The level of the collagen cross-link pyridinoline reflects the improvement of cutaneous lesions in one case of skin alveolar echinococcosis

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Abstract Cutaneous parasitic lesions, associated with a dense fibrous reaction, markedly improved under albendazole treatment in one case of supraumbilical skin localization of alveolar echinococcosis. Since collagen cross-linking increases during fibrogenesis and contributes to the stability of fibrotic lesions, we monitored the level of the cross-links pyridinoline and pentosidine in skin lesions from this patient to determine if they would reflect the changes occurring during treatment. We looked at the deposition of cross-linked type I collagen by immunohistochemistry and also measured the serum concentrations of pentosidine and of a fragment of type I collagen (ICTP), which contains a site of pyridinoline formation. Albendazole treatment did not affect either the collagen content of skin lesions or the serum concentrations of ICTP and pentosidine, but it led to a pronounced decrease in pyridinoline level concomitant with the disappearance, observed by immunohistochemistry, of extensively cross-linked fibrotic type I collagen. The follow-up of collagen cross-linking by pyridinoline in skin tissue thus appears to be useful in reflecting the improvement of fibrotic skin diseases during therapy.

Introduction Alveolar echinococcosis (AE) is a rare and severe parasitic disease that is caused by the intrahepatic growth of Echinococcus multilocularis larvae and leads to dense fibrosis. We have recently reported an exceptional case of cutaneous localization of AE of the liver. Albendazole therapy was effective in this case and led to a marked regression of the fibrous cutaneous lesions (Bresson-Hadni et al. 1996).

Type I collagen is a major constituent of dermis. Specific cross-linking of collagen leads essentially to the formation of two mature products, pyridinoline and histidinohydroxylysinonorleucine, resulting from post-translational modifications initiated by lysyl oxidase. Histidinohydroxylysinonorleucine is the major mature cross-link of healthy skin (Yamauchi et al. 1988), but pyridinoline increases in skin in pathological situations such as hypertrophic scars (Moriguchi and Fujimoto 1979); diabetes (Buckingham and Reiser 1990); chromomycosis (Ricard-Blum et al. 1993), a chronic fungal infection leading to extensive dermal fibrosis; and lipodermatosclerosis (Brickmann et al. 1996). Furthermore, pyridinoline increases in fibrotic hepatic lesions in human (Ricard-Blum et al. 1992, 1996a) and experimental liver AE (Ricard-Blum et al. 1995). Another, nonspecific pathway of collagen cross-linking involves nonenzymatic glycosylation that leads to advanced glycation end-products that are capable of forming intra- and intermolecular cross-links. One of them, pentosidine, results from irreversible posttranslational modifications of collagen (Sell and Monnier 1989) and increases exponentially with age in human skin (Sell and Monnier 1990).

We thus investigated the cross-links pyridinoline and pentosidine to establish if they were involved in the stabilization of fibrotic collagen deposits in the skin lesions and if their skin levels were modified by albendazole treatment. Fully cross-linked type I collagen molecules were localized in the lesions by immunohistochemistry.
with an antibody directed against a fragment of type I collagen, the C-terminal telopeptide (ICTP), which contains a site of pyridinoline formation (Risteli et al. 1993). We also measured the serum concentrations of pentosidine and ICTP, which is released into the circulation as a degradation product of mature type I collagen (Risteli et al. 1993), to determine if they would reflect the changes occurring in the skin lesions during the treatment.

**Patients and methods**

**Skin biopsies and sample preparation**

Skin biopsies were obtained before treatment and at different time points after the beginning of albendazole therapy during the follow-up of a patient referred to Besançon University Hospital with a supraumbilical skin localization of AE (Bresson-Hadni et al. 1996). Informed consent was obtained from the patient and the study was conducted according to the guidelines of the declaration of Helsinki (revised version, 1989). Serum samples were collected and stored under protection from light at −20 °C until analysis. Skin samples were minced, extracted with four changes of phosphate-buffered saline, and freeze-dried as previously described (Ricard-Blum et al. 1996a). Acid hydrolysis of tissue samples was performed at a final concentration of 5 mg/ml in deaerated 6 N HCl at 105 °C for 20 h. Serum samples for pentosidine analysis were hydrolyzed by the addition of an equal volume of 12 N HCl.

**Pyridinoline and pentosidine analysis**

Part of the hydrolysates were prefractionated by partition chromatography on CF1 cellulose columns (Black et al. 1988) before measurement of pyridinoline by reversed-phase high-performance liquid chromatography (HPLC; Colwell et al. 1993). Pyridinoline was detected by its natural fluorescence and the column was calibrated with purified pyridinoline (a gift from Dr. S.P. Robins, Rowett Research Institute, Aberdeen, Scotland). The pyridinoline content was expressed in moles per mole of collagen, assuming a molecular weight of 300,000 for the collagen molecule. The collagen content of skin biopsies, expressed in grams per 100 g of skin (dry weight), was determined by the measurement of hydroxyproline in skin hydrolysates by a colorimetric method (Woessner 1961).

For pentosidine analysis, skin and serum hydrolysates were prefractionated on a cation exchanger (Takahashi et al. 1993) and analyzed by reversed-phase HPLC (Sell and Monnier 1989). Pentosidine was detected by its natural fluorescence and the column was calibrated with purified pentosidine (a gift from Dr. V.M. Monnier, Institute of Pathology, Case Western Reserve University, Cleveland, Ohio, USA). The pentosidine content was expressed in millimoles per mole of collagen or in picomoles per milliliter of serum. We have previously established the reference interval of the assay in healthy adults (Ricard-Blum et al. 1996a).

**Histology and immunohistochemistry**

The skin biopsies were formalin-fixed and paraffin-embedded. The polyclonal anti-ICTP antibody was made in rabbits and purified by immunoabsorption (Risteli et al. 1993). Histological staining with hematoxylin-eosin-safron was performed on one section. Serial sections (5 µm thick) were deparaffinized, rehydrated, and quenched for endogenous peroxidase activity in 3% hydrogen peroxide for 20 min. The sections were then incubated with normal goat serum and with the anti-ICTP antibody (diluted 1:50) for 3 h at room temperature. The reaction was visualized with a biotinylated goat anti-rabbit IgG, an avidin-biotinylated horseradish peroxidase complex, and amino-ethyl-carbazole as the substrate (Vector Laboratories, Burlingame, Calif., USA). The sections were counterstained with hematoxylin.

**Measurement of serum ICTP**

The concentration of ICTP in serum was measured by an equilibrium radioimmunoassay (Orion Diagnostica, Oulunsalo, Finland) in duplicate 100 µl samples. We have previously established the reference range of the assay in healthy adults (Ricard-Blum et al. 1996b).

**Results**

**Clinical and pathological evolution of the skin lesions due to AE**

There was a remarkable improvement in the patient’s status during the course of the treatment; albendazole therapy was very effective and led to impressive regression of the cutaneous lesions (Bresson-Hadni et al. 1996). Viable parasitic vesicles were no longer observed in the biopsy by histological examination at 18 months after the beginning of the treatment. At this point the dermis remained infiltrated by postinflammatory fibrosis with discrete, nonspecific, cellular infiltrates.

**Cross-linking of skin collagen by pyridinoline and pentosidine**

The collagen content of the skin did not vary significantly throughout the albendazole treatment (Table 1). The pyridinoline level decreased by about 70% between 1 and 18 months of treatment but did not change significantly thereafter (Table 1). A concomitant decrease was observed in pentosidine, which was no longer detectable in the skin lesions after 18 months of treatment (Table 1). The pyridinoline/pentosidine ratio was 285/1 at 1 month after the beginning of the treatment, and the number of pyridinoline residues per collagen molecule remained higher than the number of pentosidine residues during the whole follow-up period.

**Table 1 Changes occurring during albendazole therapy in the concentrations of total collagen and of the cross-link pyridinoline and pentosidine in skin lesions of a patient with a cutaneous localization of AE**

<table>
<thead>
<tr>
<th>Duration of treatment (months)</th>
<th>Collagen (g/100 g skin)</th>
<th>Pyridinoline (mmol/mol collagen)</th>
<th>Pentosidine (mmol/mol collagen)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50.93</td>
<td>0.336</td>
<td>1.179</td>
</tr>
<tr>
<td>18</td>
<td>47.06</td>
<td>0.114</td>
<td>Not detected</td>
</tr>
<tr>
<td>27</td>
<td>57.05</td>
<td>0.100</td>
<td>Not detected</td>
</tr>
</tbody>
</table>