Plasma from pregnant rats has anti-tumoural activity

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Abstract. Plasma from 12-day pregnant rats showed a marked anti-tumoural activity when added to short-term cultures of rat Yoshida AH-130 cells. Indeed, 24 h after the addition of 10% pregnant rat plasma resulted in a 34% decrease in cell number as compared with cells without any serum additions or those that were added with virgin plasma. The decrease in cell number was associated with the presence of a marked aneuploid peak, as indicated by flow cytometry analysis, which suggests that pregnant plasma induces apoptosis in this particular tumour cell line. Interestingly, addition of human chorionic gonatrophin to the cell cultures did not exert any effects either on cell content of cell cycle distribution, therefore suggesting that the presence of high concentrations of this hormone in pregnant plasma is unlikely to be the cause of the increased cell death.

Introduction

For a long time, it has been speculated as to whether the pregnant condition is protected against some forms of tumour growth. The controversy is in part due to the relatively short period of human pregnancy and the preexistence of tumours before conception took place. In a very elegant study, Lunardi-Iskandar *et al* (1) demonstrated that both human and murine serum were able to inhibit growth in a Kaposi's sarcoma cell line; this type of malignancy has a high occurrence in men with AIDS. The same authors concluded that the inhibitory effect on tumour growth was due to the beta-chain of human chorionic gonadotrophin (hCG). In spite of this interesting study, there is a lack of investigations concerning tumour growth inhibition by plasma from pregnant subjects or experimental animals and the possible mediators of this effect.

Bearing all this in mind, the aim of the present study was to investigate if plasma from pregnant rats had any effect on the growth of the highly-deviated rat AH-130 hepatoma, in order to test its ability to interfere with tumour enlargement.

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Material and methods

Animals. All animals (female Wistar rats weighing 60-80 g) were fed *ad libitum* on a chow diet consisting (by weight) of 54% carbohydrate, 17% protein and 5% fat (the residue was non-digestible material) with free access to drinking water, and were maintained at an ambient temperature of 22±2˚C, with a 12h-light/12h-dark cycle (lights on from 08:00 h). They were mated at 60 days of age (180 g body weight approx.). Day 0 of pregnancy was considered when the presence of spermatozoa in vaginal smears was detected. Twelve-day-old pregnant rats were used in all the experiments.

Short-term cultures. AH-130 cells obtained from untreated rats were counted and seeded in culture dishes at a concentration of 10^6/ml in RPMI supplemented with 10% FCS, in the absence or in the presence of pregnant rat plasma (10%), and maintained for 24 h in a 5% CO_2 atmosphere. At the end of the incubation period, cells were counted and analysed either morphologically or by flow cytometry.

Flow cytometry. Flow cytometry analysis was carried out using an Epics Elite flow cytometer (Coulter Electronics Corporation, Hialeah, FL). Excitation took place using a standard 488 nm air-cooled argon-ion laser at 15 mW power. The instrument was set up with the standard configuration. Forward scatter (FSC), side scatter (SCC) and propidium iodide red fluorescence (675 nm bandpass filter) were used. Optical alignment was performed on an optimized signal from 10 nm fluorescent beads (DNA-check, Coulter Electronics Corp.). Propidium iodide was applied simultaneously with RNAse (DNAse-free) after methanol fixation. Chicken erythrocytes and peripheral rat blood leukocytes were used as calibration standard for DNA ploidy. Time was used as a control of the stability of the instrument in a fluorescence vs. time dot blot. The cell cycle analysis was carried out using Multicycle Software (Phoenix Flow Systems, San Diego, CA, USA). The variation coefficient of the diploid peaks varied from 4.5-6.6, the number of nuclei analysed usually being 20,000.

Chemicals. All chemicals were either obtained from Roche (Barcelona, Spain) or Sigma (St. Louis, MO, USA).

Statistical analysis. Statistical analysis of the data was performed by means of the Student's t-test.

Results and Discussion

Lunardi-Iskandar *et al* (1) showed that both human and murine plasma had an important inhibitory action on the growth of a Kaposi's sarcoma (KS) cell line. They attributed this anti-tumoural activity to the beta-chain of hGC since addition of this protein to the KS cell line, instead of serum, also resulted in growth inhibition. Interestingly, KS occurs more often in men than in women and is often associated with AIDS. The aim of our study was to find out if the inhibitory activity of pregnant plasma could be extended to other experimental tumour cell lines and see if hGC was also involved in the mediation of this effect. Bearing this in mind, we used short-term cultures of Yoshida AH-130 cells, a very fast growing and undifferentiated rat tumour.

The results presented in Fig. 1 clearly show the inhibitory action of rat pregnant plasma. In comparison with the cells that were grown in the presence of virgin rat plasma, there is a 34% decrease in growth. Interestingly, the treatment with the pregnant plasma also induced important changes in the cell cycle distribution of the Yoshida AH-130 cells as measured by flow cytometry (Table I). Indeed, the addition of the pregnant plasma induced an important increase in the aneuploid peak (Table I), suggesting the the inhibitory effect may be mediated by apoptosis. Bearing in mind previous investigations, we decided to analyse if hGC was involved in this growth inhibition. Therefore, we cultured Yoshida AH-130 cells in the presence of different concentration (0, 10 and 100 IU/ml) of recombinant hGC and found that this hormone did not significantly alter the number of cells (Fig. 2) neither at 24 or 48 h. The hormone did not induce any changes in cell cycle distribution (data not shown).

The role of hGC in human cancer is, however, controversial. Some studies have suggested that hGC is very effective in protecting the rat mammary gland against carcinogenesis. The protective effect of hGC in pregnancy seems to be due to gland differentiation and synthesis of inhibins by the mammary epithelial cells (2). Similarly, Russo *et al* (3) have shown (using the induction of mammary tumours with 7,12-dimethyl-benz(a)anthracene in a rat model) that hGC administration reduces tumour incidence in a manner comparable to that of a completed pregnancy. Epidemiological studies (population-based, case-control of breast cancer) suggest that administration of hGC is effective in reducing breast cancer risk in a similar manner to that of a term pregnancy (4). However, there are clear inconsistencies regarding the role of hGC in preventing cancer. In fact, Acevedo *et al* (5) have shown that hCG, the hormone of pregnancy and development that seems to have chemical and physiological properties of growth factors (6), is a common phenotypic characteristic of cancer in which the presence of membrane-associated hCG has been found in more than 85 cancer cell lines of different types and origins (7,8) and in cells isolated from human malignant tumour tissues (5), a vaccine against hGC having been suggested to be used in patients with cancer.

Other mediators could be involved in our model. Thus, Klein *et al* (9) have shown that a fraction from a human placental extract (EAP) inhibited growth in soft agar of a human lung squamous adenocarcinoma cell line and also of ras-transfected murine cells. The placenta contains various

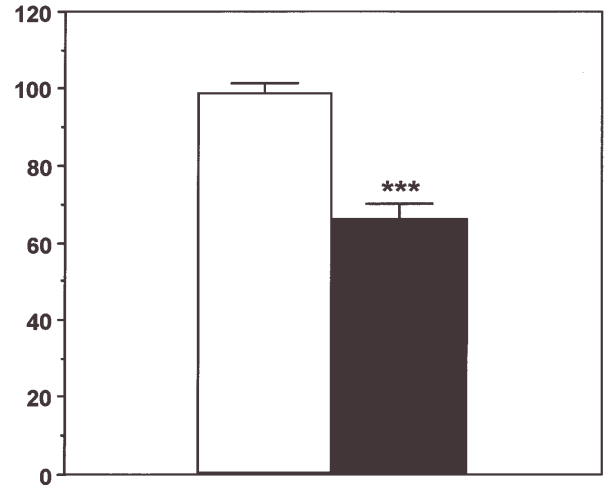

Figure 1. Effects of pregnant serum on total cell number. The results (mean ± SEM of 5 different experiments) are expressed as percentage of control incubations (incubations without addition of plasma). Open bars, incubations made with virgin plasma; black bars, incubations made with pregnant plasma. ***p<0.001 vs. control values.

Table I. Flow cytometry analysis of Yoshida AH-130 cells.

Additions	A_0	G_0/G_1	S	G_2M
None	4.5±2.8	59.2±7.7	27.6±7.9	13.3±1.5
Virgin plasma	3.0±3.1	56.3±0.2	30.9±0.4	12.8±0.6
Pregnant plasma	9.9±5.1	65.3±5.4	22.7±6.7	11.4±1.4

Cell cycle distribution analysis, DNA content (mean ± SEM) is expressed as a percentage of controls. A_0 represents the aneuploid peak.

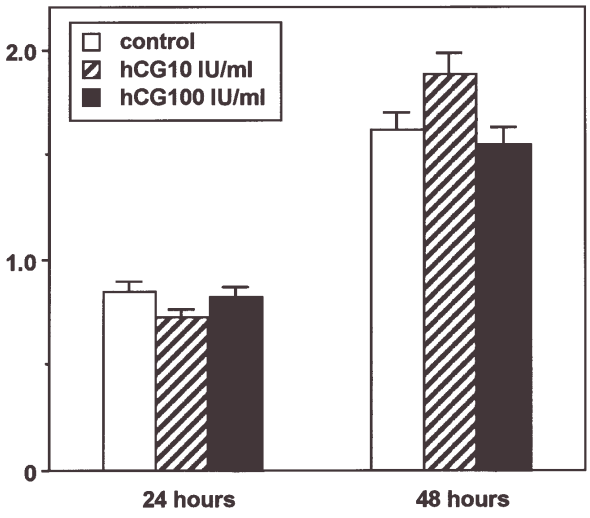

Figure 2. Effects of hCG on total cell number. The results (mean ± SEM of 3 different experiments) are expressed as million of cells. The incubations were made with different concentrations of hCG in the medium: 0 (control), 10 and 100 IU/ml for either 24 or 48 h.

growth factors controlling cell differentiation and proliferation with extreme accuracy (10). One of these factors is leukemia inhibitory factor (LIF), which seems to have a role in the growth and differentiation of the trophoblast (11). In fact, the trophoblast, an epithelial cell line of fetal origin that forms the physical barrier between the mother and developing conceptus, becomes a component of the host immune system during pregnancy and releases cytokines such as CSF-1, GM-CSF, TNF-α, TGF-ß, IL-6 and LIF (12).

In conclusion, this study clearly demonstrates that pregnant plasma contains some compound(s) that are able to inhibit considerably the growth of the fast-growing rat AH-130 ascites hepatoma. Future work will concentrate on elucidating the nature of this compound that could have an important application in either the prevention or erradication of human tumours.

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