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Estrogen-induced Changes in Protein Binding of Bupivacaine during in Vitro *Fertilization*

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Background: **Patients having *in vitro* fertilization (IVF) procedures that use gonadotropin-releasing hormone agonist down-regulation undergo hormonal manipulation of estrogen concentrations to induce oocyte maturation. After achieving minimal estrogen concentrations (baseline), stimulation increases estrogen concentrations to supraphysiologic levels, leading to egg retrieval. The isolated effect of estrogen on protein binding has not previously been reported. This study was conducted to measure the effect of estrogen concentrations on protein binding of two concentrations of bupivacaine, 1 μg/ml and 5 μg/ml, corresponding, respectively, to systemic concentrations expected after administration of epidural anesthesia and associated with bupivacaine toxicity. Serum proteins were measured to address the mechanism.**

Methods: **Twenty-nine healthy women undergoing IVF procedures were enrolled and venous samples were drawn at times of minimal and maximal estrogen concentrations. The percentage of free bupivacaine was determined at fixed concentrations of 1 and 5 μg/ml. Serum albumin and α_1-acid glycoprotein concentrations were measured at baseline and at retrieval in a group of 24 women.**

Results: **The percentage of free bupivacaine increased between times of minimal and maximal serum estrogen concentrations, corresponding to decreased protein binding. Concentrations of serum albumin and α_1-acid glycoprotein decreased between baseline and retrieval times.**

Conclusions: **Patients undergoing IVF procedures demonstrate a decrease in protein binding of bupivacaine from baseline concentrations. These changes may be explained by a decrease in albumin and α_1-acid glycoprotein. During anesthesia for egg retrieval, clinicians should consider the implications of increased free fraction of drug, especially for highly protein-bound agents. (Key words: Anesthetics, local: bupivacaine. Hormone, female: estrogen. *In vitro* fertilization. Pharmacokinetics: protein binding.)**

THE influence of estrogen level on serum-free drug concentrations may have important clinical implications, particularly when patients are receiving more than one drug that is highly protein bound. Because clinical effects depend primarily on free concentration, a knowledge of factors that influence drug binding is critical when interpreting dose–effect relations.

Estrogen has been reported to significantly decrease albumin synthesis.[1] Previous studies from our group have reported that pregnancy is associated with significant decreases in protein binding to local anesthetics throughout pregnancy, presumably due to the effects of gestational hormones.[2] Patients undergoing *in vitro* fertilization (IVF) procedures present a unique opportunity to study the isolated effects of estrogen on protein binding. Patients who use gonadotropin-releasing hormone agonists (GnRH-a) for pituitary down-regulation undergo hormonal manipulation that ablates progesterone and results in estrogen ranging from essentially zero at baseline to supraphysiologic concentrations at the time of retrieval. The current study was designed to assess the effects of these large changes in estrogen concentration on the protein binding of bupivacaine, measured at two concentrations that would correspond, respectively, to the systemic concentration after appropriate administration of epidural anesthesia (1 μg/ml) and a systemic concentration associated with bupivacaine toxicity (5 μg/ml).[3,4]

Materials and Methods

The study was approved by our hospital's human research committee, and written informed consent was

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obtained from all participants. Patients classified as American Society of Anesthesiologists (ASA) physical status 1 who were scheduled to undergo IVF procedures were enrolled. Twenty-nine patients had estradiol and bupivacaine binding assays performed on their serum. Twenty-four additional IVF patients had serum albumin (n = 24) and α_1-acid glycoprotein (n = 14) assays performed as described below at times of baseline and retrieval. All patients were taking only medications involved in the IVF protocol.

The IVF protocol stimulation regimen used at the Brigham and Women's Hospital has been reported previously.[5] Briefly, human menopausal gonadotropins (Pergonal; Serono Laboratories, Randolph, MA; or Humegon; Organon Inc., North Orange, NJ; or FSH, Metrodin; Serono Laboratories) and GnRH-a leuprolide acetate (Lupron; Tap Pharmaceutical, Deerfield, IL) are administered in the mid-luteal or follicular phase of the menstrual cycle. Administration of this gonadotropin-releasing hormone agonist reduces estrogen and progesterone concentrations. This hormonal down-regulation is confirmed by serial hormonal measurements. Acceptable baseline hormonal concentrations for initiating the IVF cycle are $<$ 1.4 ng/ml progesterone and $<$ 50 pg/ml estradiol. After down-regulation, as confirmed by ultrasound and estradiol (E2) measurements, stimulation is initiated. Patients receive 10,000 IU human chorionic gonadotropin when at least two follicles exceed 18 mm in diameter and the E2 is $>$ 600 pg/ml. Transvaginal ultrasound-guided oocyte retrievals occur 48 h later.

Venous blood samples were drawn at two points: first at the time of maximum down-regulation (baseline) when baseline progesterone concentrations were $<$ 1.4 ng/ml and baseline E2 was $<$ 50 pg/ml; and second, before ultrasound-guided oocyte retrieval, when estrogen concentrations were maximal.

Venous blood samples were collected in plain glass blood collection tubes, allowed to clot, and then centrifuged to obtain serum. Serum was separated and stored at -20°C until required for protein binding determination. Serum rather than plasma was used to avoid any possible effects of *in vitro* lipolysis, as previously reported as a result of gestational hormone concentrations.[6,7] After thawing at room temperature, microliter quantities of bupivacaine HCl solution were added to serum samples to obtain concentrations of either 1 μg/ml or 5 μg/ml. Samples were adjusted to physiologic *p*H (7.40) with appropriate microliter quantities of 0.1 N HCl or 0.1 N NaOH and gently agitated at room temperature for 1 h. Serum water was obtained from an aliquot of serum using an ultrafiltration technique previously described[8] (Amicon MPS-1 with YMT membranes; Amicon Corp., Danvers, MA), with centrifugation at 2000*g* for 40 min.

Bupivacaine concentrations were determined using a gas chromatographic technique similar to that previously described.[9] Each sample was separated into serum and serum water and assays were examined at the 1 μg/ml and 5 μg/ml concentrations. Serum water assays were used to determine the free bupivacaine concentrations. The coefficients of variation for the assays were 6% for the serum studies at each concentration; the coefficient was 6% and 15% for the ultrafiltrate of serum concentrations at the lower and higher concentrations, respectively.

The Reproductive Endocrinology Division of the clinical chemistry laboratories of the Brigham and Women's Hospital analyzed the estradiol and progesterone concentrations by radioimmunoassay in accordance with the hospital's standard protocol. Albumin serum concentrations were measured by the clinical chemistry laboratories of the Brigham and Women's Hospital using the bromcresol green dye binding method of analysis. The α_1-acid glycoprotein concentrations were measured by the MetPath Clinical Laboratories, Quest Diagnostics, San Juan Capistrano, California, in accordance with standard protocols.

Statistical analysis was done using analysis of variance for repeated measures. Statistical significance was assumed at $P < 0.05$.

Results

Thirty-three women undergoing IVF procedures were prospectively enrolled in the study; 29 women completed the IVF cycle and had both baseline and retrieval samples analyzed. At baseline, the mean estrogen concentration was 19.2 pg/ml, which is consistent with our clinical laboratory's values for postmenopausal women. Just before retrieval, on the last day of gonadotropin stimulation, the mean estrogen concentration was 1,973.2 pg/ml, with a range of 689–4,882 pg/ml. Total serum concentrations of bupivacaine were not significantly different between baseline and retrieval samples at both concentrations used (table 1). The percentage of free bupivacaine in the serum of these women significantly increased between times of minimal and maximal estrogen concentrations at both lower (1 μg/ml) and higher (5 μg/ml) concentrations of bupivacaine (fig.

Table 1. Mean (± SD) Percent Free Bupivacaine at Therapeutic (1 μg/ml) and toxic (5 μg/ml) Serum Concentrations

	Baseline	Retrieval
Estrogen (E2) (pg/ml)	19.2 ± 9.7	1973.2 ± 1107.7*
% Free bupivacaine		
1 μg/ml	2.9 ± 1.0	6.4 ± 4.1*
5 μg/ml	9.1 ± 4.6	16.1 ± 6.0*
Serum bupivacaine concentration		
1 μg/ml	.94 ± 0.06	.93 ± 0.06
5 μg/ml	4.57 ± 0.40	4.62 ± 0.49

* Indicates $P < .0001$.

1). Serum albumin and α_1-acid glycoprotein levels significantly decreased between times of baseline and retrieval (table 2).

Discussion

The effect of estrogen on protein binding of bupivacaine has not been reported before. Several articles have described alterations in serum protein electrophoresis after either estrogen administration or during pregnancy.[10-12] However, in the patients in whom estrogen administration was a part of oral contraceptive use, expected systemic concentrations would be much lower than those seen after an IVF stimulation cycle. In addition, in the articles published, systemic estrogen concentrations were not documented, making it difficult to assess the extent of any effects.

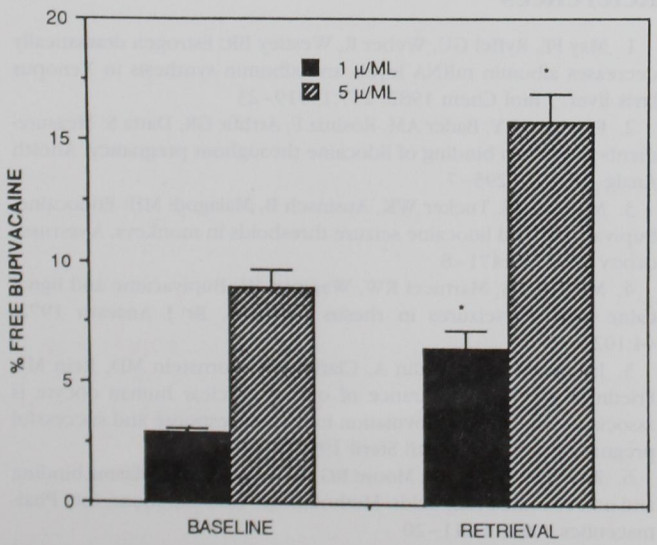

Fig. 1. Mean percentage of free bupivacaine at baseline and at the time of egg retrieval at therapeutic (1 μg/ml) and toxic (5 μg/ml) serum concentrations. Error bars represent SEMs. *Significant difference from baseline values.

Table 2. Mean (± SD) Serum Albumin and α_1-acid Glycoprotein Concentrations at Times of Baseline and Retrieval

	Baseline	Retrieval
Albumin (g/dl) (N = 24)	3.98 ± 0.65	3.77 ± 0.69*
α_1-acid glycoprotein (mg/dl) (N = 14)	71.8 ± 14.2	61.6 ± 17.4*

* Indicates $P < 0.05$.

The patient having IVF procedures presents a unique opportunity to study estrogen effects. These patients undergo hormonal manipulation that ablates progesterone and results in estrogen concentrations ranging from a mean of 19.2 pg/ml at baseline to supraphysiologic concentrations at the time of retrieval (table 1). In addition, each patient can be used as her own control when baseline and retrieval values are compared. The mean estradiol concentrations at the time of retrieval in our patients approaches and not infrequently exceeds peak estradiol concentrations during pregnancy (600-3,000 pg/ml at full term).[13]

Previous studies from our group have reported that increases in gestational hormones have been associated with a significant decrease in protein binding to local anesthetics throughout pregnancy. Our group recently published a study defining the pattern of protein binding throughout gestation to therapeutic serum concentrations of lidocaine.[2] For the current study involving IVF patients, bupivacaine was chosen as the local anesthetic. Lidocaine has a relatively high hepatic extraction ratio and intermediate protein binding; bupivacaine has a low hepatic extraction ratio and is highly protein bound. Therefore, hepatic extraction of bupivacaine is more likely to be altered by changes in protein binding.

Bupivacaine is known to have a nonlinear binding pattern.[14] Bupivacaine binds to both albumin (a low-affinity, high-capacity binding site) and α_1-acid glycoprotein (a low-capacity, high-affinity binding site). The relative influence of these two binding sites depends on the concentration of bupivacaine, with α_1-acid glycoprotein predominating at lower concentrations and albumin at higher concentrations.

Increasing progesterone concentrations have not

been shown to alter the protein binding of bupivacaine[15]; therefore, the role of estrogen in this regard was addressed. The mechanism of the estrogen effect on the protein binding of bupivacaine is unclear. Displacement of bupivacaine from binding sites on albumin is probably unlikely because in animal models albumin has been shown to have virtually no binding affinity for estradiol.[16]

Our study shows an inverse relation between serum estrogen concentrations and both serum albumin and α_1-acid glycoprotein levels. With the dramatic increases in estrogen concentrations produced by the IVF procedure, significant decreases in serum albumin and α_1-acid glycoprotein were seen. Estrogen was previously associated with decreases in albumin production.[1] The mechanism of this effect involves a significant decrease in mRNA transcription and production of serum protein, with synthesis decreased in parallel with the mRNA levels for the first 8 days after an estrogen injection and remaining decreased for a short period after mRNA amounts return to normal. Estradiol has also been shown to inhibit transcription of the human glycoprotein hormone α-subunit gene.[17] Long-term administration of estradiol in mice inhibits transcription of the gene encoding the α-subunit of pituitary glycoprotein hormones. Thus the decrease in protein binding with higher estrogen concentrations is due to hormonally induced decreases in the concentrations of α_1-acid glycoprotein and albumin.

Decreases in albumin at full term have been documented in the parturient[18]; changes in α_1-acid glycoprotein are more equivocal, with both increases[18] and decreases[10,12] reported to exist in full-term parturients. We found that high estrogen levels would be associated with a decrease in this glycoprotein. As noted previously, bupivacaine binds to albumin and α_1-acid glycoprotein in relative percentages dependent on bupivacaine concentration.

Our data show that the same serum bupivacaine concentration is associated with a significant increase in the percentage of free bupivacaine from baseline to the time of retrieval, when estrogen concentrations are maximal. These results correspond to a decrease in protein binding, due to a decrease in serum proteins. Although the magnitude of these changes is small, the percentage of change in free drug is large. This effect should be considered when administering any drug dependent on protein binding, either for anesthetic or any other therapeutic purpose, to IVF patients. Because many of these patients receive various intravenous agents at the time of retrieval, the effects may be significant. For example, alterations resulting in binding of diazepam may manifest as an increased incidence of drug-related side effects.[19] In addition, decreases in protein binding will lead to increased free drug concentrations of d-tubocurarine, metocurine, propranolol, sufentanil, and lidocaine.[20] Diphenylhydantoin, meperidine, quinidine, and desipramine have all been reported to increase the free concentration of bupivacaine, presumably through a drug-displacement mechanism.[21]

In conclusion, IVF patients have a significant increase in the free fraction of bupivacaine between baseline and time of oocyte retrieval at 1 μg/ml and 5 μg/ml concentrations of drug. This change corresponds to a decrease in protein binding due to a decrease in serum protein concentrations. The clinical effects in these patients of an increase in free fraction of drugs dependent on protein binding should be considered when administering protein-bound drugs to women undergoing ovarian hyperstimulation. Because the clinical effects depend on free drug concentration, alterations in systemic free drug concentration with estrogen level may have important clinical implications. This is particularly true when patients with high estrogen concentrations receive more than one drug that is highly protein bound. A knowledge of factors that influence drug binding is necessary to be able to interpret dose-effect relations.

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