

# Effect of Sex Hormone Binding Globulin on the Development of Ovarian Cancer in a Mouse Model

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**Abstract:** Sex hormone-binding globulin (SHBG) is the major carrier protein of testosterone and estradiol in the blood. Although studies on the role of SHBG in ovarian cancer are inconclusive, substantial evidence indicates that SHBG-steroid complexes can play a direct role in the intracellular transport of steroids to cancer cells. SHBG is synthesized in the adult liver of many species, excluding rodents. Adult mouse and rat livers do not produce SHBG, and yet most experimental models of ovarian cancer employ these species. Data reported here indicate that SHBG has a major stimulatory effect on the growth of human ovarian cancer in athymic mice. The effect is observed in subcutaneous and intraperitoneal OVCAR-3 xenografts. Tumor doubling times were  $9.98 \pm 0.14$  days and  $17.20 \pm 0.64$  days when OVCAR-3 cells were implanted with and without SHBG, respectively ( $P = 0.023$ ). The magnitude of SHBG effect depended on the age of mice and it was most prominent in the development of intraperitoneal solid tumor deposits. Levels of circulating CA-125 were also age- and tumor size-dependent. Biodistribution studies of  $^{125}\text{I}$ -SHBG indicated a prolonged retention of the protein in solid tumor deposits. Estimated half-lives of  $^{125}\text{I}$ -SHBG were  $2.3\times$  longer in solid tumors as compared to nonadherent cancer cells in the peritoneal lavage. The normal tissue distribution of  $^{125}\text{I}$ -SHBG was similar in control and OVCAR-3-bearing mice. These data suggest that currently used models of ovarian cancer in mice, including carcinogenesis and drug evaluation studies, are imperfect because of the lack of SHBG production in these species.

## INTRODUCTION

Most of the attention in assessing the role of SHBG in carcinogenesis is focused on its ability as a carrier protein to regulate the bioavailability of testosterone in prostate cancer [1, 2]. Recent evidence suggests that SHBG, independent of its role as a carrier protein [2, 3], has a more complex function in cell signaling by direct binding to its receptor, inducing synthesis of cAMP and initiating downstream signaling [4-7].

The role of SHBG in ovarian cancer is largely undefined. Population-based case-control studies found no links between several genetic variants of SHBG and ovarian cancer risk [8]. No association between serum levels of SHBG and ovarian cancer risk was identified [9, 10]. Nevertheless, the pathophysiology of ovarian cancer supports a strong connection with androgens. It is still unclear, which of the hormone-regulated mechanisms are critical in the pathogenesis of ovarian cancer [11, 12]. It is possible that the receptor-mediated action of SHBG serves as an additional control mechanism, which alters the effects of sex hormones in cancer cells. It is of great interest from the standpoint of clinical applications that in some cancers SHBG levels in blood are inversely associated with cancer risk [13, 14]. SHBG bound to the membrane of breast cancer MCF-7 cells can inhibit the estradiol-induced cell proliferation [15, 16]. In contrast, the growth of human

prostate cancer ALVA-41 cells is stimulated by dihydrotestosterone and estradiol only in the presence of SHBG [7].

The investigation of these uncertainties of SHBG effects on the cancer development and the potential role of SHBG in the clinical management of cancer is complicated by the fact that *in vivo* studies are typically conducted in mice. Livers of most adult mammals secrete SHBG into the blood, where it circulates as the major sex hormone binding protein. In rodents, adult livers do not produce SHBG and as a result, the amounts of circulating SHBG are virtually undetectable. This deficiency of the mouse model has a significant negative impact on the progress of studies to define the SHBG biological activity as well as the drug evaluation studies [17]. Although a transgenic mouse able to secrete human SHBG has been generated [18], little is known about the susceptibility of this animal to cancer. In studies reported herein, we have established the stimulatory effect of human SHBG on the growth of androgen receptor-positive human ovarian cancer OVCAR-3 cells in athymic mice.

## MATERIALS AND METHODOLOGY

### Reagents, Buffers and General Procedures

SHBG was purchased from Lee Biosolutions, Inc. (St. Louis, MO). RPMI-1640 medium was from GIBCO<sup>®</sup> Invitrogen Cell Culture (Carlsbad, CA). [ $^{125}\text{I}$ ]NaI in  $1 \times 10^{-5}$  NaOH (pH 8-11) was obtained from PerkinElmer (Waltham, MA). Radioactivity was measured with Minaxi  $\gamma$ -counter (Packard, Waltham, MA), a dose calibrator (Capintec Inc., Ramsey, NJ), and for the HPLC effluent with the sodium iodide crystal flow-count detector (Bioscan, Washington, DC) connected in-line with the UV detector. Variable

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wavelength detectors UVIS-205 (Linear, Irvine, CA) and UV-116 (Gilson, Middleton, WI) were used. Quality control analyses of radiolabeled protein were done on TSK-GEL-3000SW column (7.5 cm × 30 cm, Tosohaas, Montgomeryville, PA) with 0.1 M sodium phosphate, 0.1 M sodium sulfate, pH 7.3 as the eluant, and the SDS-PAGE electrophoresis on precast Ready Gel Tris-HCl (4–20% resolving gel, 4% stacking gel, 10-well, 30 µl, 8.6 × 6.8 cm, Hercules, CA). Cancer Antigen CA-125 EIA kit was purchased from Panomics, Inc. (Fremont, CA).

### Mice

Athymic NCr-*nu/nu* mice were from the National Cancer Institute at Frederick (Frederick, MD). Mice were allowed to acclimate for no less than 5 days after arrival to the UNMC facilities. All protocols involving mice were approved by the UNMC Institutional Animal Care and Use Committee.

### *In vitro* Cell Survival Assay

OVCAR-3 cells were plated in a 96-well plate at 3,000 cells/well and allowed to attach for 24 h. Medium was removed and replaced with fresh medium containing 0 µg/mL (n=24), 10 µg/mL (n=18) and 100 µg/mL (n=18) SHBG. After 48 hrs in culture, spent medium was removed and cell proliferation was determined using CellTiter 96<sup>®</sup> Aqueous One Solution Cell Proliferation Assay (Promega, Madison, WI). Optical density at 492 nm was read using Opsys MR<sup>™</sup> 96-well microplate reader (Dynex Technologies, Chantilly, VA).

### Radioiodination of SHBG

SHBG was radioiodinated using the Iodogen method [19]. All radioiodinations were performed on site as follows: into a glass test tube coated with 0.1 mg of 1,3,4,6-tetrachloro-3 $\alpha$ ,6 $\alpha$ -diphenylglycouril (Iodogen; Sigma-Aldrich, St. Louis, MO) was added 0.2 mL of 0.1 mg/mL SHBG in PBS, pH 7.2 and 0.01 mL of iodine-125 (~1.2 mCi; 44 MBq). The mixture was incubated at room temperature for 10–15 min. The reaction progress was measured using instant thin layer chromatography with ethanol/PBS (1: 9; v/v) as the elution system. <sup>125</sup>I-SHBG was purified on a 2-mL Zeba Desalt Spin column (Pierce Biotechnology, Rockford, IL). Typically, <sup>125</sup>I-SHBG was obtained in ~50% radiochemical yield and the specific activity of ~30 mCi/mg. Before administration, <sup>125</sup>I-SHBG was diluted in 0.01 M phosphate buffer, pH 7.2 at 25°C, 0.0027 M potassium chloride and 0.137 M sodium chloride (PBS) containing 0.1% mouse serum (Sigma-Aldrich, St. Louis, MO) to yield the injection dose volume of 0.3 mL per mouse. Triplicate standards equivalent to 0.001 mL of the injected dose were prepared in 0.1 mL PBS/0.1% mouse serum and counted in a  $\gamma$ -counter alongside the necropsy samples.

### Biodistribution of <sup>125</sup>I-SHBG

Athymic NCr-*nu/nu* female mice were injected intraperitoneally (IP) with OVCAR-3 cells propagated as the IP xenografts. Cells were injected in 0.4 mL of serum-free RPMI-1640 medium. Six weeks later mice received IP injection of <sup>125</sup>I-SHBG in 0.3 mL PBS containing 0.1% mouse serum. The average injected dose was 13.4±0.6 µCi

(0.5±0.02 MBq). Mice were killed 1 h and 24 h after <sup>125</sup>I-SHBG injection. Mice without tumors injected with <sup>125</sup>I-SHBG were used as controls.

### OVCAR-3 Xenografts

Intraperitoneal model: athymic NCr-*nu/nu* female mice aged approximately 2, 6 and 12 months received IP implants of 1×10<sup>7</sup> OVCAR-3 cells either with SHBG or without. Mice were observed two times per week until first visible tumors were detected. From this point, mice were weighed 2–3 times per week and the presence of tumors was noted. The experiment was terminated when the estimated tumor burden reached ~10% body weight.

Subcutaneous (SQ) model: athymic 6–8 weeks old NCr-*nu/nu* female mice received SQ injection of 5×10<sup>6</sup> OVCAR-3 cells in 0.1 mL PBS supplemented with SHBG. Control mice received identical inoculum of OVCAR-3 cells in PBS but without SHBG. Mice were observed two times per week until measurable tumors developed. From this point, SQ xenografts were measured 2 times per week. The experiment was terminated when the estimated tumor burden reached ~10% body weight.

### Necropsy

Mice were euthanized according to the AVMA guidelines [20]. Blood was collected *via* a cardiac puncture. Hematocrit was determined by standard techniques. HemoCue<sup>®</sup> was used to measure hemoglobin levels (Ängelholm, Sweden). The remaining whole blood was used to prepare serum without additives, as required for the determination of CA-125. Serum was stored at -80°C until use. The peritoneal cavity was lavaged with 2 mL PBS to recover nonadherent OVCAR-3 cells and ascites. Tissues were dissected, rinsed in PBS and their wet weight was determined. For the biodistribution studies, dissected tissues were placed in  $\gamma$ -counter tubes and their radioactive content was determined.

### CA-125 Measurements

Serum prepared from a whole blood specimen without additives was stored at -80°C until ready to use. All reagents and serum were brought to room temperature and the protocol provided with the kit was followed. Standard curves were constructed using the reference standard set provided with the kit. Optical density at 450 nm was read using Opsys MR<sup>™</sup> 96-well microplate reader (Dynex Technologies, Chantilly, VA).

### Statistical Analyses

Summary statistics were performed using a two-sided, unpaired Student's t-test with a significance level of P=0.05. Tumor doubling times (T<sub>D</sub>) were calculated using the curve fitting function of SigmaPlot/SigmaStat (Systat Software, Inc. Point Richmond, CA).

## RESULTS

### *In vitro* Studies

The *in vitro* growth of OVCAR-3 cells did not appear to be influenced by SHBG. The quantity of formazan product,

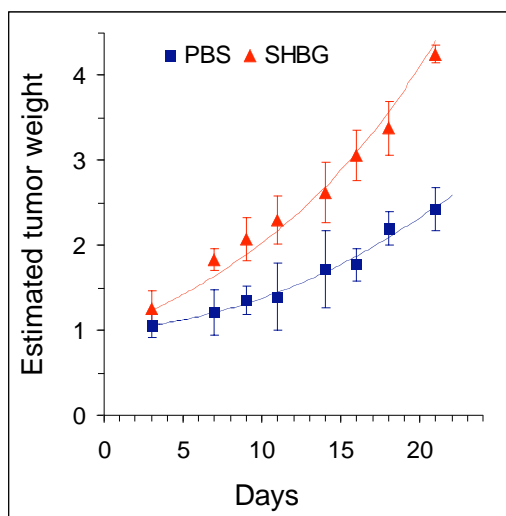
which is directly proportional to the number of living cells and is measured by the amount of 492 nm absorbance, did not indicate any notable changes in the OD in cells treated with SHBG compared to untreated controls. At the highest concentration of SHBG (0.1 mg/mL), only ~3% more growth was observed compared to OVCAR-3 cells grown in medium without SHBG and this increase was within the experimental error. OD values were as follows:  $0.469 \pm 0.011$ ,  $0.475 \pm 0.011$ , and  $0.482 \pm 0.009$  for 0, 0.01, and 0.1 mg SHBG/mL medium, respectively.

### Subcutaneous OVCAR-3 Xenografts

Only three out of five mice implanted SQ with OVCAR-3 cells in PBS developed SQ OVCAR-3 tumors whereas during the same period all mice injected with OVCAR-3 cells in PBS containing 0.01 mg SHBG developed SQ tumors. Tumors in PBS group were also significantly smaller. The average tumor weights at necropsy were  $0.21 \pm 0.07$  g and  $0.56 \pm 0.11$  g in PBS and SHBG groups, respectively.

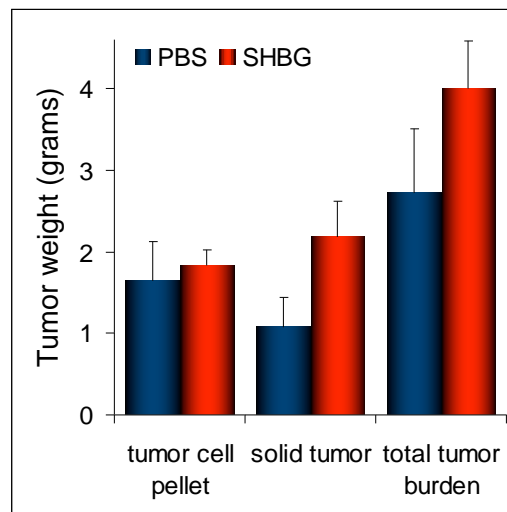
### Intraperitoneal OVCAR-3 Xenografts

OVCAR-3 cells implanted IP in medium containing SHBG developed tumors approximately twice as fast as tumors implanted without SHBG. Tumor doubling times estimated from the tumor growth curves were  $9.98 \pm 0.14$  days and  $17.24 \pm 0.64$  days, when OVCAR-3 cells were injected with SHBG and PBS, respectively (Fig. (1)). The accelerated growth rate of OVCAR-3 tumors in SHBG-treated mice was also evident from the necropsy data shown in Table 1 and Fig. (2). The average total tumor burden in PBS-treated mice was  $2.72 \pm 0.50$  g (median 2.39 g) compared to  $4.01 \pm 0.58$  g (median 3.89 g) in SHBG-treated animals, calculated collectively for all age groups. This



**Fig. (1).** Intraperitoneal OVCAR-3 tumor growth curves. Tumor weights were estimated from the increase of the total body weight relative to the body weight 38 days after the OVCAR-3 implant when mice began to develop palpable tumors (day 0). OVCAR-3 co-injected with SHBG (triangles) develop IP tumors faster with the estimated doubling time  $T_D = 9.98 \pm 0.14$  days. Control mice injected with OVCAR-3 and PBS (squares), in place of SHBG, develop slower with  $T_D = 17.20 \pm 0.64$  days.

difference derives primarily from the solid tumor burden (Table 1). Effects of SHBG on the growth of nonadherent OVCAR-3 cells collected with the IP lavage was less pronounced. The average cell pellet weight in PBS mice was  $1.64 \pm 0.48$  g compared to  $1.83 \pm 0.19$  g in SHBG-treated mice and this difference was not statistically significant.



**Fig. (2).** Average total tumor burden calculated collectively for all age groups in a given treatment scheme. Color bar represents the average tumor weight; the line is sem.

Six weeks after the OVCAR-3 implant, several mice in SHBG group were euthanized. The rapid increase in their body weights and distended abdomen indicated rapidly growing OVCAR-3 tumors and ascites. The necropsy revealed that in addition to copious ascites containing nonadherent tumor cells, large peritoneal deposits of solid tumors also formed. Multiple mesenteric tumors were observed along the entire length of the intestine. The intestine was not perforated. In most mice, large pancreatic tumors associated with the pyloric node and many smaller solid tumor nodules associated with renal, mesenteric, and lumbar or sacral lymph nodes were observed. Sporadically, when the solid tumor burden was  $>3$  g, both ovaries and uterus were also invaded. A similar tumor distribution was observed in the PBS-treated mice; however, solid tumor nodules were fewer and significantly smaller.

All mice in the SHBG group had copious ascites containing abundance of nonadherent cancer cells and frequent solid tumors. In contrast, in the PBS group, the overall tumor burden was much less and 20% of mice did not develop measurable OVCAR-3 tumors within the time frame of this study. OVCAR-3 cells exhibited the least aggressive behavior in the 58-days old mice and on an average, tumors took two weeks longer to reach the size similar to tumors in mice 207 days or 361 days of age at time of the OVCAR-3 implant (Table 2).

The difference in the growth of OVCAR-3 and its dependence on the animal age were also reflected in the levels of circulating CA-125 (Fig. (3)). Elevated CA-125 levels were observed in all mice bearing OVCAR-3 xenografts as compared to control mice without OVCAR-3 implants. Normal healthy mice do not have any measurable CA-125 in serum. Mice implanted with OVCAR-3 cells co-

**Table 1. Weight of OVCAR-3 Xenografts Collected at Necropsy in Mice Treated with either PBS (Control) or SHBG. Cancer Cell Pellets were Collected in a Peritoneal Lavage. Solid Tumors were Dissected during Necropsy. (Average Tumor Weights in grams and (sem) are Listed)**

Mice Age at Implant (days)	Treatment	Cancer Cell Pellet (g)	<i>P</i> value PBS vs. SHBG	Solid Tumor (g)	<i>P</i> value PBS vs. SHBG	Total Tumor (g)	<i>P</i> value PBS vs. SHBG
58	PBS	1.34 (0.42)	0.139	0.95 (0.29)	0.002	2.28 (0.67)	0.011
	SHBG	1.95 (0.05)		2.39 (0.22)		4.34 (0.20)	
207	PBS	2.29 (1.89)	0.692	1.92 (1.18)	0.689	4.21 (2.85)	0.972
	SHBG	1.67 (0.68)		2.64 (1.46)		4.31 (1.99)	
361	PBS	1.44 (0.23)	0.162	0.14 (0.03)	0.016	1.58 (0.26)	0.028
	SHBG	1.85 (0.18)		1.20 (0.31)		3.05 (0.48)	

injected with SHBG had significantly higher serum concentrations of CA-125 compared to PBS-treated mice. For example, in 361-days old mice, when OVCAR-3 cells were injected with PBS CA-125 at necropsy was  $251 \pm 17$  U/mL whereas when OVCAR-3 was co-injected with SHBG CA-125 levels were at  $402 \pm 47$  U/mL ( $P=0.012$ ), i.e., 60% higher. The amount of CA-125 was at the lowest level in the 58-days old mice bearing OVCAR-3 tumors when cells were implanted with PBS in place of SHBG and in this age group, CA-125 levels were not statistically different from the SHBG-treated tumors (Fig. (3);  $P=0.242$ ).

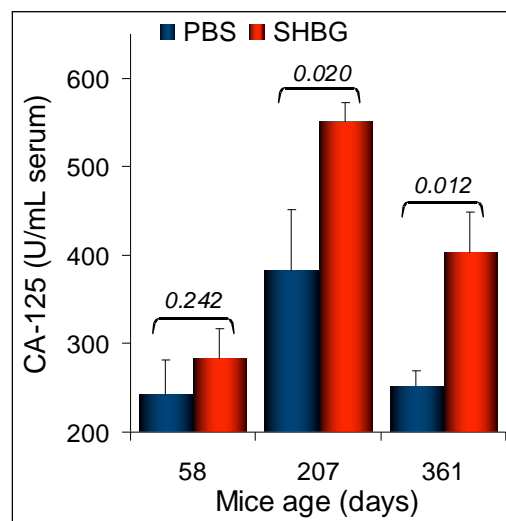
**Table 2. Percent of Mice with Palpable Tumors and Ascites at 45 Days and 54 Days after the IP Implant of OVCAR-3 Cells**

mice age	Days after IP OVCAR-3 implant	
	45	54
<b>PBS</b>		
361 days	50%	*
207 days	67%	67%
58 days	0%	0%
<b>SHBG</b>		
mice age	45	54
361 days	100%	*
207 days	75%	75%
58 days	0%	100%

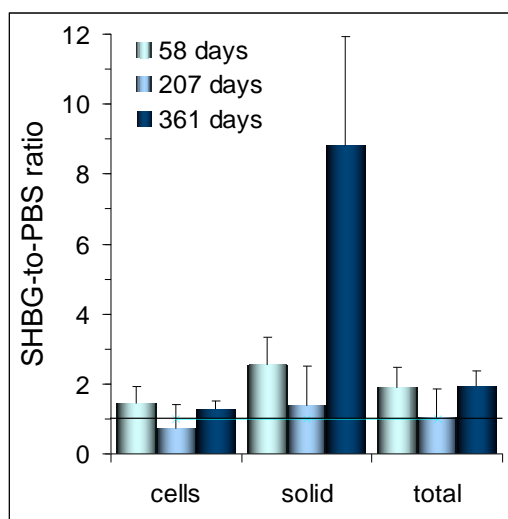
\*All mice in this age group were killed on day 45.

Although tumors were larger in younger animals (Table (1)), the magnitude of the SHBG effect on the growth of IP OVCAR-3 in 58-days- versus 361-days-old mice is similar, i.e., at necropsy, the total tumor burden in SHBG-treated mice was  $\sim 1.9 \times$  larger compared to PBS-treated tumors (Fig. (4); bars labeled total). However, this effect of SHBG did not hold in the “middle age” mice (age 207 days on the day of OVCAR-3 implant). In this group of mice, there was more

variability in the tumor development, which was also less dependent on the treatment. The average solid tumor weight at necropsy was  $1.92 \pm 1.18$  g and  $2.64 \pm 1.46$  g in PBS and SHBG groups, respectively in mice aged 207 days on the day of OVCAR-3 implant (Table 1). CA-125 levels in these mice, however, differed significantly between SHBG- and PBS-treated groups (Fig. (3)). Serum of PBS-treated mice in this age group contained  $383 \pm 69$  U CA-125/mL, whereas SHBG-treated mice had CA-125 levels at  $550 \pm 23$  U/mL (Fig. (3)).



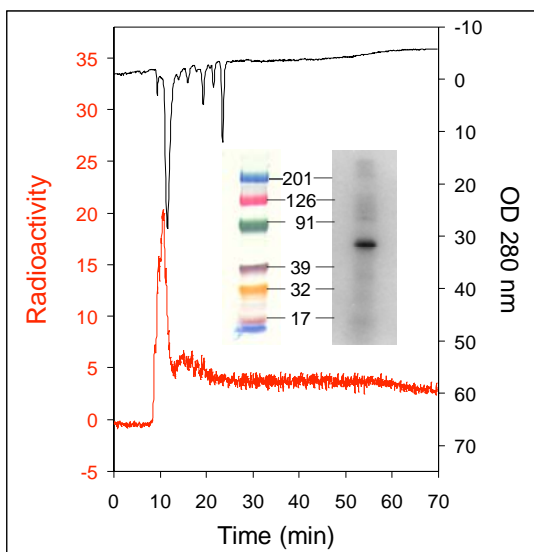
**Fig. (3).** Effects of SHBG on OVCAR-3 tumor development in 2, 6, and 12 months old mice as reflected by the Cancer Antigen 125 (CA-125) levels in serum. OVCAR-3 tumors were implanted IP at the indicated age of the animal. Serum was prepared from whole blood specimen without additives. Co-injection of SHBG significantly increases levels of CA-125 in serum. Numbers in italics above the bars represent *P* values for a two-tailed, homoscedastic Student's *t*-test for comparison of PBS to SHBG means.



**Fig. (4).** Differences between IP OVCAR-3 tumor development expressed as the ratio of the tumor weights lavaged (cells) and dissected during necropsy (solid tumor) in SHBG-treated mice to PBS-treated control mice on the day of necropsy. Ratios calculated for the total tumor burden is also shown.

#### Biodistribution of $^{125}\text{I}$ -SHBG in OVCAR-3-Bearing Mice and Control Mice

The radioiodination of SHBG with  $^{125}\text{I}$  was carried out using the Iodogen method. The purification on a Zeba column produced  $^{125}\text{I}$ -SHBG in ~50% radiochemical yield and the specific activity of 30 mCi/mg. Fig. (5) shows the quality control data for  $^{125}\text{I}$ -SHBG. The HPLC analyses were done on the size exclusion column. The red HPLC trace is the output from the radioactivity detector. The black line is the output from the UV detector connected in series. The



**Fig. (5).** Quality control analyses of  $^{125}\text{I}$ -SHBG used in biodistribution studies. HPLC of  $^{125}\text{I}$ -SHBG was done on the size exclusion column. (red trace is from the radioactivity detector; black is from the UV detector). The inset is the SDS-PAGE autoradiogram alongside the molecular weight markers. The radioactive band (black) corresponds to  $^{125}\text{I}$ -SHBG.

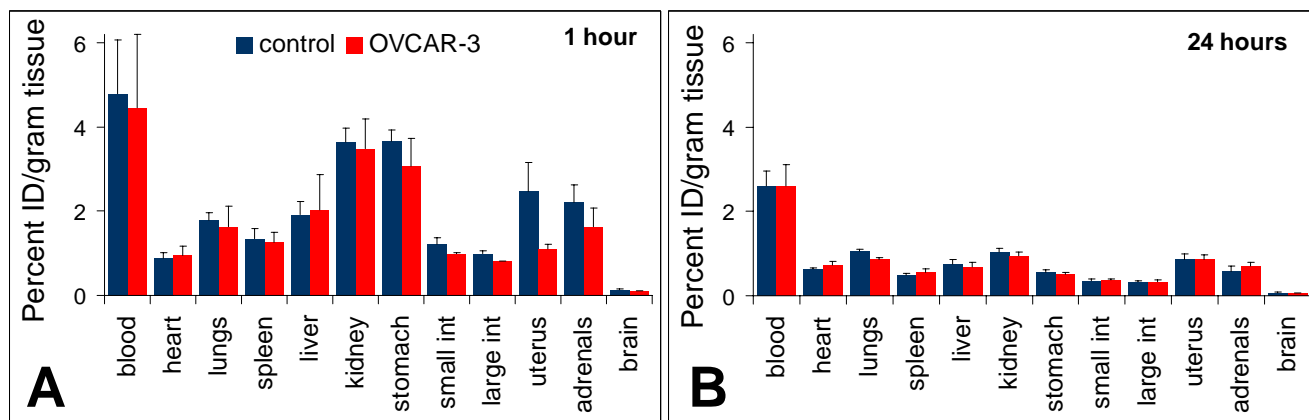
radioactive material elutes at the retention time, which corresponds to the molecular weight of the original material. This chromatographic property indicates that the size of the protein is not affected by the radioiodination procedure. The inset is the autoradiography of the gel (SDS-PAGE) run under denaturing conditions. Color bands are the molecular weight markers run alongside the  $^{125}\text{I}$ -SHBG aliquot. The SDS-PAGE autoradiogram shows a single radioactive protein band with a molecular weight of approximately 45,000 corresponding to one-half subunit of the SHBG homodimer. The HPLC and SDS-PAGE data confirm that intact SHBG was recovered after radioiodination.

Biodistribution was conducted in athymic mice bearing OVCAR-3 IP xenografts and age-matched control mice without tumors.  $^{125}\text{I}$ -SHBG was injected IP in PBS containing 0.1% mouse serum. Groups of mice were killed at 1 h and 24 h after administration of the radiotracer. Fig. (6A) and (6B) show the uptake of  $^{125}\text{I}$ -SHBG in several normal tissues and blood expressed as a percent-injected dose per gram tissue. There were no significant differences in normal tissue uptake between tumor carrying and control mice with the exception of uterus and adrenals. Uterus dissected from the control mice at 1 h retained  $2.48 \pm 0.67\%$  ID/g compared to  $1.10 \pm 0.11\%$  ID/g in OVCAR-3-bearing mice ( $P=0.04$ ). At 24 h after administration, the uptake in both groups was identical at  $0.87 \pm 0.12\%$  ID/g. Although there is a difference in the adrenal uptake, it is less prominent and at 1 h after administration in control mice it was recorded at  $2.20 \pm 0.42\%$  ID/g versus  $1.63 \pm 0.44\%$  ID/g in OVCAR-3 mice ( $P=0.16$ ). The differences in the uterus and adrenals uptake are most likely attributable to the lower intraperitoneal levels of  $^{125}\text{I}$ -SHBG in OVCAR-3-bearing mice because of the uptake of  $^{125}\text{I}$ -SHBG by tumor cells.

Tumor uptake of  $^{125}\text{I}$ -SHBG and the amount of  $^{125}\text{I}$  recovered in the peritoneal lavage are shown in Fig. (7A) and (7B). Half-lives of  $^{125}\text{I}$ -SHBG in tumors were estimated as 7.2 h and 16.8 h in nonadherent cancer cells and solid tumor deposits, respectively, assuming mono-exponential clearance curves. The blood clearance half-life was ~30 h and there was no difference between the control and OVCAR-3-bearing mice. At 24 h after administration, the supernatant from the peritoneal lavage of control mice did not contain any measurable amounts of radioactivity whereas OVCAR-3-bearing mice still had ~1.8% ID associated with the lavage as shown in Fig. (7B).

#### DISCUSSION

The biological activity of SHBG in cancer is still considered principally in the context of SHBG ability to regulate the bioavailability of sex hormones by binding them and preventing their diffusion across the cancer cell membranes. The inverse correlation of SHBG with some cancer risks is often attributed to this function of SHBG. Studies reported here reveal the stimulatory effect of SHBG on the growth of ovarian cancer in immunodeficient mice. This effect is more pronounced in older mice. In human subjects the ovarian cancer onset and risk is also age-dependent, i.e., the risk for ovarian cancer increases with age. Moreover, younger patients have approximately two times higher 5-year survival rates. SHBG appears to influence the growth of solid tumors to the greater extent



**Fig. (6).** Biodistribution of  $^{125}\text{I}$ -SHBG in normal tissues of athymic mice. OVCAR-3 cells were implanted IP and tumors were allowed to develop for 6 weeks. Control mice were age-matched.  $^{125}\text{I}$ -SHBG, 0.5 MBq/mouse, was IP injected 45 days after OVCAR-3 implant in PBS containing 0.1% mouse serum. Biodistribution was conducted 1 hour (A) and 24 h (B) after  $^{125}\text{I}$ -SHBG administration.

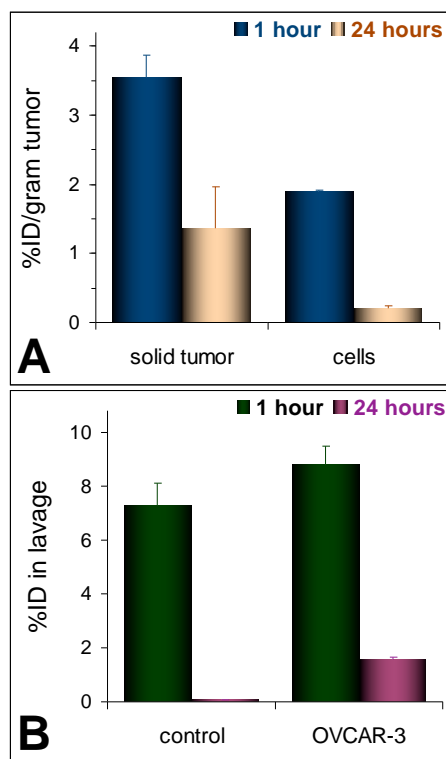
than nonadherent cancer cells in peritoneal ascites. This observation is in line with the biodistribution studies of  $^{125}\text{I}$ -SHBG in OVCAR-3-bearing mice. Although the distribution of  $^{125}\text{I}$ -SHBG in healthy mice and OVCAR-3-bearing mice did not reveal any major differences in the uptake by normal tissues, there was a noteworthy retention of  $^{125}\text{I}$ -SHBG in the solid peritoneal tumors. The retention of this protein in tumor indicates that a single dose of SHBG injected

alongside cancer cells can have a powerful and prolonged stimulatory effect on the growth of ovarian cancer. Moreover, there is a persistent presence of the IV injected  $^{125}\text{I}$ -SHBG in circulation indicating the bioavailability of this protein over a period of several days. The reported half-life of  $^{125}\text{I}$ -SHBG in *Macaca mulatta* is ~4 days [21].

Adult mice livers do not produce SHBG, therefore the enhanced ovarian cancer growth when cancer cells are co-injected with SHBG, can be directly attributed to the interaction of SHBG with cancer cells. This *in vivo* effect of SHBG on OVCAR-3 tumors is contrary to the apparent lack of any effects of SHBG in cells grown *in vitro*. The disparity of SHBG effects in cells grown *in vitro* and *in vivo* suggests a possible dual role for SHBG in the regulation of ovarian carcinoma: the classic activity of this protein as the extracellular binding reservoir for testosterone and estradiol and the second function as the growth factor. *In vivo* studies suggest that in some tissues both forms of the sex hormone, free and bound to albumin or SHBG, may be taken up by the tissue [22]. On the other hand, *in vitro* SHBG is believed to keep sex steroids inactive and to participate in controlling the amount of free hormones entering cells by passive diffusion [15, 16]. Therefore, the stimulatory effects of SHBG observed *in vivo* are probably canceled out *in vitro* by the deprivation mechanisms responsible for the inhibitory activity of SHBG in cell culture [23]. Moreover, sex hormones have been shown to have either no effect on the *in vitro* growth of human ovarian cancer cells, despite the abundant expression of the cognate receptors [24, 25], or in some instances they exert a significant inhibitory effect [26, 27].

## CONCLUSIONS

Sex hormone binding globulin stimulates the growth of human ovarian cancer in athymic mice. Data reported here indicate that *in vitro* models may be insufficient to illuminate the effects of SHBG on cancer cells in a multifactorial *in vivo* system. These data also indicate that the lack of SHBG in mice may be detrimental to the evaluation of the biological effects of SHBG and sex hormones in ovarian cancer, as well as in the evaluation of some anti-cancer drugs.



**Fig. (7).**  $^{125}\text{I}$ -SHBG uptake and retention in the peritoneal cavity and OVCAR-3 tumors. A. Percent injected dose (ID)  $^{125}\text{I}$ -SHBG per gram extirpated OVCAR-3 solid tumors and cells recovered in the peritoneal lavage. B. Radioactivity collected with the supernatants of the peritoneal lavage in control mice and OVCAR-3-bearing mice.



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