PostScript

LETTERS

Cystic fibrosis transmembrane conductance regulator (CFTR) is expressed in human bone

Mutations within the CFTR gene are central to the pathophysiology of cystic fibrosis. CFTR encodes a chloride channel that is located primarily on epithelial cell membranes and is responsible for the regulation of transmembrane chloride and other ion transport. Recent studies indicate a potential association between mutation of the CFTR gene and osteoporosis in patients with CF. Dif *et al*[1] reported an abnormal skeletal phenotype in CFTR-null mice with striking osteopenia, reduced cortical width and thinning of the trabeculae, while in a study of adults with CF, the ΔF508 mutation was shown to be an independent risk factor for low bone mineral density.[2] An association between CFTR mutations and bone disease might be mediated either indirectly by effects of the mutations on other systems (for example, the endocrine system), or it could be due to abnormally functioning CFTR in bone cells. However, to date, CFTR has not been reported in bone cells. We have therefore investigated the expression of CFTR in human bone using both in situ and in vitro approaches.

Immunolocalisation of CFTR was performed in human neonatal bone sections, primary human osteoblasts, an osteoblastic cell line (MG63) and osteoclasts cultured from peripheral blood mononuclear cells.[3] T84 colonic carcinoma cells were used as a positive control. The primary antibody was mouse IgG_{2A} anti-human CFTR (C-terminus specific) mono-clonal antibody (clone 24-1) (R&D Systems, Abingdon, UK).[4] CFTR in bone sections was visualised using a HRP-DAB staining technique. For cell cultures, an FITC-conjugated goat anti-mouse IgG secondary antibody was added for visualisation. Neonatal rib bone was collected post-mortem from six full-term infants (3 boys, 2 girls and 1 of unknown sex) who had no evidence of growth retardation or skeletal abnormalities. These samples were obtained with informed parental consent after approval by the local research ethics committee.

In neonatal bone sections, strong CFTR expression was detected in osteoblasts on forming surfaces (fig 1A) and in osteocytes newly incorporated into bone, whereas more deeply embedded osteocytes showed no CFTR expression (data not shown). Osteoclasts, defined by their multinucleate appearance and associated resorption pits, also expressed CFTR (fig 1B). In addition, CFTR expression was seen in an uncharacterised population of haematopoietic cells within the bone marrow. No CFTR expression was seen in resting, proliferating or hypertrophic chondrocytes in the growth plate.

In primary osteoblast cultures, immunofluorescence was confined to the cytoplasm of osteoblastic cells and was localised to small areas, giving a speckled appearance (fig 1C). This speckled appearance was also seen in the MG63 osteoblastic cell line (data not shown). In cultured osteoclastic cells, immunofluorescence was also confined to the cytoplasm (fig 1D). T84 cells showed positivity for CFTR within the cytoplasm, with strong perinuclear staining (data not shown). In addition, Western blot analysis using the mouse IgG_{2A} anti-human CFTR (C-terminus specific) monoclonal antibody (clone 24-1) confirmed the presence of CFTR in the osteoblastic cell line and in primary human osteoblasts (data not shown).

Our results show, for the first time, the presence of CFTR in human osteoblasts, osteocytes and osteoclasts. Although there are known limitations regarding the sensitivity and specificity of the anti-human CFTR (clone 24-1) antibody, the inclusion of appropriate positive and negative controls and confirmation of our findings by Western blot analysis in osteoblastic cells support this conclusion. The functional significance of these findings and their implications for bone disease associated with cystic fibrosis require further study. Other chloride channels are known to be essential for bone cell function,[5] and our finding that CFTR is expressed by normal bone cells suggests that it might also have a physiological role.

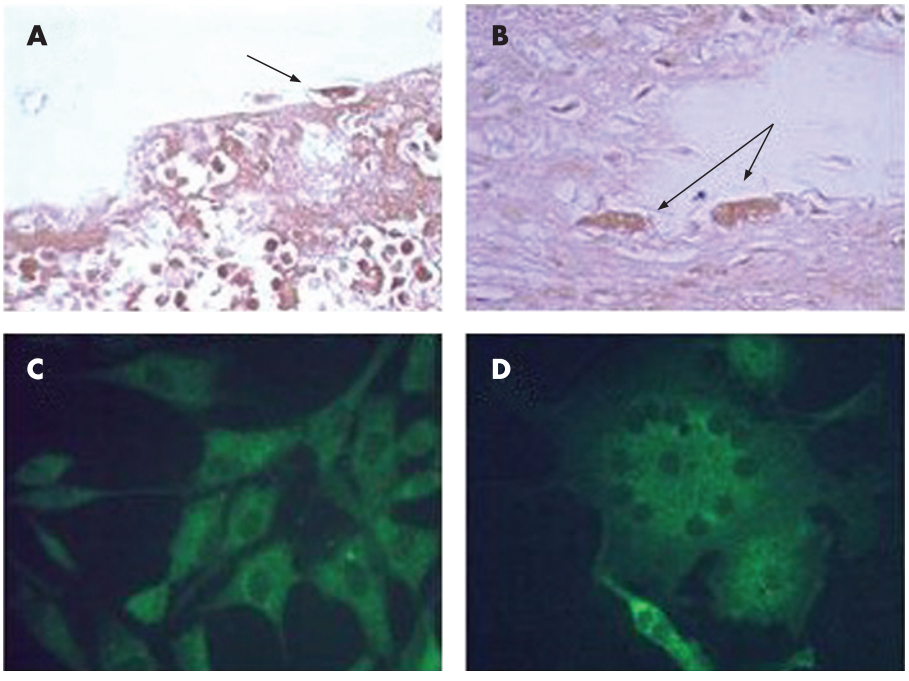

Figure 1 CFTR expression immunolocalised in human neonatal bone, cell lines and primary cells with C-terminus specific human anti-CFTR antibody as indicated by a brown DAB reaction (A, B) and green FITC immunofluorescence (C, D). (A) Osteoblasts at a forming surface (arrow). (B) An osteoclast (arrow) within a resorption pit showing positive staining. (C) Human osteoblasts (4 months, passage 8) with cytoplasm staining positive for CFTR. (D) Human multinucleate osteoclast with cytoplasm staining positive for CFTR.

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Obstructive sleep apnoea in patients with type 2 diabetes

An increased body mass index (BMI) is a risk factor for type 2 diabetes mellitus and obstructive sleep apnoea (OSA). The question whether this is just a shared risk factor or whether there is a deeper relationship has been addressed by West *et al*[1] who used an initial screening Berlin questionnaire followed by overnight oximetry in selected respondents. OSA was found to be highly prevalent in this patient group. Although BMI was the best predictor of OSA, type 2 diabetes conferred a significant extra increase in the likelihood of having OSA after allowing for BMI, age and neck size.

We have examined the risk of OSA in a district general hospital diabetes clinic. We used the Berlin questionnaire and assessed sleepiness using the Epworth score in 63 people (30 women) with type 2 diabetes and a BMI of >30 kg/m^2. Diabetic control was assessed using HbA1C. Thirty-five patients (56%, 16 women) were found to have a high risk of OSA.

Despite the suggestion that improvement in sleep-disordered breathing using continuous positive airway pressure improves glucose intolerance in both the short and long term, no significant association was found between poor glycaemic control and the Berlin questionnaire risk group category.[2 3]

These results are similar to those of West *et al* and suggest the potential of a high burden of unrecognised OSA in people with diabetes. Furthermore, our findings are not restricted to the male population. We feel that clinicians who manage patients with type 2 diabetes should have a heightened awareness of the increased likelihood of OSA in this group. The Berlin questionnaire is easy to use and is an attractive alternative in the initial screening for OSA, particularly where access to sleep studies and oximetry is very limited.

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Screening for type 2 diabetes in patients with obstructive sleep apnoea

We read with much interest the findings in the recent paper by West *et al*[1] of the prevalence of obstructive sleep apnoea (OSA) in men with type 2 diabetes. Although there may have been selection bias in the questionnaire respondents, the findings support the hypothesis that OSA is common in this population and is likely to be underdiagnosed. OSA is known to be independently associated with an increase in the cardiovascular risk factors that comprise the metabolic syndrome,[2] including diabetes mellitus and impaired glucose tolerance.[3]

We have reviewed our data on 156 successive patients with OSA recently diagnosed by polysolmnography, 114 of whom (72 men) had glucose measurements checked at the time of diagnosis. Sixteen patients (14%) were already known to have diabetes or impaired glucose tolerance. Although only five newly diagnosed cases of diabetes were identified, a further two had a single raised fasting glucose level and four had raised non-fasting glucose levels. Thus, a total of 11 patients (9.6%) were identified by the screening process as potentially having diabetes or impaired glucose tolerance.

Unsurprisingly, the patients with diabetes or impaired glucose tolerance had higher mean body mass indices (37 vs 33.2 kg/m^2), but there seemed to be little difference in either the Epworth score (11.8 vs 9.9) or in the apnoea-hypopnoea index (22.6 vs 24.4). These data support active screening of patients with newly diagnosed OSA for diabetes in order to allow earlier recognition and treatment.

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