Radiosensitizing Effect of Medroxyprogesterone Acetate on Endometrial Cancer Cells in Vitro

HANS HUBER, MD, PHD, PETER HUSSLEIN, MD, WERNER MICHALICA, MD, AND PETER WAGENBICHLER, MD

From clinical experience it is known that medroxyprogesterone acetate (MPA) can increase the radiosensitivity of adenocarcinomas of the corpus uteri. This study investigates this phenomenon *in vitro*. Primary explants of highly differentiated adenocarcinomas were irradiated with or without pretreatment with MPA and compared with an untreated control group and to a group treated with MPA only. Cell culture itself was performed on an agarose medium in order to prevent overgrowth by fibroblasts. Untreated samples formed 43 ± 5 clones, explants treated with MPA only produced 39 ± 5 clones, a difference which was not statistically different; samples irradiated without pretreatment produced 16 ± 8 and samples after combined treatment 9 ± 3 clones (all values $\bar{x}$ ± SD). This numeric reduction of cell growth through preirradiation treatment with MPA was statistically significant. The effect of MPA as a radiosensitizer may be due to its potential to prolong the radiosensitive G_2 phase of the cell cycle. This effect of MPA may be useful also in other hormone-dependent tumors.

Cancer **54:999–1001, 1984.**

THE FACT that hormonal therapy recently has been included into the oncologic armantarium can be considered an important innovation. Clinical experience in various types of malignancies has been so convincing that today hormonal therapy can already be considered an integral part of oncologic treatment.

In gynecology, progesterone derivatives have been used successfully in the treatment of hormone-sensitive tumors. Their mechanism of action seems to be based on the antiproliferative effect of progesterone, which has been demonstrated and described repeatedly.[1,2]

Experimental evidence for this effect has been gained in rats in whom an adenocarcinoma of the breast was implanted followed by application of medroxyprogesterone acetate (MPA) as the progestational agent. The treated animals showed significant regression of tumor growth when compared with controls or animals treated otherwise.[3] In addition, *in vitro* experiments showed that addition of MPA to cell cultures incubated with labeled thymidine led to a reduction of thymidine incorporation and thereby to decreased proliferation.[4]

However, in addition to this well-established antiproliferative action the suspicion was raised from clinical observations that MPA might also serve as a "radiosensitizer" for certain tissues.[5] A group of 20 patients with highly differentiated adenocarcinomas of the corpus uteri was treated for 5 weeks with MPA before radiotherapy. A significantly improved radiation effect after pretreatment with MPA could be demonstrated in comparison to irradiation alone. Since this cylindric epithelium is normally poorly sensitive to irradiation[6] this constitutes a clinically important observation. In the current study we have tried to find *in vitro* evidence to support this finding and have, therefore, irradiated cell cultures *in vitro* alone and after treatment with MPA.

Materials and Methods

Originally we started our cultures in Falcon tubes with Ham F 10. This led to an overgrowth of the cultures by fibroblasts. Therefore, tissue cultures were performed according to the "plaque-technique" described by Jacoby and Pastan.[7] This technique not only prevents overgrowth by fibroblasts, but also significantly facilitates evaluation through counting of the plaques.

Briefly, a Difco Bacto agarose was mixed with 20 ml of calf-serum, 20 ml trytosephosphate, and 80 ml twice-concentrated Glasgow MEM medium at 44°C, and was applied into an agarose-concentration of 0.5%. Seven milliliters of this medium were pipeted into a 5 cm Petri dish and formed the "base layer." Two parts of the 0.5% agarose described above were then mixed with a portion of the culture medium containing a monocell suspension of the cells to be cultured; 1.5 ml was applied as "upper

From the First Department of Gynecology and Obstetrics (Chief Professor Dr. E. Gitsch), Vienna University, Spitalgasse 23, A-1097 Vienna, Austria.

Address for reprints: DDr. H. Huber, 1. Universitäts-Frauenklinik, Spitalgasse 23, A-1097 Vienna, Austria.

Accepted for publication June 22, 1983.

TABLE 1. Number of Clones After *in Vitro* Culture of Highly Differentiated Endometrial Adenocarcinomas

	$\bar{x} \pm SD$	No. in sample of the population
Controls	43 ± 5	6
MPA alone	39 ± 5	6
Irradiation alone	16 ± 8*	8
Irradiation + MPA	9 ± 3*	7

* $P < 0.05$; Student's t-test, $t = 2.177$.
$\bar{x}$: mean of the sample; SD: standard deviation; MPA: medroxyprogesterone acetate.

layer" on top of the base layer; for successful growth at least 10^3 cells had to be dispersed.

Monocell suspensions were obtained by trypsinization of tissue specimens in a magnetic stirrer. First, tissue fragments were washed in a 0.25% trypsin solution diluted with calcium-free and magnesium-free Balanced salt solution; the supernatant was decanted and warm trypsin in the same concentration was added by means of a magnetic stirrer. This was done four to five times until the suspension contained single cells; then the solution was immediately incubated with calf-serum to neutralize the trypsin effect. After three washings and after counting, the monocells were dispersed into the Petri dish as described above. The cultures were incubated for 14 days with CO_2; at that time colonies measuring approximately 0.1 to 0.2 mm in diameter were counted under the stereomicroscope.

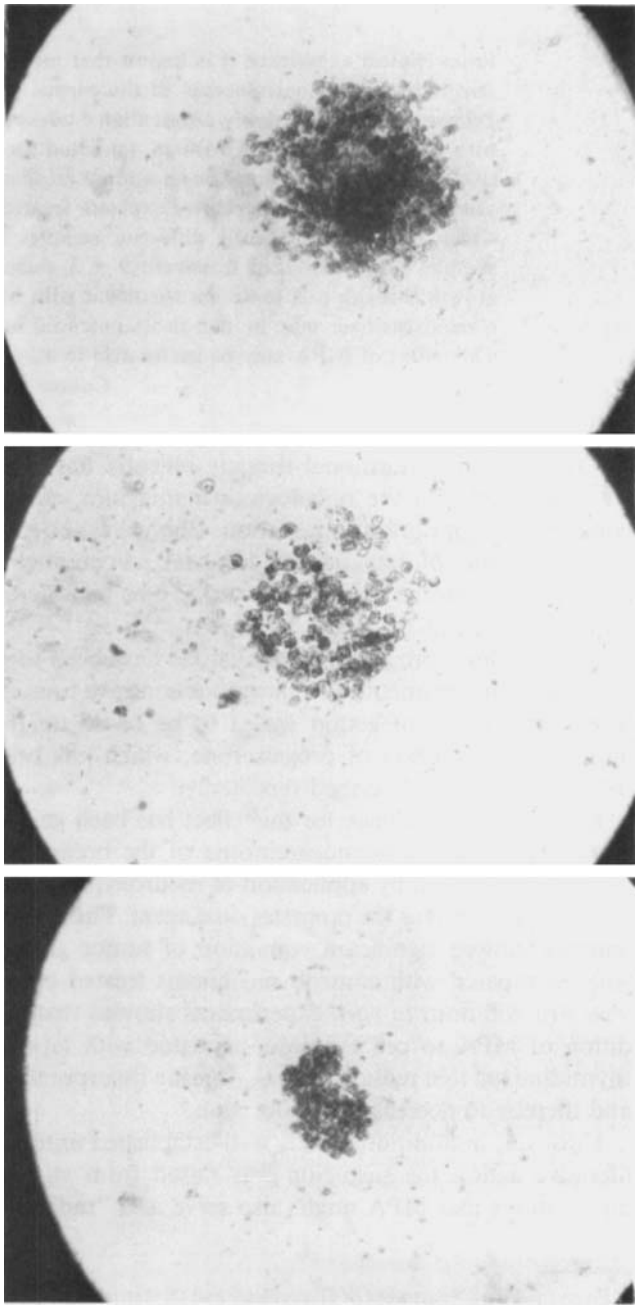
Twenty-four explants of highly differentiated adenocarcinomas from six different patients (from two to six explants per patient) were cultured according to the method described above. The tissue was obtained by dilatation and curretage in all instances. Each piece of tissue was divided into two parts; one was used for histology and the other for *in vitro* cultures. The explants were equally distributed at random to the different treatment groups. Six explants were given MPA in crystalline form into their culture medium in a concentration of 10 mcg per ml and were not irradiated later on. Fifty percent of the remaining explants were offered MPA in the same concentration, and were irradiated with 200 rad on the third day after initiation of cell culture together with those explants not receiving MPA.

Six additional tissue samples from two patients were cultivated as controls according to the same technique and using the same cell concentration but without addition of MPA and without irradiation.

Results

The overall results are shown in Table 1. Cell cultures of the control group produced 43 ± 5 clones, the low variability confirming the reproducibility of the method. The six explants not irradiated but treated with MPA revealed 39 ± 5 clones per explant, which is not statistically different from the control group.

Of the nine cell cultures irradiated without addition of MPA, one culture revealed only two clones. This sample was excluded since we assumed a primary defect of cell growth already after dissemination of the cells. The remaining eight samples produced 16 ± 8 colonies each.



FIGS. 1A–1C. Individual clones of adenocarcinomas of the endometrium cultured in Agarose-gel *in vitro*. (A, top) no treatment, (B, center) irradiation with 200 rad, (C, bottom) irradiation with 200 rad after pretreatment with MPA (10 μg/ml).

Since this amounts to approximately 37% of the 43 clones of the samples not irradiated, irradiation with 200 rad was more or less equivalent to the average lethal radiation dose.[8]

Of the nine samples irradiated after addition of MPA, two samples failed to grow; we did, however, not consider this as a radical cytotoxic effect of the combined treatment but rather assumed that unknown factors may have inhibited cell growth primarily when the cultures were implanted. Therefore, the two samples were excluded from our results. In the seven remaining cultures 9 ± 3 clones were found, which is significantly different from the 16 ± 8 colonies of the irradiation alone (Table 1).

Apart from this reduction in numeric growth the colonies were also growing with reduced speed, *e.g.*, the diameter of the clones was smaller in the combined treatment group, a parameter which, however, still needs confirmation (Fig. 1).

The tissue cultures of fibroblasts in the Falcon tubes did not show any "radiosensitizing" effect of MPA.

Discussion

The current study demonstrated that the addition of MPA to tissue cultures of adenocarcinomas of the corpus uteri increases their susceptibility to irradiation *in vitro;* significantly less clones were found in the group where MPA preceded irradiation when compared with irradiation alone. Although *in vitro* studies have to be interpreted cautiously, the fact that clinical observation of this radiosensitizing effect of MPA preceded our experimental confirmation indicates that radiosensitization seems to be a true phenomena.

That this effect seems to be receptor mediated is indicated by the fact that it could not be observed in fibroblast cultures that lack progesterone receptors and therefore are no target organ for MPA.

According to Hustin, progesterone and its derivatives interfere with the cell cycle by prolonging the late G_2 phase[2]; since the results of Kärcher[9] show that the G_2 phase is the phase of the cell cycle which is specifically sensitive to irradiation, this could explain the improved irradiation effect of pretreatment with MPA.

In summary, the results of the current study constitute *in vitro* evidence to substantiate the observation of Bonte and coworkers,[5] who in 1970 showed that MPA can act as a radiosensitizer of adenocarcinomas of the corpus uteri *in vivo.* Since various other cancerous tissues are hormone responsive, it may be conceivable that MPA pretreatment could be useful in other tumors as well. Since increased susceptibility of surrounding tissues, *e.g.*, skin, is unlikely because of the lack of progesterone receptors the observed improved radiation effect after pretreatment with MPA should be considered for clinical use.

REFERENCES

1. Trams G. Autoradiographic studies on the influence of gestagens on oestrogen-induced incorporation of 3H-uridine and 3H-thymidine into nucleic acids of the rat uterus. *Gynecol Invest* 1973; 4:155–164.

2. Hustin J. Morphology and DNA content of endometrial cancer under progesteron treatment. *Acta Cytol* 1976; 20:556–558.

3. Di Marco A. The antitumor activity of 6-alpha-methyl-17-alpha-acetoxy-progesterone (MPA) in experimental mammary cancer. In: Jacobelli S, di Marco A, eds. Role of Medroxy-Progesterone in Endocrine-Related Tumor. New York: Raven Press, 1980; 2.

4. Di Marco A. The antitumor activity of 6-alpha-methyl-17-alpha-acetoxy-progesterone (MPA) in experimental mammary cancer. In: Jacobelli S, di Marco A, eds. Role of Medroxy-Progesterone in Endocrine-Related Tumor. New York: Raven Press, 1980; p. 6.

5. Bonte J, Decoster JM, Ide P. Radiosensitization of endometrial adenocarcinoma by means of medroxyprogesterone. *Cancer* 1970; 25:907–910.

6. Soost HJ, Baur S. Gynäkologische Zytodiagnostik, 4. Auflage: Völlig Neu Bearbeitete. Berlin, Heidelberg, New York: Springer, 1980; 267.

7. Jacoby WB, Pastan JH. Cell Culture. New York, San Francisco, London: Academic Press, 1979; 300.

8. Adams RLP. Cell Culture for Biochemists. Amsterdam: Elsevier, 1980; 159.

9. Kärcher KH. Aktuelle Probleme der klinischen Strahlentherapie. Berlin, Heidelberg, New York: Springer, 1970; 7.