PIVKA-II, Canine Tissues, Anticoagulant Poisoning

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PIVKA-II is an aberrant form of vitamin K that has been demonstrated to be increased in human coagulation disorders and in some neoplastic diseases. In veterinary medicine, PIVKA-II levels have been demonstrated to be useful for distinguishing anticoagulant poisoning from other coagulopathies. In forensic pathology, there is the need to distinguish malicious poisoning from other causes of death and, in some cases, identifying poisoned dogs from dogs that died as a result of other coagulative disorders can be challenging. This study evaluated the usefulness of the immunohistochemical expression of PIVKA-II in hepatic and renal tissues in order to identify patients with coagulative disorders due to clinical condition or the ingestion of anticoagulants substances.

PIVKA-II anticoagulants poisoning canine

1. Introduction

Vitamin K, discovered by Dane Henrik Dam in 1930, is an essential cofactor in the carboxylation of hepatic coagulation factors (F), i.e., prothrombin (FII), FVII, FIX, and FX; proteins C, S, Z, and M; and several extrahepatic vitamin-K-dependent proteins [1]. Vitamin K is an essential cofactor for the γ-carboxylation of key glutamic acid residues in blood clotting proteins by vitamin K-dependent carboxylase [2]. Acute vitamin K deficiency is classically characterized by deranged coagulation because of insufficiency of vitamin K-dependent coagulation factors, but it can also be induced by action of vitamin K antagonists such as warfarin, used therapeutically as oral anticoagulants.

Coumarin anticoagulants are considered as antivitamin K [3]. During catalysis, vitamin K is converted from the active form to vitamin K 2,3-epoxide, which must be recycled to the active form by vitamin K epoxide reductase (VKOR) to maintain the coagulation cycle. 4-Hydroxycoumarins antagonize VKOR, preventing vitamin K recycling and resulting in an accumulation of an abnormal form of coagulation protein called des-γ-carboxyprothrombin (DCP) or proteins induced by vitamin K antagonism (PIVKA-II) [4].

PIVKA-II concentrations increase when hepatic vitamin K stores are sufficiently low in order to prevent the effective γ-carboxylation of factor II when the vitamin K epoxide reductase activity is reduced or when the γ-glutamyl carboxylase GGCX activity is insufficient. Elevated PIVKA-II precedes any subsequent change in PT [5].

PIVKA-II is also considered a retrospective indicator of vitamin K status [2]. All of these applications have been extensively investigated in human medicine where PIVKA-II serum concentrations are demonstrated to be

increased in coagulation disorders and in some neoplastic disease such as in hepatocellular carcinoma [6][7][8][9] and pancreatic cancers [10]. During the process of malignant transformation in hepatocytes, PIVKA-II is produced as a defect in posttranslational carboxylation [11]. In this process, PIVKA-II loses its normal prothrombin function but may take on an important role promoting malignant proliferation in hepatocellular carcinoma [11][12]. In canine species, in addition to the above mentioned causes of vitamin K deficiency, there are also superwarfarin-type vitamin K antagonists [13] that are widely employed as rodenticides, which are among the most common causes of poisoning in dogs worldwide [14].

The Istituti Zooprofilattici Sperimentali (IIZZSS) laboratories are part of the state veterinary laboratories that fall under the umbrella of the Ministry of Health, and are designated as the local reference laboratories for monitoring and conducting toxicological analyses, in close collaboration with judicial authorities. According to the provisions of Law No. 189 of 2004 and the Ministerial Order of 18 December 2008, and following amendments, the use of poisons and the killing of animals are illegal and are defined as a crime. In veterinary medicine, PIVKA-II was first investigated by Mount and colleagues in 2003, who measured PIVKA-II levels in plasma, demonstrating that this is a diagnostically useful method for distinguishing anticoagulant poisoning from other coagulopathies [15].

Although in human medicine the PIVKA-II expression in tissues has been evaluated for its importance as a prognosticator in cancer, to the best knowledge of the authors, PIVKA-II has not been previously investigated in the veterinary field, so the aim of this work is to consider the PIVKA-II expression in canine tissues as a useful diagnostic tool to distinguish whether the cause of death was as a result of anticoagulant poisoning or some other conditions.

2. Abnormal Prothrombin (PIVKA-II) Expression in Canine Tissues as an Indicator of Anticoagulant Poisoning

To the best of the authors' knowledge, this is the first study to examine PIVKA-II expression in canine tissues. PIVKA-II is an abnormal prothrombin generated by liver cells under conditions of reduced vitamin K or in the presence of a vitamin K antagonist [13][16]. Since the 1990′s, PIVKA-II has been widely used as a human marker of hepatocellular carcinoma, demonstrating that an aberrant form of vitamin K can be produced by a lack of vitamin K induced by anticoagulant administration, coagulation disorders, and neoplastic modification of the hepatic tissue [16][17][18][19]. This aberrant prothrombin is used as an indicator of blood coagulability in human medicine, and the same role was demonstrated in canine specie by Mount and colleagues [15]. In their study, they demonstrated that a PIVKA-II test on canine plasma samples was diagnostically useful for distinguishing anticoagulant poisoning from other coagulopathies [15].

Recent studies have indicated that, in <4% of animals, cause of death was poisoning, sometimes via the simultaneous use of harmful and toxic substances with the intention to maximize pain in the victim (Di Blasio et al., 2020). More often, pet owners who found their animals dead requested forensic necropsy suspecting a malicious poisoning. It is important to rapidly and accurately screen suspected deadly cases of rodenticide poisoning in order to act and prosecute perpetrators properly.

In some cases, toxicological substances can easily be evaluated by chemical examination of the gastric content and other tissues, but sometimes, in very acute or chronic cases, it can be challenging. Histological exams could contribute to discovering whether fatal cases are related or unrelated to anticoagulant poisoning, particularly where gross lesions are mixed or confused by concurrent pathologies. The use of another method could be of some help to better improve the accuracy when establishing the cause of death.

Based on what has been described by Mount and colleagues, who investigated the PIVKA-II expression in the plasma of dogs, we investigated the expression of the aberrant form of vitamin K (PIVKA-II) in the tissues of animals in which poisoning was suspected to be de cause of death, observing that it could be expressed in the hepatic and renal tissue of dogs that died as a result of anticoagulant poisoning (100% of the cases), but also in some subjects that died from coagulative disorders or having hepatic degeneration.

It is not surprising that in cases of hepatic degeneration, PIVKA-II is weakly expressed due to liver dysfunction induced coagulative disorders [20]. This result appears to be different from that observed in some studies carried out on human hepatic carcinomas tissues, in which PIVKA-II seemed to be strongly expressed in the neoplastic cells and was negative in the adjacent cirrhotic hepatic tissue [16][21]. On the contrary, our results are in agreement with that observed in a previously cited study carried out on canine sera, which demonstrated that the PIVKA-II test can also be increased by deficiencies in factors II, VII, and X of heredity and acquired coagulopathies [15], and with what was described in a study carried on feline sera, which also observed a prolonged PIVKA clotting time in patients with hepatic pathologies [22]. As in those studies a PIVKA-II cut-off value in serum was assessed to be indicative of anticoagulant poisoning, it could be possible that in our cohort of cases, a correlation with poisoning would have been found if a quantitative method of evaluation had been adopted. In addition, we found the PIVKA-II expression in hepatic or renal tissues in the case of DIC. This is not surprising, because the PIVKA test is widely used to in human medicine as a screening test to differentiate whether or not vitamin K deficiency is present in various clinical cases, such as patients with liver diseases, disseminated intravascular coagulation, and other clinical conditions [19][23][24][25][26]. The main limitation of this study is the lack of sera collected before death in the animals of this study, and consequently the lack of correlation between the PIVKA-II level in the serum and biochemical serum enzyme levels of the main indicators of both hepatic and renal dysfunction; thus, further intra vitam studies are necessary to better understand the role of PIVKA-II. In addition, further studies are required to evaluate if the PIVKA-II test can also be used on the plasma collected during necroscopical examination in canine species, as described by Rutty and colleagues in 2003 for autopsy investigation in human forensic pathology [26].

Moreover, the response to anticoagulants is quite variable, resulting in different commercially available substance classes, dose-related toxicities, animal species, or repeated exposure, including liver bioaccumulation. Therefore, further studies are required for establishing if PIVKA-II could potentially be employed as an effective predictive biomarker in the diagnosis of rodenticide poisoning in dogs as well as in other animal species.

3. Conclusions

This study demonstrates the PIVKA-II expression in the canine tissue of animals with coagulopathies induced by anticoagulant substances with a high sensitivity but a low specificity, because it is also present in tissues of dogs with pathologies induced by coagulative disorders.

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