Effect of protease inhibitors on parameters of bovine pregnancy-associated glycoprotein (bPAG) radioimmunoassay

A. AYAD[1], N.M. SOUSA[1], J.L. HORNICK[2], M. IGUER-OUADA[3], J.F. BECKERS[1]

[1]Laboratory of Animal Endocrinology and Reproduction; [2]Nutrition Unit, Faculty of Veterinary Medicine, University of Liege, B-4000, Belgium
[3]Department of Organism and Populations Biology, Faculty of Life and Nature Sciences, University A. Mira, 06000 Bejaia, Algeria

Introduction

- The binucleate cells of the trophoblast produce pregnancy specific or associated glycoproteins (bPAG, bPSPB or PSP60) (Sasser *et al*., 1986; Green *et al*., 2000).
- They are members of the aspartic proteinase family, having high sequence homology with each other as well as with pepsin, pepsinogen, chymosin, cathepsin D and E and renin (Xie *et al*., 1991)
- Radioimmunoassay for PAG detection in serum or plasma samples is currently used as a specific serological method for pregnancy diagnosis in cattle from days 28 (Szenci *et al*., 1998) to 30 (Humblot *et al*., 1988) after breeding.

AIM

The aim of this study was to test the possible effect of protease inhibitors on parameters of bPAG radiommunoassay.

Materials and Methods

- In the RIA system, 67 kDa PAG preparation was as used tracer (labeled with ^{125}Iodine according to Chloramin T method) (Greenwood *et al*., 1963) and as standard.
- Different compounds were added to the basic buffer (Buffer 1) namely Tris-BSA, Buffer 2 (Tris-BSA containing Pepstatin A at 2.0 mg/L; Sigma-Aldrich Co., St. Louis, MO, USA), Buffer 3 (Tris-BSA containing Phenylmethylsulphonylfluoride at 0.068 g/L; PMSF, Sigma-Aldrich Co.), Buffer 4 (Tris-BSA containing Ethylenedinitrilo-tetra-acetic acid at 14.8 g/L; EDTA, Merck), Buffer 5 (Tris-BSA with protease inhibitor cocktail composed by Pepstatin A at 2.0 mg/L, PMSF at 0.068 g/L, EDTA at 14.8 g/L), and Buffer 6 (Tris-BSA, pH adjusted to 7.6 with a solution 90 vol ethanol: 10 vol acetic acid).
- All buffers were tested six times for specific and non-specific binding of the tracer (B_0 and NSB, respectively) in the PAG RIA-497 system.
- The radioimmunoassay was performed according to method of Perényi *et al*. (2002).

Results

Buffer tested	Assay parameters	1st test (%)	2nd test (%)	3rd test (%)
Buffer 1	NSB/T	1.32 ± 0.17[a]	1.68 ± 0.47[a]	1.33 ± 0.13[a]
	B_0/T	30.9 ± 1.70	26.6 ± 0.80[a]	27.4 ± 1.30[a]
Buffer 2	NSB/T	1.31 ± 0.07[a]	1.56 ± 0.35[a]	1.45 ± 0.11[a]
	B_0/T	31.0 ± 0.70	26.4 ± 1.10	29.3 ± 0.90
Buffer 3	NSB/T	1.22 ± 0.09[a]	1.76 ± 0.44	1.40 ± 0.09[a]
	B_0/T	31.1 ± 1.20	26.2 ± 1.10	27.7 ± 0.70
Buffer 4	NSB/T	1.36 ± 0.13[a]	1.47 ± 0.31[a]	1.45 ± 0.11[a]
	B_0/T	30.7 ± 1.20[a]	25.9 ± 0.70	27.8 ± 1.10
Buffer 5	NSB/T	1.37 ± 0.11[a]	1.59 ± 0.36[a]	1.38 ± 0.09[a]
	B_0/T	31.4 ± 0.70	26.1 ± 0.80	28.8 ± 1.20
Buffer 6	NSB/T	1.43 ± 0.19[a,b]	1.68 ± 0.33[a]	1.36 ± 0.13[b]
	B_0/T	31.9 ± 0.60	26.8 ± 0.80	28.3 ± 1.40

NSB/T = Non-specific binding/total tracer added (mean ± SD); B_0/T = Tracer bound in the zero standards/total tracer added (mean ± SD)
[a,b] Values with similar superscripts in the same row are not statistically different in NSB/T and B_0/T parameters ($P > 0.05$).

Conclusion

In conclusion, the study clearly shows that the buffer Tris-BSA remains already good and that these protease inhibitors did not improve the parameters of bPAG radioimmunassay.

The Life Science Summit, Biomedica, March 21st-22nd, 2007, Aachen, Germany.