.62±9.75	27.22±2.62	30.38±2.18	17.08±1.90

Table 2. Effect of colchicine on the intravascular/extravascular location of uterine eosinophils in rats with or without estrogen treatment

	Estrogen-treated rats	Colchicine-treated rats	Estrogen- and colchicine-treated rats	Control rats
Percent of eosinophils located intravascularly and non-attached to the endothelial lining	8.65±0.94	100*	17.62±1.40	100*
Percent of eosinophils located intravascularly and attached to the endothelial lining	13.40±1.76	-	61.74±0.57	-
Percent of eosinophils located in the extravascular space	77.93±2.32	-	20.64±2.25	-

* The few eosinophils found were located intravascularly and nonattached to the endothelial lining.

Table 3. Effect of colchicine on the endometrial-myometrial-mesometrial location of uterine eosinophils after estrogen treatment

	Estrogen-treated rats	Estrogen- and colchicine-treated rats
Percent of eosinophils located in the endometrium	31.96±2.22	20.40±1.00
Percent of eosinophils located in the myometrium	55.60±0.51	35.22±1.61
Percent of eosinophils located in the mesometrium	12.44±0.88	44.38±1.16

Table 4. Effect of colchicine in the proportion of intravascular/extravascular eosinophils in myometrium and mesometrium after estrogen treatment

	Estrogen-treated rats	Estrogen- and colchicine-treated rats
Proportion of intravascular/extravascular eosinophils in the myometrium	0.210	2.96
Proportion of intravascular/extravascular eosinophils in the mesometrium	0.620	4.649

control rats without any treatment and in colchicine-treated rats, the few eosinophils found were located in the intravascular space and none of them attached to the endothelial lining (table 2). In estrogen-treated animals, most eosinophils are located in the uterine muscular layers; in colchicine + estrogen-treated animals, most eosinophils are located in the mesometrium (table 3). In estrogen-treated animals, the proportion of extravascular/intravascular eosinophils is much higher in the myometrium than in the mesometrium; in colchicine + estrogen-treated rats this proportion is slightly higher in the mesometrium (table 4). Colchicine drastically decreases the estrogen-induced increase in uterine wet weight (table 1).

Discussion. The disassembly of microtubules by colchicine[10] was suggested as the mechanism to block the migration of leukocytes to the gout[11]. These properties ascribed to colchicine could explain the suppression of estrogen-induced migration of eosinophil leukocytes to uterine stroma and therefore also block estrogen-induced uterine edema.

There are other possible explanations for the suppression of both uterine eosinophilia and uterine edema by colchicine. It has been proposed that colchicine, at a concentration necessary to disaggregate cytoplasmic microtubules, inhibits the release of enzymes mediating the inflammatory response by increasing cGMP levels[12]. The inhibition of prostaglandin E_1 synthesis, release and/or effects[8] might also account for the interaction of colchicine with uterine eosinophilia and edema. It was previously proposed that eosinophils recognize uterine blood vessel endothelial lining after estrogen stimulation[9]. The present results show that colchicine does not block the recognition of uterine endothelial lining by eosinophils, which are attracted to it in estrogen + colchicine-treated animals, but blocks their migration through the vascular wall and also blocks eosinophil migration from the mesometrium to myometrium through uterine stromal extravascular space. Therefore, we suggest that prostaglandin E_1 suppression by colchicine and/or inhibition by colchicine of cGMP increase are not the main mechanisms of interaction by colchicine with eosinophil migration in estrogen action. Instead, we propose that the suppression of cellular mobility by colchicine is the main mechanism of interaction with estrogen-induced uterine eosinophilia which was proposed to mediate edema.

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Allotransplantation of rat parathyroid glands: Effects of organ culture and transplantation into the adrenal gland[1]

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Summary. Allotransplantation of fresh, 1 or 2 week cultured parathyroid glands from Wistar rats (AgB^2) to Fischer rats (AgB^1) resulted in prompt rejection of the transplant in the muscle site; whereas transplantation into the adrenal site offered slightly prolonged survival, suggesting that the latter is a privileged transplantation site.

Culturing of tissues for 3–50 days prior to transplantation has been shown to prolong survival of allografts in some studies[3–7], while other studies have not shown such beneficial effects[8,9]. Transplantation of tissues into several privileged sites such as brain[10], anterior chamber of the eye[11], skin island[12], testis[13], liver[14], spleen and thymus[15] has also been shown to prolong the graft survival.

The present studies1. evaluated the effect of prior culturing for 1 or 2 weeks and 2. evaluated the adrenal gland (AG) as a privileged recipient site for allotransplantation of parathyroid glands (PTG) across a major histocompatibility barrier in inbred rats. Evaluation of the AG as a privileged site was prompted by a fortuitous observation by us in which pieces of a human parathyroid adenoma transplanted into the AG of a rat showed evidence of function for 80 days.

Materials and methods. Wistar furth rats (AgB^2) were used as donors and Fischer 344 rats (AgB^1) as recipients. All recipient rats were surgically parathyroidectomized several days prior to transplantation. Parathyroidectomy was confirmed by serum calcium (Ca) value of less than 7.0 mg/100 ml after an 18-h fast. After removal from the donor, the PTG were cultured at 37 °C in minimum essential Eagle's medium supplemented with 0.03% glutamine and 5% heat inactivated pooled rat serum. The media were changed 3 times a week. The cultures were gassed daily with 95% O_2 and 5% CO_2. For transplantation into the muscle, PTG were placed into a 5×5 mm pocket, created in the right thigh muscles and secured with 4–0 silk. For transplantation into the AG, laparotomy was performed under anesthesia and the left AG exposed. The PTG to be transplanted were placed inside a 22 gauge spinal needle,