Eur J Cancer Clin Oncol, Vol. 23, No. 5, pp. 573–576, 1987.
Printed in Great Britain

0277-5379/87$3.00+0.00
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Letter to the Editor

Effects of Serum Type on Steroid Receptor Binding Sites and Response to Progestin of Cultured Human Breast Cancer Cells

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RECENT experiments on the mode of action of a progestin on human breast cancer cells have provided evidence for diverse effects and mechanisms [1]. Growth inhibitory effects appeared to be mediated by both progestogen and glucocorticoid receptors (PR and GR) while the mechanism of a growth-promoting effect remains to be elucidated. The finding that progestin-binding activity of cultured breast cancer cells (MCF-7) can be modulated specifically by addition of dextran–charcoal-treated calf serum [2] suggested that a model may be available for further studies of progestin action through mechanisms not involving PR. We therefore investigated the effects of charcoal-stripped newborn calf serum (SNBCS) on concentrations of PR, GR and oestrogen receptor (ER) binding sites and on the growth inhibitory actions of medroxyprogesterone acetate (MPA) on MCF-7 cells.

Cells were maintained, harvested and counted, sera treated and receptor binding sites estimated by means of whole cell assays as described previously [1]. Foetal and newborn sera (FCS and NBCS) from single batches (Gibco, Paisley, Scotland), stripped of small molecules as described previously [1] were used throughout.

STEROID RECEPTOR BINDING SITES

Estimates of ER, PR and GR binding sites varied widely, even between flasks of the same passage, and remained high in the presence of SNBCS (Table 1). These variations are extremely unlikely to be attributable to the assay procedure since control experiments using this whole cell assay gave a coefficient of variation consistently less than 10%. As the results are expressed per unit weight of DNA, variations in cell recovery should not influence the final value. However, receptor synthesis does vary relative to the degree of confluence. Cells were generally assayed as the culture approached confluence but it was not possible to ensure the same degree of confluence in all cases. Mean levels for ER (± S.D.) were 0.53 ± 0.33 (FCS), 0.67 ± 0.89 (SFCS) and 1.81 ± 0.89 (SNBCS) pmol/mg DNA, while corresponding levels for PR were 2.7 ± 1.1, 10 ± 11 and 3.9 ± 2.6 pmol/mg DNA and for GR 0.51 ± 0.33, 1.8 ± 1.9 and 1.1 ± 0.83 pmol/mg DNA. Estimates of PR were unusually high for passage 102 following its first passage with SFCS and SNBCS, but there was no reason for rejecting these values.

GROWTH INHIBITORY RESPONSE TO MPA

MCF-7 cells respond to low concentrations of MPA with growth inhibition [1], suggesting a progestin receptor-mediated effect. A glucocorticoid action of the drug has also been demonstrated [1]. In the experiments reported here cells grown and passaged up to 4 times in media containing charcoal-stripped FCS (SFCS) showed responses to 10 nM MPA (Table 2). Replacement of SFCS with charcoal-stripped NBCS (SNBCS) did not

Accepted 22 August 1986.

Table 1. Effect of 24 standard or investigational agents on ^{125}I-EGF binding

Drug	Concentration (μg/ml)	^{125}I-EGF-binding (% Control ± S.D.)	*P* value
Actinomycin D	0.01	78 ± 5	0.010
m-AMSA	0.50	73 ± 4	0.003
Ara C	10.00	116 ± 14	0.277
Bisantrene	0.50	87 ± 6	0.118
Bleomycin	0.20	88 ± 3	0.016
Cisplatin	0.20	80 ± 5	0.043
Dacarbazine (DTIC)	0.10	83 ± 3	0.006
Diaziquone (AZQ)	0.30	87 ± 6	0.101
Doxorubicin	0.04	87 ± 8	0.188
2-FLAMP	0.10	106 ± 6	0.326
5-Fluorouracil	6.00	74 ± 3	0.002
Hexamethylmelamine	1.00	111 ± 3	0.015
4-Hydroperoxy-cyclophosphamide	3.00	80 ± 5	0.015
Interferon A	1000.00*	104 ± 3	0.237
Melphalan	10.00	86 ± 5	0.053
Menogaril	0.02	87 ± 5	0.057
Methotrexate	10.00	116 ± 13	0.220
MGBG	10.00	75 ± 3	0.001
Mitomycin C	0.10	97 ± 4	0.545
Mitoxantrone	0.05	97 ± 5	0.631
Thioguanine	1.00	84 ± 2	0.004
Vinblastine	0.50	100 ± 15	0.991
Vincristine	0.01	84 ± 3	0.007
VP 16	3.0	87 ± 3	0.011

*International Units.

MCF-7 cells were cultured in six-well plates. When the cells were nearly confluent, EGF binding studies were performed in the presence of the specified concentrations of each drug. The results are expressed as percentage of untreated control. Numbers represent the mean and standard deviation of triplicate determinations.

as an index of membrane integrity.

After a 30 min preincubation, colony survival was reduced by each drug in a dose-dependent manner (Fig. 1). Trypan blue exclusion was not affected by drug treatment, indicating that cell membranes were not grossly altered. EGF binding was moderately increased by cisplatin at very low concentrations, but no consistent effect on EGF-binding was observed for the other four drugs. However, when the drug preincubation time was prolonged to 2 hr, a significant reduction in EGF receptor binding as well as colony survival was observed with two of the five drugs, vinblastine and cisplatin (Figs. 2D and 2E). At the highest concentrations tested, binding was reduced to 69% of control with vinblastine and to 32% of control with cisplatin. The reduction in binding with these two agents was reproducible in multiple experiments. Inhibition of binding required higher concentrations than inhibition of colony growth. 4-Hydroperoxy-cyclophosphamide and 5-fluorouracil inhibited colony survival, but had no effect on trypan blue exclusion or EGF binding (Figs. 2B, 2C). Doxorubicin led to moderate increase in EGF binding at the highest concentration (Fig. 2A).

Effect of cisplatin on receptor affinity and concentration

We next investigated the effects of cisplatin, the most potent drug, on EGF receptor binding in more detail. Binding studies were repeated at 0°C to prevent ligand degradation and hormone-receptor internalization. Scatchard analysis [34] of binding data obtained from cells incubated with constant tracer amounts of ^{125}I-EGF and increasing concentrations of unlabeled EGF revealed that the major effect of cisplatin was a 3-fold reduction in binding affinity (Fig. 3). Receptor concentration was not significantly altered. Identical data were obtained from Scatchard analyses of binding studies using increasing concentrations of ^{125}I-EGF (data not shown).

DISCUSSION

Our studies indicate that binding of EGF to target cells can be disturbed *in vitro* by pretreatment with high concentrations of vinblastine and cisplatin. The effect is time dependent and was observed after exposure to the drug for 2 hr; simultaneous incubation or preincubation with drug for 30 min did not result in consistent changes in EGF receptor binding. Scatchard analysis suggests that the affin-

Table 2. Growth-inhibitory effects of MPA on MCF-7 cells grown with SFCS and SNBCS

Serum	Passage	Passage with given serum	Days grown	Treatment	No. of cells (% of control)	P*
SFCS	29	1	7	Control	100 ± 12	
				10nM	65 ± 3	<0.01
	32	1	6	Control	100 ± 9	
				1nM	47 ± 4	<0.001
	74	1	5	Control	100 ± 9	
				10nM	62 ± 3	<0.001
	75	1	6	Control	100 ± 9	
				10nM	50 ± 6	<0.001
	76	2	11	Control	100 ± 14	
				10nM	26 ± 2	<0.001
	77	3	9	Control	100 ± 6	
				10nM	14 ± 1	<0.001
	78	4	13	Control	100 ± 5	
				10nM	8 ± 1	<0.001
	80	1	6	Control	100 ± 10	
				10nM	23 ± 7	<0.001
	83	1	7	Control	100 ± 5	
				1 nM	46 ± 1	<0.001
SNBCS	57	3	8	Control	100 ± 46	
				10nM	64 ± 15	NS
	59	5	6	Control	100 ± 12	
				10nM	120 ± 40	NS
	61	7	10	Control	100 ± 12	
				10nM	76 ± 5	<0.02
	76	2	7	Control	100 ± 8	
				10nM	42 ± 3	<0.001
	77	3	12	Control	100 ± 6	
				10nM	23 ± 3	<0.001
	78	4	9	Control	100 ± 13	
				10nM	40 ± 5	<0.001
	80	6	13	Control	100 ± 6	
				10nM	22 ± 2	<0.001

*Student's *t*-test: difference from control.

dextran–charcoal in their laboratory [2] and twice in ours [1] and more of any factor(s) responsible for the changes observed by Eckert and Katzenellenbogen may have been removed by our treatment. However, it seems most likely that the quality of sera varies from batch to batch and that the serum we investigated did not display the property described. The authors did not indicate whether their results could be obtained with different batches of foetal and calf sera or were confined only to a single set of each.

Attenuation of PR synthesis in breast cancer cells by bovine serum factors does not appear to be a reproducible phenomenon. The relevance of such attenuation to human breast cancer and its treatment remains to be established.

Acknowledgements—We are indebted to Dr. Marc Lippman (National Cancer Institute, Bethesda MD) for MCF-7 cells, to Upjohn Company for MPA, and to St. Mary's Hospital for financial support.

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