Simple Biochemical Method to Assess Progestin Effects on Human Endometrial DNA Synthesis and Its Application to Endometrial Carcinoma

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ABSTRACT

Endometria of normal histology from postmenopausal women receiving either estrogen or estrogen plus a progestin have been analyzed for nuclear estradiol receptor, epithelial DNA synthesis, isocitric dehydrogenase, and estradiol dehydrogenase activities. Epithelial DNA synthesis correlated positively with nuclear estradiol receptor and negatively with both the dehydrogenases; this result was obtained regardless of whether the enzyme activity was related to the protein or DNA content of the samples. Thus, either of the dehydrogenases might provide an index of progestin effects on proliferative activity in endometrial carcinomata.

Provera administered in vivo had no effect on either dehydrogenase activity in soluble estradiol receptor-poor carcinomata, whereas both dehydrogenase activities were high in some but not all soluble estradiol receptor-rich tumors. The enzyme activities in Provera-treated tumors have been compared with those in normal epithelium and endometrium from postmenopausal women taking estrogen plus progestin. The activities of both dehydrogenases were lower in soluble estradiol receptor-rich carcinomata than in either endometrium or epithelium from estrogen plus progestin-primed, normal postmenopausal women. This may indicate suboptimal progestin effects in the patients with carcinoma, and potential reasons for this are discussed.

INTRODUCTION

Adenocarcinoma of the human endometrium arises in the epithelium, and progestins are widely used in its treatment with approximately one-third of the patients benefiting from such treatments (3, 18, 23). Clinically, it is desirable to distinguish between the responsive and unresponsive patients and offer treatment to the former only. Biochemical analysis of the tumor may help determine progestin responsiveness and, ideally, an assay of progestin inhibition of growth is required. Measurements of DNA synthesis either biochemically or autoradiographically could form the basis of such an assay but are not practical on a routine basis because of their complexity and the requirement of fresh tissue. A biochemical component that correlates with epitherial DNA synthesis and which is simple to measure in frozen tissue would circumvent these problems.

We have been monitoring progestin effects on normal, postmenopausal endometrium (7, 25) and, in this paper, present information on the progestin responsiveness of 3 indices, REN,2 estradiol dehydrogenase, and isocitric dehydrogenase, and their correlation with epithelial DNA synthesis. Steroid receptor assays are widely used to predict the hormone sensitivity of breast cancer (21), although REN estimation has had limited use (1, 13). Data have also been published for endometrial carcinoma (6, 14, 16, 17), but information on their prognostic value is sparse (2, 26). Estradiol dehydrogenase has been suggested as an index of progestin sensitivity of endometrial carcinoma (16, 24), whereas isocitric dehydrogenase has not. We feel that, because of the simplicity of the assay, the latter enzyme may have certain advantages over the other types of assay as an index of possible progestin sensitivity of endometrial carcinomata.

MATERIALS AND METHODS

Endometria of normal histology were obtained by suction curettage (5) from postmenopausal women receiving p.o. estrogen either with or without progestin for symptoms associated with the climacteric. The treatment regimens have been described elsewhere (7, 12, 25) as have the methods of biochemical analysis (10, 12).

Tumor samples were obtained either at diagnostic curettage or at hysterecotomy. The samples were frozen until analyzed. Where stated, the women had taken medroxyprogesterone acetate (Provera; Upjohn Limited, Crawley, Sussex, England) p.o. (10 to 300 mg/day) for at least 3 days prior to the collection of tissue. Maximal effect of p.o. progestins on endometrial DNA synthesis, receptor concentrations, and enzyme activities is achieved after 3 to 6 days of treatment (25).

The comparative data for normal, postmenopausal epithelium and endometrium included in Table 4 have been taken from work published previously (9, 11).

Because all of the biochemical data were log normally distributed, all statistical correlations and comparisons were assessed on the logged data.

RESULTS

Normal Endometria. Data from women receiving either estrogen or estrogen plus progestin were combined for the statistical comparisons presented in Table 1. Although there were clear-cut differences in biochemical parameters between the 2 treatment groups (25), we wished to see if linear correlations existed regardless of hormone treatment.

Highly significant correlations were found between epithelial DNA synthesis and each of REN, isocitric dehydrogenase, and estradiol dehydrogenase activities (Table 1). Likewise, REN content correlated with the 2 dehydrogenase activities.

If a biochemical test for progestin sensitivity of DNA synthesis is to be used, it is important to know if the assays should be related to protein or DNA content. The enzyme and receptor data presented in Table 1 were calculated per mg DNA. The correlations were still present if the enzyme activities were expressed per mg soluble protein, although the statistical significance of the correlation between estradiol dehydrogenase and epithelial labeling index was decreased (Table 2). Regardless of
the method of expressing the enzyme activities, isocitric dehydrogenase correlated better with labeling index and REN than did estradiol dehydrogenase. Thus, assay of either of the 2 dehydrogenases gives an indication of epithelial DNA synthesis and REN content and suggests that isocitric dehydrogenase may be the more reliable of the 2 enzymes.

Carcinomata. The enzyme data for endometrial carcinomata from women either receiving or not receiving p.o. Provera at the time of sampling are shown in Chart 1. Provera significantly (p < 0.05) increased the estradiol dehydrogenase activity regardless of method of calculation. No significant effect was seen with the isocitric dehydrogenase.

REN was detected in about 10% of samples and in untreated carcinomata only (Table 3). Treatment with Provera did not affect the proportion of REC-rich tumors, although it tended to decrease the number of RPC-rich samples (Table 3).

Different responses to Provera in the REC-rich and -poor tumors are compared in Table 4 with endometria and epithelia of normal histology obtained from postmenopausal women receiving hormone therapy. In REC-rich tumors, Provera induced mean isocitric and estradiol dehydrogenase activities similar to those obtained with estrogen-primed, postmenopausal endometria of normal histology. Since the tumors derive from the epithelial cells of the endometrium, a better comparison might be between tumors and normal epithelium, although the variable stromal content of tumors might invalidate that suggestion. Such a comparison indicates that, because of the predominant localization of both dehydrogenases in the epithelium of normal endometrium, progestin-treated, normal epithelium has much higher dehydrogenase activities than do progestin-treated tumor cells (Table 4).

**DISCUSSION**

Endometrial carcinomata arise in the epithelium of the uterus and are most common in postmenopausal women. In this and other studies (7, 25), we have shown that progestins are capable of inducing both isocitric and estradiol dehydrogenase activities similar to those obtained with estrogen-primed, postmenopausal endometria of normal histology. Since the tumors derive from the epithelial cells of the endometrium, a better comparison might be between tumors and normal epithelium, although the variable stromal content of tumors might invalidate that suggestion. Such a comparison indicates that, because of the predominant localization of both dehydrogenases in the epithelium of normal endometrium, progestin-treated, normal epithelium has much higher dehydrogenase activities than do progestin-treated tumor cells (Table 4).
Endometrial DNA Synthesis

Chart 1. Effect of Provera on isocitric and estradiol dehydrogenase activities of endometrial carcinomata. Isocitric dehydrogenase activity (A) is expressed as nmol NADPH/min either per mg protein (left ordinate) or per μg DNA (right ordinate). Estradiol dehydrogenase activity (B) is expressed as nmol estrone/hr either per mg protein (left ordinate) or per mg DNA (right ordinate). Number inside black box, number of samples with undetectable activity. Patients were receiving either no treatment (●, ●) or Provera (○, ○) at the time of sampling. Results are presented individually. Median value, ——. Comparisons of the effect of Provera by Student’s t test: *, p < 0.05; other comparisons were not significant.

assay is very simple and does not involve the use of isotopes. From the point of view of determining progestin effects on DNA synthesis, the results presented here indicate that both enzyme activities are inversely correlated with normal epithelial DNA synthesis to a highly significant degree so that either enzyme activity could be used to predict the response of DNA synthesis to progestins.

We have assayed normal epithelial DNA synthesis by [3H]-thymidine labeling of fresh endometrium. Because of the frequent inability to obtain fresh tumor samples for direct measurement of DNA synthesis, we have measured dehydrogenase activities of the more readily available frozen tumors. Provera increased both dehydrogenase activities in REC-rich but not REC-poor tumors. The division between REC-rich and -poor samples at 20 fmol per mg protein was based on our experience with the same assay with breast tumors, in which this cut-off value gave the best distinction between endocrine-responsive and -unresponsive tumors (8). No correlations were obtained between Provera-induced enzyme activity and RPC values, possibly because of the transfer of RPC into the nucleus and thus loss to the assay. These data are compatible with the view that Provera can induce dehydrogenase activities in some REC-rich but not REC-poor tumors and that this should also reflect effects on tumor DNA synthesis. The progestin effect on REC-rich tumors was evident with both dehydrogenases when their activities were calculated per mg protein, but only the estradiol dehydrogenase data attained statistical significance when the results were calculated per mg DNA. The reason for this is uncertain given the better correlations between DNA synthesis and enzyme activity expressed per mg DNA than protein with normal endometria. The aneuploidy associated with some tumors (19) might explain the poorer DNA results with tumors.

Our results were obtained with carcinomata from women receiving Provera. The alternative approach of in vitro testing would appear to be less desirable, given the inability of progestins to induce estradiol dehydrogenase activity in explant cultures of tumors (20). It is clear that some REC-rich tumors do not respond well to...
short-term Provera. This may be due to cellular defects beyond
REC but might also be caused by the low estrogenic environment
existing in the postmenopausal women from whom most of
these tumors were taken. Progestins are more effective in many
cells after estrogen priming, and the same may be true for
endometrial carcinoma. It is noteworthy that the only premeno-
apausal tumor thus far encountered gave high activities of both
enzymes after Provera. Also, comparison of tumor and normal
epithelium indicates suboptimal progestin effects in the former
tissue. The antiestrogen, tamoxifen, might increase the sensitiv-
ity to progestins, since it can increase RPC in human endome-
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ACKNOWLEDGMENTS

We are grateful to Sarah Murdoch, Rosemary Johnson, and M. Sherif for
the preparation of the autoradiograms.

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