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Invited review

Treating canine Cushing's syndrome: Current options and future prospects

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ABSTRACT

Naturally occurring hypercortisolism, also known as Cushing's syndrome, is a common endocrine disorder in dogs that can be caused by an adenocorticotrophic hormone (ACTH)-producing pituitary adenoma (pituitary-dependent hypercortisolism, PDH; 80–85% of cases), or by an adrenocortical tumor (ACT; 15–20% of cases). To determine the optimal treatment strategy, differentiating between these two main causes is essential. Good treatment options are surgical removal of the causal tumor, i.e. hypophysectomy for PDH and adrenalectomy for an ACT, or radiotherapy in cases with PDH. Because these options are not without risks, not widely available and not suitable for every patient, pharmacotherapy is often used. In cases with PDH, the steroidogenesis inhibitor trilostane is most often used. In cases with an ACT, either trilostane or the adrenocorticolytic drug mitotane can be used. Although mostly effective, both treatments have disadvantages. This review discusses the current treatment options for canine hypercortisolism, and considers their mechanism of action, efficacy, adverse effects, and effect on survival. In addition, developments in both adrenal-targeting and pituitary-targeting drugs that have the potential to become future treatment options are discussed, as a more selective and preferably also tumor-targeted approach could have many advantages for both PDH and ACTs.

Introduction

Hypercortisolism, often referred to as Cushing's syndrome, was first described by the neurosurgeon Harvey Cushing in 1932 (Cushing, 1969), and is characterized by chronically increased circulating glucocorticoids. Hypercortisolism can be either iatrogenic, caused by glucocorticoid administration, or occur naturally, caused by excessive endogenous cortisol production (Galac et al., 2010a).

Naturally occurring hypercortisolism is a common endocrine disorder in dogs, with an incidence of 1–2 cases per 1000 dogs per year (Willeberg and Priester, 1982; O'Neill et al., 2016). In 80–85% of cases, the condition is caused by an adenocorticotrophic hormone (ACTH)-secreting pituitary adenoma (pituitary-dependent hypercortisolism; PDH). In the remaining 15–20%, it is most often caused by a cortisol-secreting adrenocortical tumor (ACT), which is classified as an adrenocortical carcinoma in the majority of cases (Labelle et al., 2004; Galac et al., 2010a). Rare causes of hypercortisolism in dogs include ectopic ACTH syndrome (Galac et al., 2005) and food-dependent hypercortisolism (Galac et al., 2008).

Diagnosis

The diagnosis of hypercortisolism should be based mainly on the dog's medical history and clinical signs. Hypercortisolism usually occurs in middle-aged to older dogs (Kooistra and Galac, 2012; O'Neill et al., 2016). The most common clinical signs include polyuria, polydipsia, polyphagia, central obesity, hepatomegaly, panting, muscle atrophy, progressive bilateral alopecia, and systemic hypertension. Other clinical signs include hyperpigmentation, calcinosis cutis and insulin-resistant diabetes mellitus (Galac et al., 2010a; Behrend et al., 2013; O'Neill et al., 2016). Additionally, the pituitary tumor or ACT can induce mass-occupying effects. In cases with a large pituitary tumor, these effects include neurological signs such as anorexia, lethargy, and altered behavior. In cases with an ACT, these effects develop secondary to metastases or invasion of the ACT into the phrenicoabdominal vein or caudal vena cava (Galac et al., 2010a; Behrend et al., 2013).

When there is clinical suspicion of hypercortisolism, the results of a complete blood count (CBC), serum biochemistry panel, urinalysis and blood pressure measurement may further support the diagnosis. Abnormalities that can be found in these tests include the presence of a stress leukogram, increased serum

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alkaline phosphatase (ALP) activity, and low urine specific gravity. None of these findings are pathognomonic, but can be supportive of hypercortisolism (Behrend et al., 2013).

Endocrine tests should be used to further confirm the suspicion of hypercortisolism. It is important to only test for hypercortisolism in dogs with a high degree of clinical suspicion to decrease the chance of false-positive results (Gilor and Graves, 2011). The recommended screening tests are the low-dose dexamethasone suppression test or the urinary corticoid:creatinine ratio (UCCR). The UCCR can also be combined with the high-dose dexamethasone suppression test (HDDST). When the hypercortisolism is suppressible (>50%) by dexamethasone the dog is diagnosed with PDH. When the hypercortisolism is non-suppressible, further differentiation requires measurement of plasma ACTH concentration and/or diagnostic imaging. A CT or MRI scan is preferred to determine the size and contour of the pituitary and adrenal glands, and in case of an ACT also to detect vascular invasion and to screen for metastases (Galac et al., 2010a; Kooistra and Galac, 2012; Behrend et al., 2013). Moreover, pituitary tumors and ACTs can coexist (Greco et al., 1999; Beatrice et al., 2018), which could be missed without complete imaging. Differentiating between the two main causes of hypercortisolism is essential when choosing the optimal treatment strategy (Behrend et al., 2013).

Comparative pathobiology

Many similarities exist between hypercortisolism in dogs and humans, including the clinical signs, diagnostics, and medical care (De Bruin et al., 2009; Kooistra et al., 2009). Consequently, new insights in human hypercortisolism can advance the understanding of and treatment for canine hypercortisolism, and vice versa. In this review we will therefore not only focus on current treatment options for canine hypercortisolism, but also on advancements in the treatment of human hypercortisolism. Additionally, we discuss promising drugs that might develop into future treatment options.

Therapy

The goals of treating canine hypercortisolism would optimally be to eliminate the source of either ACTH or autonomous cortisol excess, to achieve normocortisolism, to eliminate the clinical signs, to reduce long-term complications and mortality, and to improve the quality of life. Surgical removal of the causal tumor or radiotherapy are currently the only treatment options that have the potential to eliminate the source of either ACTH or autonomous cortisol excess. However, these options are not without risks, not widely available and not appropriate for every patient. Pharmacotherapy is a commonly used treatment that aims to eliminate the clinical signs of the condition. A combination therapy of medical treatment with radiotherapy is also possible (Galac et al., 2010a; Pérez-Alenza and Melián, 2016).

Without treatment, dogs with PDH have a median survival time of 359 days (95% confidence interval (CI), 271–829) (Kent et al., 2007) to 506 days (95% CI, 292–564) (Nagata et al., 2017). There are no data on the survival of dogs with an ACT without treatment.

Surgery

Hypophysectomy

Hypophysectomy in dogs is performed using a transsphenoidal approach where the entire pituitary gland is removed (Meij, 2001; Meij et al., 2002). In a recent study with a large cohort of 306 dogs with PDH that underwent hypophysectomy (van Rijn et al., 2016), 91% of the dogs were alive after 4 weeks, of which remission was confirmed in 92%. Of the dogs that were in remission, disease recurrence was observed in 27%. The median survival time was 781 days (range, 0–3808 days) and the median disease-free interval of the dogs that were in remission was 951 days (range, 31–3808 days).

Replacement therapy after hypophysectomy consists of lifelong administration of glucocorticoids and thyroxine, and temporary administration of desmopressin, a synthetic vasopressin analogue (Meij, 2001; Hanson et al., 2005; Galac et al., 2010a). The main complications of hypophysectomy are perioperative death, transient mild postoperative hypernatremia, transient reduction or cessation of tear production, prolonged or permanent diabetes insipidus, and recurrence of hypercortisolism (Meij, 2001; Meij et al., 2002).

Factors that negatively influence the prognosis include a high pituitary height/brain area (P/B) value, old age, high preoperative circulating ACTH concentration, and high pre- and postoperative UCCRs (Hanson et al., 2007; van Rijn et al., 2015, 2016). Although a high P/B value is a negative prognostic indicator, hypophysectomy remains a good treatment option also for large pituitary tumors (Fracassi et al., 2014; van Rijn et al., 2016). The main limitation of hypophysectomy is that it is available only in large veterinary centers with an established team of experienced surgeon(s), anesthetist(s), critical care specialist(s) and endocrinologist(s), with consequently high initial costs (Pérez-Alenza and Melián, 2016).

Adrenalectomy

Adrenalectomy is recommended for dogs with uni- or bilateral ACT. Adrenalectomies were traditionally performed as ventral or paracostal open laparotomies. Perioperative mortality rates were quite high in initial studies (Scavelli et al., 1986), but improved in later studies (van Sluijs et al., 1995; Anderson et al., 2001; Kyles et al., 2003; Schwartz et al., 2008; Massari et al., 2011) and are as low as 6–8% in most recent studies (Lang et al., 2011; Mayhew et al., 2014). Adrenalectomy can also be performed laparoscopically. Laparoscopic adrenalectomy has been used in human medicine since the early 1990s and has recently been gaining interest and shown to have benefits in veterinary medicine as well (Naan et al., 2013; Mayhew et al., 2014).

Reported median survival times for dogs undergoing adrenalectomy range from 778 days (range, 1–1593) (Anderson et al., 2001) to 953 days (range, 0–1941) (Massari et al., 2011). When dogs survive the perioperative period, the long-term survival is good (Anderson et al., 2001; Lang et al., 2011). The main complications that can occur include minor to severe hemorrhage, hypotension, tachycardia and peri-operative death (van Sluijs et al., 1995; Lang et al., 2011; Massari et al., 2011; Mayhew et al., 2014). The tumor capsule can rupture, possibly more often in laparoscopic than in open adrenalectomies, but does not commonly lead to tumor regrowth (Mayhew et al., 2014). The main complications that can occur postoperatively include pancreatitis and thromboembolism (van Sluijs et al., 1995; Anderson et al., 2001; Mayhew et al., 2014). The reported hypercortisolism recurrence rate varies between 12% (Anderson et al., 2001) and 30% (van Sluijs et al., 1995), which can be either because of regrowth of the ACT or metastases.

Adrenalectomy is not recommended in patients that have metastases or extensive vascular invasion, which is why thorough presurgical diagnostic imaging is imperative. Vascular invasion does not necessarily exclude patients from undergoing adrenalectomy, since some studies indicate that tumor invasion in the caudal vena cava does not affect perioperative mortality (Kyles et al., 2003; Lang et al., 2011), and techniques to remove the tumor thrombus have improved (Mayhew et al., 2018). However, when the vascular invasion is extensive, in particular when the tumor invasion in the vena cava extends beyond the hepatic hilus, the perioperative mortality rates can increase (Barrera et al., 2013).

Interestingly, when patients with vascular invasion survive the perioperative period, their long-term survival is not worse than that of patients without vascular invasion (Lang et al., 2011; Barrera et al., 2013).

Radiotherapy

Pituitary radiotherapy

Radiotherapy (RT) can be useful to decrease tumor size and reduce neurological signs in large pituitary tumors (Kent et al., 2007; Herrtage and Ramsey, 2012; Pérez-Alenza and Melián, 2016). Usually, a total dose of 36–48 Gy is administered in 3–4 Gy fractions, which requires the dog to be under anesthesia on approximately twelve occasions. The tumor size decreases after RT in most cases, but the time to effect and whether it diminishes the clinical signs of hypercortisolism can vary considerably between patients (Goossens et al., 1998; de Fornel et al., 2007; Kent et al., 2007; Sawada et al., 2018). Temporary or permanent additional pharmacotherapy may therefore be required to manage hypercortisolism. Adverse effects that can occur after RT are pituitary hemorrhage and otitis media (Sawada et al., 2018). The median (±SD) survival time was 539 days (±51) in one study (de Fornel et al., 2007), and was not reached in a study with a median follow-up time of 702 days (range, 27–1927) (Kent et al., 2007).

In humans with PDH, radiotherapy is currently primarily applied as single-session, focused stereotactic radiosurgery (SRS) using a Gamma Knife, where image-guided precisely-targeted radiation is applied at high-dose fractions. The use of SRS is well tolerated in humans and may also result in shorter response times than conventional RT (Mehta et al., 2017). The same technique has recently been applied to dogs with pituitary tumors and showed promising results (Mariani et al., 2015; Zwingenberger et al., 2016).

Adrenal radiotherapy

Information on the use of RT in canine ACTs is limited: only one study on RT in canine ACTs has been published so far (Dolera et al., 2016), in which nine dogs with ACTs with vascular invasion were enrolled. The ACTs showed progressive shrinkage in varying degrees in all dogs, consistent with a partial response (Dolera et al., 2016). More research is required to determine the efficacy of RT in dogs with ACTs.

In humans, RT is sometimes used for postoperative treatment of adrenocortical carcinomas (ACCs). Although earlier publications reported that human ACCs are resistant to RT, more recent studies show that RT can improve local tumor control (Fassnacht et al., 2006; Sabolch et al., 2011, 2015). Postoperative RT could potentially be useful for canine ACTs, particularly in patients where additional prognostic tools indicate a high risk of recurrence.

Pharmacotherapy – adrenal-targeting drugs

Pharmacotherapy is often used to control the clinical signs of hypercortisolism. Trilostane is the drug of choice for dogs with PDH, and in case of an ACT either trilostane or mitotane can be used.

Trilostane

Trilostane is a synthetic steroid analogue that competitively inhibits the steroidogenic enzyme 3β-hydroxysteroid dehydrogenase (3βHSD) (Potts et al., 1978), which is required for the production of all classes of adrenocortical hormones (Fig. 1). Trilostane therefore inhibits both cortisol production, which results in a loss of negative feedback and a compensatory increase in plasma ACTH concentration (Witt and Neiger, 2004), and aldosterone production, which causes a compensatory increase in plasma renin activity (Galac et al., 2010b; Reid et al., 2014). Additionally, trilostane possibly also inhibits other enzymes in the steroidogenesis cascade, such as 11β-hydroxylase (CYP11B1) (Sieber-Ruckstuhl et al., 2006, 2008).

Trilostane is registered for the medical management of both canine PDH and cortisol-producing ACTs, but most studies on the use of trilostane have been performed in dogs with PDH. Trilostane is absorbed rapidly from the gastrointestinal tract. Because administration with food significantly increases the rate and extent of absorption, trilostane should always be given with food (Ramsey, 2010).

There is a marked variation in the optimal trilostane dose, and the current recommendations are to start with much lower dosages than originally recommended by the manufacturer, which can be equally effective but induce fewer adverse effects than higher dosages (Feldman, 2011; Feldman and Kass, 2012; Cho et al., 2013). Larger dogs generally need a lower dose per kg body weight than smaller dogs (Feldman and Kass, 2012). Because the duration of cortisol suppression is less than 12 h in most dogs, administrating trilostane twice daily can improve the clinical response while keeping the total daily dose relatively low, and significantly reducing the adverse effects (Bell et al., 2006; Vaughan et al., 2008; Feldman, 2011; Arenas et al., 2013; King and Morton, 2017). The authors of this review advise to start treatment for PDH with an initial dose of 0.5–1 mg/kg twice daily. If twice daily treatment is undesirable for financial or practical reasons, the initial dose should be 1–2 mg/kg once daily.

Within weeks, an adequate dose of trilostane can increase the dog's activity and reduce polyuria, polydipsia and polyphagia. More time is needed to observe notable improvements on the skin and hair coat, which can take months. The hair coat can sometimes initially appear to worsen, due to shedding of telogen hairs and dry skin scales (Pérez-Alenza and Melián, 2016).

Trilostane is usually well tolerated, but the main adverse effect that can occur is transient hypocortisolism (shortage of glucocorticoids), possibly combined with or followed by complete hypoadrenocorticism (shortage of both glucocorticoids and mineralocorticoids). A recent study reported that the chance of a dog having at least one episode of clinical hypocortisolism within the first 2 years of trilostane treatment is approximately 15% (King and Morton, 2017). In most dogs, the adverse effects resolve once trilostane treatment is withdrawn. In such cases continuation of treatment with a lower dose is recommended when clinical signs of hypercortisolism recur. However, in some dogs, the hypoadrenocorticism can be permanent, which is possibly the result of adrenal necrosis, and can be fatal in severe cases (Chapman et al., 2004; Ramsey et al., 2008; King and Morton, 2017). One study found at postmortem examinations that adrenal necrosis was present in four out of six dogs with PDH that were treated with trilostane (Reusch et al., 2007). Subsequent studies suggested that it is not trilostane but rather the increased ACTH production resulting from a loss of negative feedback that causes this adrenal necrosis (Galac et al., 2010b; Burkhardt et al., 2011). The reported median survival times of dogs with PDH treated with trilostane range from 662 days (range, 8–1971) (Barker et al., 2005) to 852 days (range, 2–3210) (Fracassi et al., 2015).

For successful management of PDH with trilostane, frequent monitoring is essential. In the last decade efforts have been made to identify the best method to monitor trilostane therapy. In all methods, evaluation of the clinical signs is the first step. The preferred monitoring method is the use of the ACTH-stimulation test, which monitors the adrenal glands' reserve capacity to secrete cortisol (Neiger et al., 2002; Ruckstuhl et al., 2002). The timing of the ACTH-stimulation test is crucial since this influences the results (Bonadio et al., 2014), and the recommendation is to coincide the test with the maximal trilostane action (2–4 h after trilostane administration) (Griebsch et al., 2014). Despite its

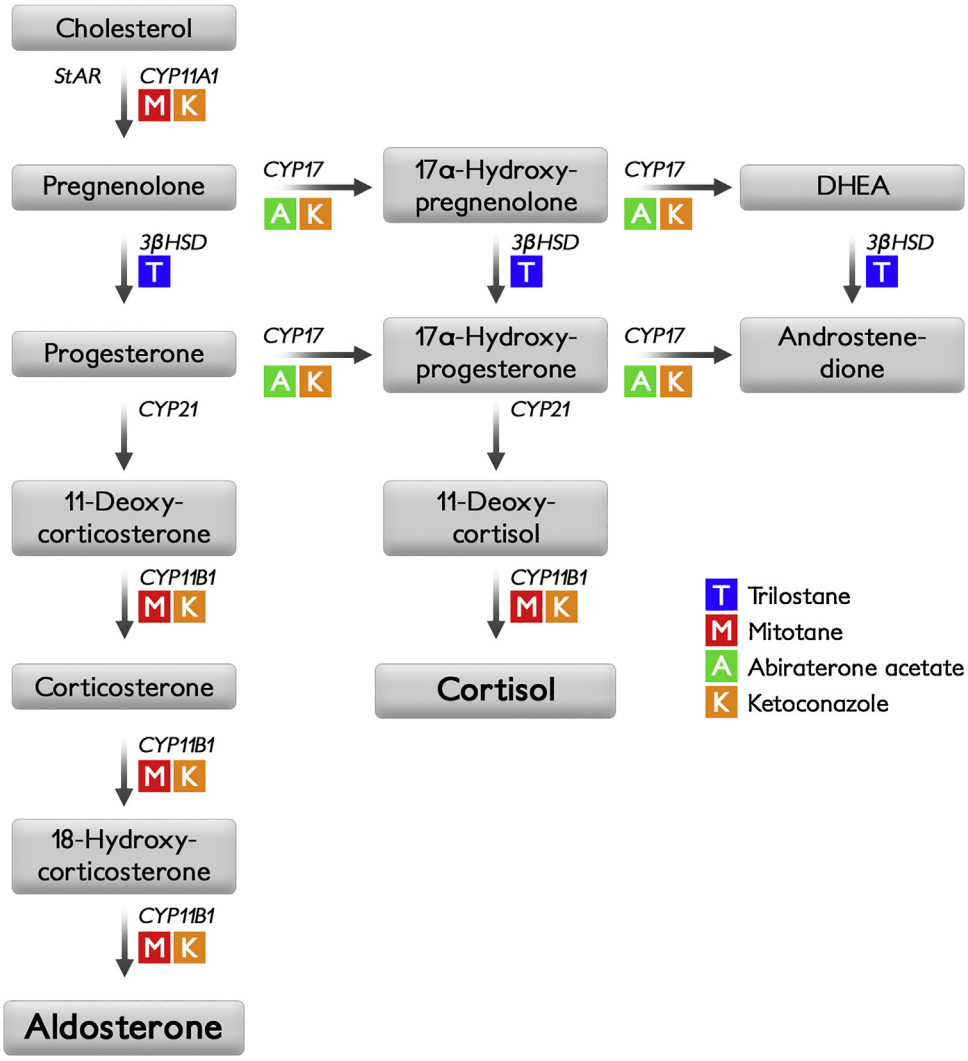

Fig. 1. Schematic overview of the site of action of steroidogenesis inhibitors. StAR, steroidogenic acute regulatory protein; CYP11A1, cytochrome P450 cholesterol side-chain cleavage enzyme; 3βHSD, 3β-hydroxysteroid dehydrogenase; CYP17, 17α-hydroxylase/17,20-lyase; CYP21, 21-hydroxylase; CYP11B1, 11β-hydroxylase.

widespread use, the ACTH stimulation test has never been validated as a monitoring tool for trilostane therapy, and there are some concerns regarding the variation in results depending on the timing of the test and whether this reflects clinical control (Midence et al., 2015; MacFarlane et al., 2016). Moreover, tetracosactide (synthetic ACTH [1–24]) is not easily available in all countries. A recently proposed alternative method is to measure the pre-pill cortisol (Pre-Vetoryl Cortisol; PVC) concentration and compare it to the clinical signs reported by owners. The PVC was found to better reflect the clinical control than the ACTH stimulation test (MacFarlane et al., 2016). However, even dogs with excellent clinical control can have insufficient adrenocortical reserve capacity, which can become clinically relevant when they face stress situations (King and Morton, 2017). The PVC approach is not comparable to the principle of the ACTH stimulation test: it is not a measure of the adrenocortical reserve and will therefore not reflect the safety of trilostane therapy. The applicability of this alternative method will have to be determined in future studies.

In dogs with PDH, trilostane effectively controls the clinical signs of glucocorticoid excess but does not directly affect the growth of the pituitary tumor. This is irrelevant initially in dogs with non-enlarged pituitary glands (i.e. P/B value ≤ 0.31 mm/mm^2 (van Rijn et al., 2016)), but the pituitary tumor might grow over time. In healthy dogs, the P/B value has been shown to significantly increase following trilostane therapy (Teshima et al., 2009), but this has not been studied in dogs with PDH. Nonetheless, especially in younger dogs, a control CT or MRI scan after 1 year of treatment could be considered to re-evaluate the pituitary size.

In dogs with a cortisol-secreting ACT it is important to remember that while these tumors are mostly malignant, trilostane will only reduce the clinical signs and not affect the growth or possible metastases of the ACT. Palliative treatment with trilostane has been shown to be effective in controlling clinical signs (Eastwood et al., 2003; Benchekroun et al., 2008; Galac et al., 2010a). The reported median survival times of dogs with ACTs treated with trilostane range from 353 days (95% CI, 95–528) (Helm et al., 2011) to 427 days (range, 101–1678) (Arenas et al., 2014). Although there is no scientific data available to support this, it is the authors' experience that dogs with an ACT can be more sensitive to trilostane treatment, which is why the authors advise to start the treatment with a relatively low initial dose of 0.5 mg/kg twice daily.

Mitotane

Mitotane (o,p'-DDD) is an adrenocorticolytic agent that leads to progressive adrenocortical necrosis and atrophy. Mitotane also inhibits the steroidogenic enzymes cytochrome P450 cholesterol side-chain cleavage enzyme (CYP11A1) and CYP11B1 (Fig. 1), which

contributes to inhibition of cortisol synthesis (Young et al., 1973; Veytsman et al., 2009), and induces other cytochrome P450 enzymes such as cytochrome P450 enzyme 3A4 (CYP3A4), which leads to increased metabolic clearance of glucocorticoids (Kroiss et al., 2011).

Although mitotane has been used to treat hypercortisolism for decades, the exact mechanism of action was poorly understood. A recent study found that one of its mechanisms of action is inhibition of sterol-O-acyl-transferase 1 (SOAT1), an enzyme that catalyzes the conversion of free cholesterol to cholesterol esters. Inhibition of this conversion increases the amount of free cholesterol in the cell, which can lead to endoplasmic reticulum stress and, subsequently, cell apoptosis (Sbiera et al., 2015). Interestingly, the dog is much more sensitive to mitotane than other species (Martz and Straw, 1980), which makes the dog an interesting candidate to further elucidate the mechanism of action.

The use of mitotane for the treatment of canine PDH has largely been replaced by that of trilostane (Galac et al., 2010a). This is mostly because trilostane is just as effective, is safer to handle and has been associated with fewer adverse effects than mitotane (Clemente et al., 2007; Ramsey, 2010). However, in case of an ACT, treatment with mitotane is still a good option because it has the added advantage that it can destroy ACT cells.

Because the goal of mitotane therapy in cases with an ACT is not only to reduce cortisol production but also to destroy as many neoplastic cells as possible, a non-selective protocol that affects the entire adrenal cortex should be considered (Kintzer and Peterson, 1997; Galac et al., 2010a). This treatment protocol consists of 50 to 75 mg/kg mitotane per day: daily for 5 days and then every other day over 40 days. For dogs of small breeds, a higher dose of up to 100 mg/kg may be required. Each daily dose should be divided into three or four portions (Rijnberk and Belshaw, 1988; Galac et al., 2010a). For sufficient absorption, mitotane should be given with food (Watson et al., 1987). Substitution therapy starts at the third day and consists of daily glucocorticoids (e.g. 2 mg/kg cortisone acetate), mineralocorticoids (e.g. 0.0125 mg/kg fludrocortisone acetate) and salt (0.1 g/kg sodium chloride), all divided in at least two portions (Galac et al., 2010a). After the initial course of mitotane has been administered, the glucocorticoid dose is reduced (e.g. 0.5–1 mg/kg cortisone acetate), but doubled for one or two days in the event of injury, severe physical stress, or anesthesia. To prevent recurrence, mitotane should be administered at the initially daily dose once weekly for at least 6 months, or even lifelong (Galac et al., 2010a).

Adverse effects of mitotane include anorexia, lethargy, weakness and diarrhea (Kintzer and Peterson, 1997). If the dog develops adverse effects, the mitotane treatment has to be temporarily discontinued, but not the substitution therapy. If these adverse effects are ignored and the owner continues to give mitotane, this can result in a potentially fatal hypoadrenocorticoid crisis (particularly if they continue to vomit or refuse substitution therapy). When the owner is given clear instructions this rarely occurs and the mitotane administration can usually be resumed after a few days (Galac et al., 2010a). Due to the cytotoxicity of mitotane, it should not be used when there are young children or pregnant women in the household (Galac et al., 2010a).

To evaluate whether the ACT has been completely destroyed, the UCCR can be measured in morning urine after the initial course of mitotane (i.e. after day 45) and every 6 months thereafter. Prior to morning urine collection for this UCCR measurement, the evening doses of glucocorticoids and mineralocorticoids should be withheld. Complete destruction of the ACT results in very low or even undetectable UCCRs. In addition to UCCR measurements, regular blood sodium and potassium measurements are required, which help to regulate the dose of mineralocorticoids that are administered (Galac et al., 2010a). The reported median survival times of dogs with ACTs treated with mitotane range from 102 days (95% CI, 43–277) (Helm et al., 2011) to 476 days (range, 61–1129) (Arenas et al., 2014) and did not differ significantly from the survival times for dogs treated with trilostane in both studies. However, these studies did not use the mitotane protocol as described herein (aimed at complete adrenocortical destruction), which might give different results.

In humans, mitotane is used in patients with nonresectable ACCs, and as adjuvant therapy after surgical resection of an ACC, especially in patients with a high recurrence risk. In the patients with nonresectable ACCs, and in the patients with local or metastatic recurrence, approximately 25–30% responds to mitotane treatment (Sbiera et al., 2015; Creemers et al., 2016). Since mitotane was shown to be a SOAT1 inhibitor, the degree of response could depend on SOAT1 expression. Indeed, Sbiera et al. (2015) showed that human ACCs with high SOAT1 expression responded better to mitotane treatment than those with low SOAT1 expression (Sbiera et al., 2015). A chemotherapy protocol where mitotane was combined with etoposide, doxorubicin and cisplatin seemed to give the best results in terms of progression-free survival times (Fassnacht et al., 2012). Prognostic indicators such as Steroidogenic factor-1 (SF-1) expression (Galac et al., 2014) (see below) could be helpful, as well as determination of the SOAT1 expression, to select dogs with a high recurrence risk that might benefit from adjuvant mitotane therapy following adrenalectomy. Although more research is required, the SOAT1 expression might provide insight into whether the ACT or its metastases will respond to mitotane.

Ketoconazole

Ketoconazole is a synthetic imidazole derivative which was originally developed as an antifungal agent. Ketoconazole inhibits multiple cytochrome P450 enzymes, including CYP11A1, 17α-hydroxylase/17,20-lyase (CYP17) and CYP11B1 (Fig. 1) (Creemers et al., 2015), thereby resulting in inhibition of cortisol production. Ketoconazole has been used for the treatment of hypercortisolism in dogs, but the percentage of non-responders was relatively large (approximately 25%) and it caused more adverse effects than trilostane (Galac et al., 2010a; Herrtage and Ramsey, 2012).

Levoketoconazole is an enantiomer of ketoconazole that has been purified from racemic ketoconazole, and has been reported to be a more potent inhibitor of cortisol production with reduced hepatotoxicity as compared to ketoconazole in vitro and in vivo in humans (Fleseriu and Castinetti, 2016; Ciato et al., 2017). It is currently under development in a phase III clinical trial in humans (Fleseriu and Castinetti, 2016; Ciato et al., 2017).

Future prospects – adrenal-targeting drugs

The main downsides of current medical adrenal-targeting treatment options include low selectivity for the glucocorticoid pathway, the possibility of overdosage, and occurrence of disruptive changes in the adrenal cortex, which necessitate careful dosing schemes and regular check-ups. In this section we provide an overview of interesting candidates that might prove to have superior selectivity, effectivity and/or tolerability when compared to currently available treatment options.

Melanocortin 2 receptor antagonists

The melanocortin 2 receptor (MC2R) is the receptor for ACTH and is expressed only in the adrenal cortex. It is a member of the melanocortin receptor subfamily of type 1 $G_{s\alpha}$-protein-coupled receptors, and is highly selective for ACTH (Mountjoy et al., 1992). A selective antagonist of the MC2R could therefore have great potential in the medical treatment of PDH, since it would directly block the excessive ACTH stimulation without having other (intra-

adrenal) effects. The selectivity of an MC2R antagonist for the MC2R is crucial, since inadvertently antagonizing or agonizing any of the other melanocortin receptors could result in multiple adverse effects (Clark et al., 2016; Ghaddhab et al., 2017). A recent study showed that two ACTH analogs, GPS1573 and GPS1574, are potent antagonists of the MC2R in vitro. However, these peptides also had some agonistic and/or antagonistic effects on other melanocortin receptors, and subsequent studies in rats showed disappointing results in vivo (Bouw et al., 2014; Nensey et al., 2016). Further developments might eventually generate a selective MC2R antagonist that could be used as a medical treatment option in dogs with PDH.

Abiraterone acetate

Recently, we showed that CYP17 is the only steroidogenic enzyme that is required for cortisol but not for aldosterone production in dogs (Sanders et al., 2016). Selective inhibition of CYP17 could therefore be an interesting treatment approach in hypercortisolism, since this would inhibit the production of cortisol but not that of aldosterone. One known CYP17 inhibitor is abiraterone acetate (Fig. 1), which is approved in the USA for use in human patients with castration-resistant prostate cancer to inhibit androgen (precursor) production by the adrenal glands (Gomez et al., 2015). Abiraterone acetate also inadvertently induced hypocortisolism in these patients (Vasaitis et al., 2011). We are currently in the process of testing the effects of abiraterone acetate on canine adrenocortical cells in vitro, and have so far obtained promising results on inhibition of cortisol production (De Wit et al., 2018).

Steroidogenic factor-1 (SF-1) inverse agonists

Steroidogenic factor-1 is an orphan nuclear receptor that regulates adrenal development, growth, and steroidogenesis (Schimmer and White, 2010). ACTH stimulates the transcriptional activity of SF-1, which increases the transcription of genes that encode steroidogenic enzymes (Chen et al., 2005). Increased SF-1 activity is therefore an important characteristic in dogs with PDH. Moreover, earlier work by our group showed that SF-1 mRNA expression was significantly higher in ACTs of dogs in which the hypercortisolism recurred within 2.5 years after adrenalectomy, than in ACTs of dogs without recurrence (Galac et al., 2014). We recently showed that one SF-1 inverse agonist compound, compound #31, is an effective inhibitor of cortisol production and SF-1 target gene expression in canine adrenocortical cells in vitro (Sanders et al., 2018). Inhibition of SF-1 activity therefore shows much promise as a possible future treatment approach in both PDH and ACTs.

Sterol-o-acyl-transferase 1 inhibitors

As mentioned previously, one recently discovered mechanism of action of mitotane is that it inhibits SOAT1 and thereby increases the amount of free cholesterol, which is toxic for the cell. More selective SOAT1 inhibitors that have the same adrenocorticolytic effects as mitotane but with fewer off-target adverse effects could possibly be interesting for a targeted treatment approach in dogs with non-operable or metastasized ACTs. In the early 1990s, researchers described that ATR-101, a SOAT1 inhibitor, potently induced selective adrenocorticolysis in healthy beagle dogs (Dominick et al., 1993). ATR-101 recently regained interest and is currently being studied as a possible future treatment for humans with ACCs (Naing et al., 2015; LaPensee et al., 2016; Burns and Kerppola, 2017). Because the effect of ATR-101 is particularly apparent in dogs (Kroiss and Fassnacht, 2016), this could be an interesting treatment approach in dogs with ACCs.

Pharmacotherapy – pituitary-targeting drugs

Medical management of PDH would ideally target the pituitary tumor. Because dopamine (DA) and somatostatin (SST) both have inhibitory functions in the pituitary gland, the main focus in research on pituitary-targeting drugs are three receptor subtypes: DA receptor subtype 2 (DRD2), and SST receptors subtype 2 (SSTR2) and subtype 5 (SSTR5). In canine corticotroph adenomas, the receptor subtype that is mainly expressed is SSTR2, while DRD2 and particularly SSTR5 are expressed at much lower levels (De Bruin et al., 2008). When comparing treatments between dogs and humans, it's important to realize that this distribution is somewhat different in human corticotroph adenomas, where the main receptors are SSTR5 and DRD2 (De Bruin et al., 2008). There are currently no pituitary-targeted drugs that are registered for use in canine PDH.

Cabergoline

Cabergoline is a DA agonist that binds to the DRD2. In line with the moderate DRD2 expression in canine corticotroph adenomas, canine corticotroph cells responded only modestly to cabergoline in vitro (De Bruin et al., 2008). However, in vivo experiments showed that 43% of dogs with PDH responded well to treatment with cabergoline, with fewer clinical signs, smaller pituitary adenomas and lower UCCRs (Castillo et al., 2008). This observed difference in efficacy could possibly be explained by the different durations of treatment (De Bruin et al., 2008).

Pasireotide

Pasireotide (SOM230) is a multiligand SST analog that binds to the SST receptors 1, 2, 3 and 5 (Weckbecker et al., 2002). In 20 dogs with PDH, pasireotide decreased the plasma ACTH concentration and improved the clinical signs, while no severe adverse effects were observed (Castillo et al., 2011). In a recent study, pasireotide was administered to dogs with macroadenomas that were also treated with trilostane or mitotane. The pituitary tumor volume decreased in six out of nine dogs, and increased in the remaining three, while no neurologic signs or grossly apparent adverse effects were observed (Lottati and Bruyette, 2018). In humans, the efficacy of pasireotide has been demonstrated, and it has been approved both in Europe and in the USA for the second-line treatment of patients with PDH when surgery has failed or is not an option (Colao et al., 2012; Simeoli et al., 2015). Due to their expression profile, a SST analog that has higher affinity for the SST2 than pasireotide could prove to be more effective in dogs, as also demonstrated during in vitro experiments (De Bruin et al., 2008).

Octreotide

Octreotide is a SST analog that binds to SSTR2 with high affinity, and to SSTR3 and SSTR5 with moderate affinity (Cuevas-Ramos and Fleseriu, 2014). In line with the high SSTR2 expression in canine corticotroph adenomas, octreotide significantly inhibited ACTH release in canine corticotroph cells in vitro, and did so more effectively than either pasireotide or cabergoline (De Bruin et al., 2008). In humans, octreotide can cause gastrointestinal side effects, but this is less well documented for dogs. For other indications such as insulinoma, octreotide is sometimes used as adjunctive treatment to inhibit insulin secretion. However, its short duration time after subcutaneous injection limits its use (Robben et al., 2006; Plumb, 2011). Recently, a new technology has been developed that increases the absorption of drug molecules through transient opening of the tight junctions of the gut epithelium, which can achieve therapeutic octreotide levels after oral ingestion in humans (Biermasz, 2017). An advantage of oral octreotide could be the lack of injection-related side effects, but there is a need for a strict twice daily fasted dosing regimen in

humans (Biermasz, 2017). Due to the high SSTR2 expression in canine corticotroph tumors, the availability of oral octreotide treatment could be an interesting treatment approach for dogs with PDH.

Dopamine/somatostatin chimeras

Although the effectivity of individual DA or SST analogs has been proven in the treatment of human pituitary adenomas, a considerable percentage of patients respond poorly or not at all to these treatments. An interesting new approach that is currently being developed is the use of DA/SST chimeras, which can cause SST and DA receptors to heterodimerize and generate a more effective hybrid receptor (Jaquet et al., 2005). This treatment approach seems very promising, and developments to produce an effective SSTR2/SSTR5/DRD2 chimera are ongoing (Culler, 2011; Ibáñez-Costa et al., 2017).

Retinoic acid

To produce ACTH, the precursor molecule proopiomelanocortin (POMC) is cleaved into multiple peptide hormones. The gene expression of POMC is regulated by many factors, including the transcription factors AP-1 and Nur77. Retinoic acid is an agent that regulates multiple cellular processes, including reducing the binding of these transcription factors to their DNA binding sites, ultimately inhibiting ACTH secretion. In 22 dogs treated with retinoic acid, investigators reported a decreased plasma ACTH concentration, decreased UCCR, resolved clinical signs, and decreased pituitary size (Castillo et al., 2006). In humans, adverse effects such as teratogenicity, mucocutaneous toxicity, defects in liver function and severe photosensitivity have been reported, which might be reduced by limiting the exposure to light (Ciato et al., 2017). A recent study showed that 9-cis RA, an active isomer of retinoic acid, activates the DRD2 promoter and thereby sensitizes pituitary adenomas for dopaminergic treatments (Occhi et al., 2014). Additionally, a synthetic retinoid analog named bexarotene has been reported to induce hypopituitarism (Atmaca et al., 2014; Occhi et al., 2014), and a phase I and II clinical trial has been initiated in humans.[1]

Conclusions

Differentiating between PDH and a cortisol-secreting ACT is essential when choosing the optimal treatment strategy. Surgical removal of the causal tumor is a good option in both cases, or radiotherapy in case of PDH, since these are currently the only treatment options with the potential to eliminate the source of either ACTH or autonomous cortisol excess. However, these options are not without risk, not generally available and not suitable for every patient. Pharmacotherapy is therefore often used, with trilostane advised in dogs with PDH, and either trilostane or mitotane in dogs with an ACT. Interesting new drugs are currently being developed and have great potential as future treatment options for canine hypercortisolism. A more selective and preferably also tumor-targeted approach could have many advantages for both PDH and ACTs.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

[1] Bush, Z., Vance, M.L., 2009. Preoperative bexarotene treatment for Cushing's disease. Identification No. NCT00845351. Retrieved from https://clinicaltrials.gov/ct2/show/NCT00845351.

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