A New ELISA for Quantification of the blood biomarker TK1 in Canine malignancies

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Abstract

Serum TK1 activity is an established marker for blood malignancies and several uncontrolled cell proliferation which is a main characteristic of cancer progression. Thymidine kinase 1 (TK1) is an ATP-dependent enzyme involved in DNA precursor synthesis and its activity is cell cycle dependent. TK1 activity of blood tumours is a marker for monitoring the progression of the disease. Furthermore, TK1 activity has also been used as a measure of monitoring chemotherapies. TK1 activities and TK1 protein levels were determined using a sandwich ELISA for sera from dogs with haematological and solid tumours.

Introduction

Here we attempt to develop a sandwich ELISA for determining TK1 protein levels in sera from dogs with haematological and solid tumours. The new robust TK210-ELISA was developed based on anti-TK1 antibodies that were produced against the C-terminal (C215) region, which served as a catcher antibody and against the active site of TK1 as a catcher antibody (MAB 528-2, provided by AroCell AB, Uppsala).

Results

The TK1 ELISA performance was evaluated and compared with the activity assay results for Canine haematological and solid tumours. TK1 protein levels in sera from dogs with haematological (n=43) and solid tumours (n=55) were significantly higher compared to healthy dogs (n=42). The new anti dog TK1 ELISA could detect increased TK1 protein levels in sera from dogs with solid tumours which was not seen with the TK activity assay.

Method

The new robust TK210-ELISA was developed based on anti-TK1 antibodies that were produced against the C-terminal (C215) region, which served as a catcher antibody and against the active site of TK1 as a catcher antibody (MAB 528-2, provided by AroCell AB, Uppsala).

Procedure for the anti dog TK1 ELISA

1. Recombinant dog TK1 and serum samples were diluted 1:1 in the serum dilution buffer and incubated at RT for 60 min.
2. Plates with coated antibody were prewashed 4 X 3 min with wash buffer.
3. The plate with prepared calibrators, controls and samples is incubated at RT for 2h.
4. Washed 4 X in wash buffer (WB). Biotinylated anti-TK1 antibody diluted in wash buffer was added and incubated at RT 60 min.
5. Streptavidine–HRP was added and incubated 60 min at RT.
6. Wash 4 X in WB. The colorometric substrate TMB was added and incubate for: 15 min.
7. The Stop solution was added and the absorbance at 450 nm recorded

ROC curve analysis of TK1-ELISA and TK1 activity assays

The ROC curve analysis of the results revealed that both assays were sensitive enough for differentiating sera from dogs with haematological tumours from healthy dogs. The new TK1 ELISA also had a higher sensitivity for dogs with solid tumours (60%) compared to the results with the TK1 activity assay (20%).

TK1 activity and protein levels in Canine Lymphoma during therapy

7 dogs with malignant lymphoma were followed during therapy and serum samples were collected after each dose. The TK1 protein levels showed similar patterns as the TK activity levels. In case of dogs in remission, both TK1 activity and TK1 protein levels reached normal values after one or two doses of therapy. 2 out of 7 dogs had increased TK1 activity and protein levels after therapy which correlated with tumour relapse in these dogs.

Conclusions

- The new anti dog TK1 ELISA could detect increased TK1 protein levels in sera from dogs with solid tumours which was not seen with the TK activity assays.
- The new TK1 ELISA was sufficiently sensitive for monitoring chemotherapies of dogs with malignant lymphoma and thus serve as a valuable tool in veterinary medicine.

References