DEVELOPMENT OF NEW IMMUNOASSAYS FOR THE QUANTIFICATION OF INFLAMMATORY RELATED CARTILAGE DEGRADATION.

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AIM

Protein nitration is a prominent feature of inflammatory processes in the joint. Our aim was to develop immunoassays specific for a breakdown product of type II collagen and its nitrated form. This peptide is composed of 9 amino acids sequence (HRGYPGLDG) which can be nitrated on the tyrosine residue [HRGY(NO_2)PGLDG].

MATERIALS AND METHODS

The amino acids sequence (HRGYPGLDG) (Coll 2-1) specific for type II collagen and its nitrated form [HRGY(NO_2)PGLDG] (Coll 2-1 NO_2) were conjugated to thyroglobulin and injected into rabbits. Two antisera (D3 and D37) were selected for their specificity and affinity and used to develop immunoassays (table 1). To establish reference values, we have collected 269 sera of healthy subjects. The Coll 2-1 and Coll 2-1 NO_2 values were expressed as mean ± standard deviation. The non-parametric test Mann-Whitney U-test was used to estimate differences between different groups of healthy patients.

RESULTS

1. Antiserum specificity.

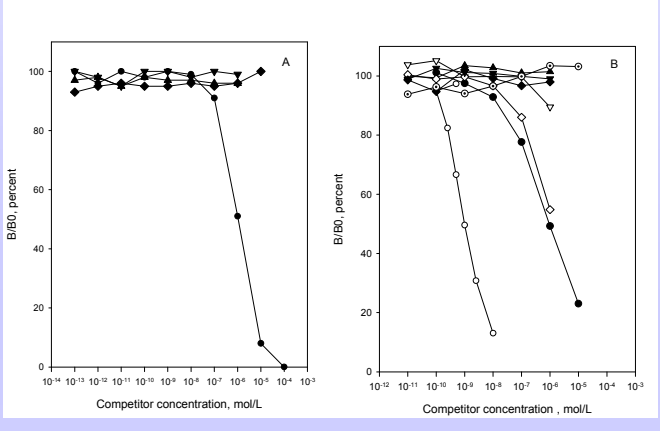

Figure 1 A and B : Competitive inhibitions of (A) D3 with Coll 2-1 (●), native type II collagen (◆), type I collagen (▲) and BSA (▼) and (B) D37 with Coll 2-1 (●), Coll 2-1 NO_2 (○), type II collagen (◆) , nitrated type II collagen (◇), type I collagen (▲), nitrated type I collagen (△), BSA (▼), nitrated BSA (▽) and 3 L-nitro-tyrosine (⊙). B/B0 represents the ratio of coated antigen bound in the presence of free antigen to the coated antigen bound in the absence of free antigen.

2. Technical performances

	COLL 2-1 IMMUNOASSAY			COLL 2-1 NO_2 IMMUNOASSAY		
	Urine	Serum	Synovial fluid	Urine	Serum	Synovial fluid
Limit of detection (nM)	17	17	17	0.025	0.025	0.025
CVs						
Intraassay	6.6	8.2	9.5	8.3	6.9	14.4
Interassay (%)	8.9	9.3	11.7	13.6	9.9	20
Linearity (%)	110.3	99.1	92.2	103.9	96.6	97
Analytical recovery (%)	121	104.7	109.9	121	121.9	ND

Not Determined

3. Reference values

The mean concentrations of Coll 2-1 and Coll 2-1 NO_2 in normal subjects (n=269) were 127.57 ± 58.17 nM and 0.31 ± 0.49 nM, respectively. When the population was stratified by 5 years brackets, Coll 2-1 and Coll 2-1 NO_2 serum levels did not significantly vary over lifetime. When subjects aged from 46 to 55 years corresponding to the early postmenopausal were removed, Coll 2-1 NO_2 level was significantly higher in premenopausal women than in postmenopausal women. Further, before 45 years old, Coll 2-1 NO_2 level in serum was significantly higher in women than in men (0.46 ± 0.86 nM vs 0.25 ± 0.27 nM, p = 0.005), but decreased to men level after 55 years old (figure 2).

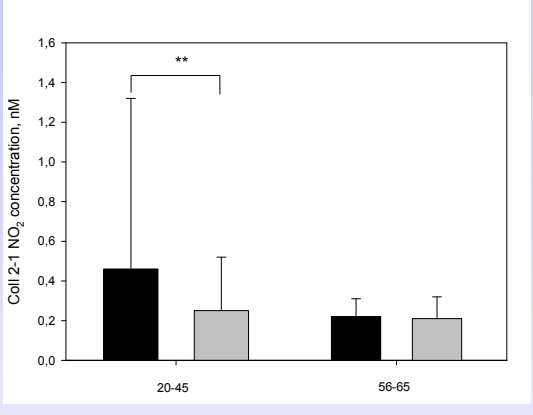

Figure 2 : Coll 2-1 NO_2 concentration

CONCLUSIONS

We have developed two rapid and sensitive competitive immunoassays for the measurement of a breakdown product of type II collagen and its nitrated form in urine, serum and synovial fluid. Coll 2-1 NO_2 level was significantly higher in women before 45 years old than after 55 years old, suggesting a relationship between oestrogen and Coll 2-1 nitration. Therefore, Coll 2-1 NO_2 could be a relevant marker for studying hormonal substitution in postmenopausal women.