

GROWTH HORMONE SERUM CONCENTRATIONS IN BITCHES WITH SPONTANEOUS MAMMARY TUMORS BEFORE AND AFTER MASTECTOMY

Concentraciones séricas de hormona de crecimiento en perras con tumores mamarios espontáneos antes y después de la mastectomía

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ABSTRACT

An autonomous non-pituitary production of growth hormone GH in the mammary gland has been reported in dogs. To assess the impact of mastectomy of tumoral glands on GH serum concentrations in clinical cases of spontaneous mammary tumors in eighteen intact bitches mastectomy of all tumoral glands was carried out. Three healthy bitches were maintained under the anaesthetic protocols for a similar period to the surgical procedure. Blood samples for GH determination were taken by peripheral venipuncture before (day -1), 2 and 11 hours and 7 and 14 days after mastectomy or anaesthesia. Tissue samples of the tumoral mammary glands of Group 1 were histologically examined. Serum GH was measured by a homologous liquid phase radioimmunoassay. Histology findings were tubular (n=2) and papillary carcinoma (n=4), tubular adenocarcinoma (n=8), carcinosarcoma (n=3) and complex carcinoma (n=1). Mean \pm SEM serum GH concentrations (ng/ml) of the treated group before and after surgery were 17.3 ± 2.8 ; 8.5 ± 1.5 ; 6.3 ± 1.1 and 6.1 ± 1.5 for -1 day, 2 hours, 7 and 14 days, respectively. The same values for the sham group were 4.2 ± 1.0 ; 5.5 ± 1.3 and 5.2 ± 1.5 for -1 day, 2 and 11 hours after anaesthesia. After surgery, serum GH showed a decrease (mean \pm SEM) of $-22.6 \% \pm 4.9$ at 2 hours, $-31.8 \% \pm 5.1$ on day 7, and $-29.7 \% \pm 9$ on day 15 while it increased to $31.1 \% \pm 08$ and $26.3 \% \pm 9$ at 2 hours and 11 hours, respectively after anaesthesia in the sham

group. It is concluded that in bitches ectopic GH is produced also by spontaneous malignant mammary gland tumors.

Key words: (growth hormone), (mammary tumor), (mastectomy), (bitch)

RESUMEN

La producción no pituitaria y autónoma de hormona del crecimiento (GH) en la glándula mamaria ha sido reportada en perros. Se determinó el impacto de la mastectomía de glándulas mamarias tumorales sobre las concentraciones séricas de GH, en casos clínicos de tumores mamarios espontáneos de dieciocho perras enteras, a las que se les realizó mastectomía de todas las glándulas tumorales. Tres perras sanas fueron mantenidas solo bajo protocolo anestésico por un período similar al del procedimiento quirúrgico. Se tomaron muestras sanguíneas por venopunción periférica para determinar GH sérica, antes (día -1), 2 y 11 horas y 7 y 14 días después de la mastectomía o de la anestesia. Se realizó el estudio histopatológico de las glándulas mamarias tumorales extirpadas en la cirugía. La GH sérica fue determinada por un radioinmunoensayo homólogo de fase líquida. La media \pm SEM (ng/ml) del grupo tratado antes y después de la cirugía fue de 17.3 ± 2.8 ; 8.5 ± 1.5 ; 6.3 ± 1.1 y 6.1 ± 1.5 para el día -1, 2 horas, 7 y 14 días respectivamente. Los mismos valores para el grupo control fueron de 4.2 ± 1.0 ; 5.5 ± 1.3 y 5.2 ± 1.5 para el día -1, 2 y 11 horas luego de la anestesia. Después de la cirugía, la GH sérica descendió (media \pm SEM) $-22.6 \% \pm 4.9$ a las 2, $-31.8 \% \pm 5.1$ al día 7, y $-29.7 \% \pm 9$ al día 15, mientras que en el grupo control se incrementó en un $31.1 \% \pm 0.8$ y $26.3 \% \pm 9$ a las 2 y 11 horas, respectivamente luego de la anestesia. La histopatología de las mamas tumorales analizadas reveló carcinoma tubular y papilar, adenocarcinoma, carcinosarcoma y carcinoma complejo. Se concluyó que en perras la GH ectópica es también producida por la glándula mamaria con tumores espontáneos malignos.

Palabras Clave: (hormona de crecimiento), (tumor mamario), (mastectomía), (perra)

INTRODUCTION

An autonomous non-pituitary production of growth hormone (GH) in the mammary gland has been reported in dogs (Concannon et al 1980; Selman et al 1993; van Garderen et al 1997; Kooistra et al 1998). In the dog endogenous progesterone and synthetic progestins are known to induce GH excess that results in characteristic signs of acromegaly (Misdorp 1991; Selman et al 1991; Selman 1994; Mol et al 1996). There is evidence that the synthetic antiprogestin RU486 inhibits that GH overproduction in acromegalic dogs (Watson et al 1987). Hypophysectomy of dogs with progestins-induced GH excess did not result in a decrease of the elevated GH concentrations in plasma (Selman et al 1994).

Mammary gland tumors are the most prevalent tumors in the female dog and hormonal factors are described to be involved in their

development (Hampe and Misdorp 1974; Emerman et al 1985; Rutteman 1990; Rutteman 1992; Gobello et al 2001). Several malignant tumor types can be identified in the bitch, with carcinomas being the predominant type (van Garderen et al 1997). In one study, immunohistochemical investigation revealed the presence of GH in 18 of 19 malignant mammary tumors (van Garderen et al 1997). Ectopic production of GH by the mammary gland was confirmed by the lowering GH plasma concentrations to basal levels within 2 hours after complete mastectomy in two bitches with progestin-induced mammary tumors and elevated GH plasma concentrations (Selman et al 1994). Immuno-histochemical staining revealed that GH immunoreactivity was localized in focal areas of hyperplastic ductular epithelium. In mammary tissue of healthy untreated female dogs no GH immunoreactivity

was found (Selman et al 1994). It was, therefore, of interest to assess the impact of mastectomy of tumoral glands on GH serum concentrations in clinical cases of spontaneous mammary neoplasia.

MATERIALS AND METHODS

Animals

Eighteen intact bitches (9 crossbred, 7 German Shepherds, and 2 Doberman), 4 to 12 years of age with spontaneous mammary tumors, which were clinically compatible with neoplasia, were included in this study (Group 1). Three healthy bitches with similar characteristics (1 crossbred, 1 Doberman, and 1 German Shepherds) were used a sham non-operated group (Group 2).

Procedure

All the bitches were premedicated with diazepam, nalbufina and atropine sulfate, and anaesthesia was induced with sodium thiopental. After endotracheal intubation anaesthesia was maintained using halothane, and oxygen, which was delivered in a closed system. The bitches were positioned in dorsal recumbency and mastectomy of all tumoral glands was carried out by routine surgical techniques (Johnston 1993). The 3 sham non-operated bitches were maintained under the same anaesthetic protocol for periods similar to the surgical procedure. Tissue samples of the tumoral mammary glands of the bitches of Group 1 were fixed in 10 % formalin for the histological study. Mammary tumors were classified according to the World Health Organization (Hampe and Misdorp 1974) into six broad classes: carcinoma, sarcoma, carcinosarcoma, benign or apparently benign tumors, unclassified tumors and benign or apparently benign dysplasias.

Blood samples for GH determination were taken by peripheral venipuncture before (day –

1), 2 and 11 hours and 7 and 14 days after mastectomy or anaesthesia for Groups 1 and 2. Samples were centrifuged at 4,000 g for 15 min. Serum was separated and stored at –20°C until hormone assays were conducted.

Hormone Assay

Serum GH was measured by a homologous liquid phase radioimmunoassay (RIA) with the materials provided by Dr. A.F. Parlow, Pituitary Hormones and Antisera Center, Harbor-UCLA Medical Center, Torrance CA, USA. The intra- and inter-assay coefficient of variation were 8.2 and 13.8, respectively. The sensitivity at 95 % binding was 0.8 ng/ml.

Statistical Analyses

Mean \pm SEM GH serum concentrations (ng/ml) were calculated for the two groups at each time point (day –1; n = 21, 2 hours (n = 17) and 11 hours (n = 3), 7 days (n = 12) and 14 days (n = 8) after mastectomy or anesthesia for Groups 1 and 2, respectively. The percentage change of GH during the experiment was calculated for each group at each time point of the protocol with the following formula: [final value (at each time point) – initial/value (day –1)] / initial value] x 100.

RESULTS

Histology findings of Group 1 were tubular (n=2) and papillary carcinoma (n=4), tubular adenocarcinoma (n=8), carcinosarcoma (n=3) and complex carcinoma (n=1).

Mean \pm SEM serum GH concentrations (ng/ml) of Group 1 before and after surgery were 17.3 \pm 2.8; 8.5 \pm 1.5; 6.3 \pm 1.1 and 6.1 \pm 1.5 for – 1 day, 2 hours, 7 and 14 days, respectively. The same values for the sham non operated group were 4.2 \pm 1.0; 5.5 \pm 1.3 and 5.2 \pm 1.5 for –1 day, 2 and 11 hours after anesthesia. The percentage changes of GH during the experiment for both groups are represented in **Figure 1**.

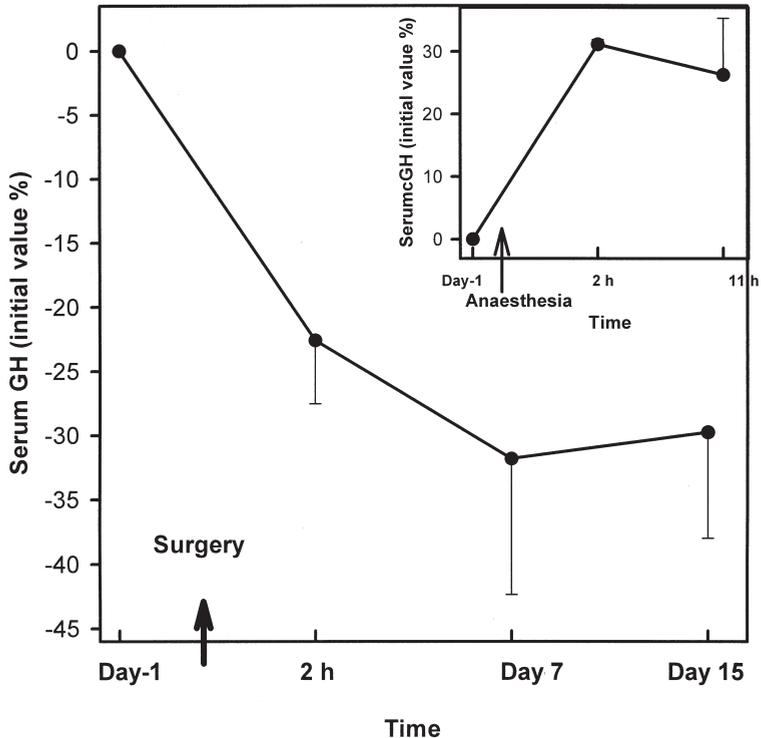


Figure 1: Percentage change of GH concentrations (ng/ml) for each day of the protocol to day –1 before and after mastectomy of neoplastic glands in eighteen intact the bitches. Inset: Same percentage 2 and 11 hours after anaesthesia in 3 bitches of a sham non- operated group. Bars on the symbols represent SEM.

CONCLUSIONS AND DISCUSSION

Mean \pm SEM serum GH concentrations at the beginning of the experiment in the operated Group were higher than our reference range for this hormone (Corrada et al 2001; Gobello et al 2002) suggesting mammary production for this hormone. In this Group removal of all tumoral tissue resulted in a decline of serum GH concentrations to our upper reference range value (Corrada et al 2001; Gobello et al 2002) while an increase for this hormone was evidenced in the sham non- operated group up to 11 hours after anaesthesia.

In humans, GH disappears from plasma with an initial half-life of about 20 to 25 min., being

the clearance rate between 100 and 150 ml/m² of body surface/min (Thorner et al 1998). In line with the present study, ectopic production of GH in the mammary gland was confirmed by lowering of plasma GH concentrations to reference range within 2h after complete mamnectomy (Selman et al 1994). Thus, dogs may very probably have a similar rapid clearance of this hormone. Conversely, post anaesthesia GH elevation in the control group may be due to the increasing effect of stress on GH serum concentration (Martin et al 1978)

The mechanisms involved in the production of elevated GH levels by tumoral mammary gland are not obvious, and information on GH regulation in the normal bitch is also limited

(Selman 1004). The local GH production may be followed by mammary IGF production, enhancing neoplastic changes in the gland (Mol et al 1997). GH production was found in the great majority of benign and malignant mammary tumors (Mol et al 1995; Mol et al 1996). The significance of production for tumor growth is not clear yet. If canine mammary tumors also preferentially originate in these structures, in which it is frequently detected GH diffusely, then it can be explained that these tumors have the potential to produce GH (van Garderen 1997).

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