Clinical Case Reports

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CASE REPORT

Association of long-term tacrolimus (FK506) therapy with abnormal megakaryocytosis, bone marrow fibrosis, and dyserythropoiesis

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Funding Information
No sources of funding were declared for this study.

Received: 13 January 2015; Revised: 13 April 2015; Accepted: 30 April 2015

Clinical Case Reports **2015; 3(7): 664–668**

doi: 10.1002/ccr3.303

Key Clinical Message

Haematopoietic abnormalities associated with tacrolimus are relatively rare with reversible pure red cell aplasia being the most common. We report for the first time, to our best knowledge, tacrolimus therapy associated with bone marrow fibrosis, abnormal megakaryocytosis, and dyserythopoiesis in a 17-year-old male treated with tacrolimus for nephrotic syndrome.

Keywords

Dyserythropoiesis, fibrosis, megakaryocytois, tacrolimus.

Introduction

Tacrolimus (FK506) is a neutral macrolide immunosuppressant commonly used in solid organ transplantation. Tacrolimus has also been shown to effectively treat steroid-resistant nephrotic syndrome [1–3]. Tacrolimus was first discovered from the fermentation broth of a Japanese soil sample that contained the bacteria *Streptomyces tsukubaensis* in 1984 by Fujisawa Research as the pure crystalline form of FR900506. The subsequent naming of FK refers to Fujisawa Kaihatsu. The name tacrolimus is derived from "Tsukuba macrolide immunosuppressant". Tacrolimus was first approved by the FDA in 1994 for the prevention of rejection after liver transplantation. It has been later extended to include kidney, heart, small bowel, pancreas, lung, trachea, skin, cornea, bone marrow, and limb transplants [4].

Tacrolimus functions as an immunosuppressant by binding to an immunophilin, FK506-binding protein (FKBP), in T lymphocytes. The complex of FKBP and tacrolimus binds to calcineurin and inhibits the phosphatase activity of calcineurin which in turn inhibits the dephosphorylation of nuclear factor of activated T cells (NFAT). This inhibition prevents the translocation of NFAT from the cytoplasm to the nucleus of the T cells and subsequently inhibits the gene transcription for interleukin 2 (IL-2), and other transcription factors which are essential to early T- cell activation. In addition, tacrolimus is thought to be able to inhibit IL-2 receptor production, mixed lymphocyte reactions, and the generation of cytotoxic T-cells [1, 2, 4].

There are a number of adverse effects associated with the use of tacrolimus, including nephrotoxicity, hyperglycemia, abnormal electrolytes, hypertrophic cardiomyopathy, and hematologic toxicity [2]. The hematopoietic abnormalities associated with tacrolimus are relatively rare. There are very few case reports of reversible pure red cell aplasia (PRCA) [5, 6] and generalized bone marrow suppression [7, 8] in transplantation recipients following the use of tacrolimus. The bone marrow suppression associated with tacrolimus appears to be reversible as all the patients in the case reports recovered after tacrolimus was discontinued.

Here we report for the first time, to our best knowledge, a case of bone marrow fibrosis, abnormal megakaryocytosis, fibrosis, and dyserythopoiesis associated with tacroli-

mus therapy in a 17-year-old patient who had received several years of treatment with tacrolimus for his nephrotic syndrome.

Case History

Patient presentation

A 17-year-old male had a past medical history of nephrotic syndrome secondary to minimal changes disease diagnosed 6 years ago for which he was treated with tacrolimus for 5 years due to nonresponse to steroids. The dose of tacrolimus was increased 4 months prior to admission. He was admitted for a 4-month history of worsening abdominal pain and diarrhea with exacerbation of symptoms for 1 week. Shortly after admission, the patient developed fever and diffuse lymphadenopathy.

Diagnostic workup

Abdominal ultrasound, hepatobiliary iminodiacetic acid (HIDA) scan, abdominal CT, and endoscopy tests were all negative. Extensive infectious workup, including testing for virus, TB, and fungi, was performed and the results were all negative. The tacrolimus level on day of admission was elevated compared to the level 4 months ago (13.3 ng/mL vs 3.7 ng/mL) but was still in the normal range. The patient was also noted to be pancytopenic on admission (WBC 3×10^3/mcL, Hb 10.7 g/dL, Plt 30×10^3/mcL). Consideration of long-term tacrolimus use was proposed as the cause. A bone marrow biopsy was performed.

Results

Bone marrow findings

The patient's bone marrow shows mildly hypercellular marrow (Fig. 1). Myelocytes were predominant with normal maturation and increased numbers of blasts were not seen. Erythroids showed mild megaloblastoid changes with mild nuclear irregularity and nuclear budding (Figs. 2 and 3). Megakaryocytes were markedly increased in numbers with various forms of abnormal morphology, including hypersegmented nuclei, hyperchromatic nuclei, detached nuclear lobes, and micromegakaryocytes. Clusters of megakaryocytes were present (Fig. 4). Focal dense collagen fibrosis and moderate to severe reticulin fibrosis was also present (Figs. 5 and 6). In addition, prominent dilated sinuses were identified. Trace iron without ring sideroblasts was seen on iron stains. Immunohistochemical stain for CMV was negative. Flow cytometric analysis showed no overtly aberrant myeloid or lymphoid populations. Chromosome analysis showed a normal male karyotype, 46, XY. Fluorescence in situ hybridization (FISH) analysis for acute lymphocytic leukemia (ALL)

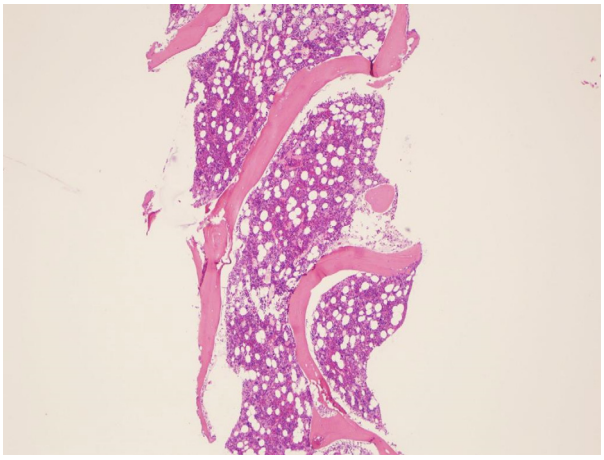

Figure 1. Mild hypercellular bone marrow (bone marrow biopsy).

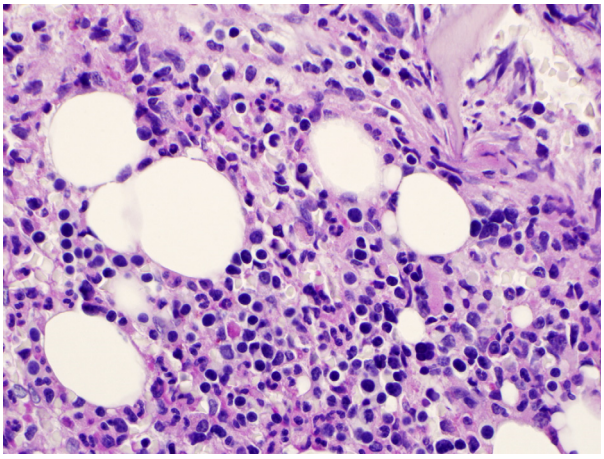

Figure 2. Mild megaloblastoid changes with mild nuclear irregularity in erythroid precursors in the bone marrow (bone marrow biopsy).

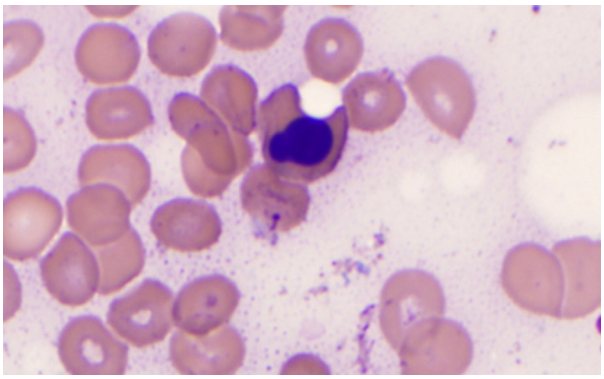

Figure 3. Nuclear budding in an erythroid precursor in the bone marrow (bone marrow aspirate).

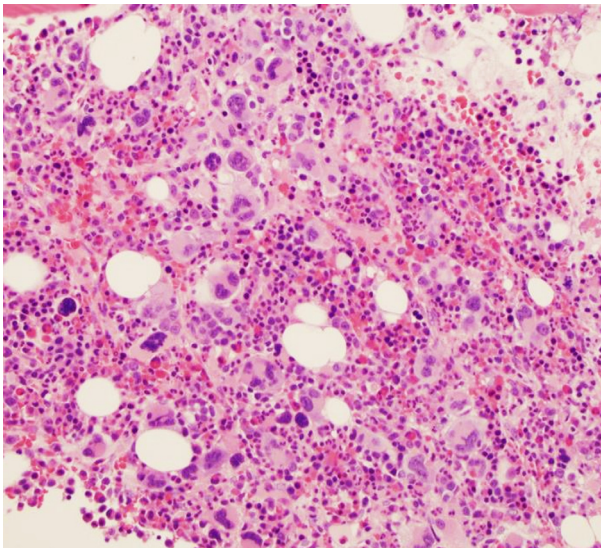

Figure 4. Striking megakaryocytosis with various forms of abnormal morphologies in the bone marrow (bone marrow biopsy).

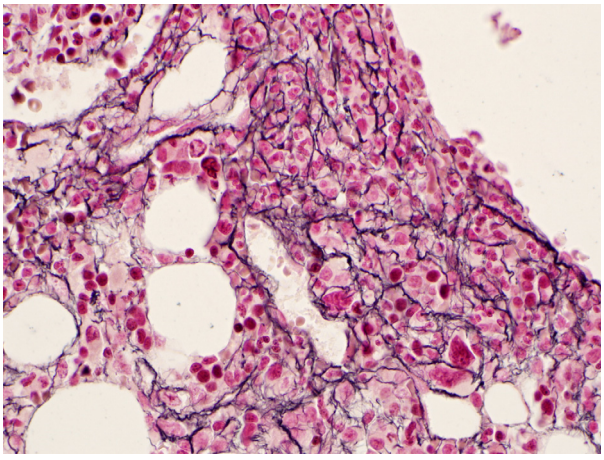

Figure 5. Reticulin fibrosis in the bone marrow (reticulin stain, bone marrow biopsy).

panel were normal with no evidence of trisomy 4, 10, or 17. There was no evidence for BCR/ABL1, MLL rearrangement, or ETV6/RUNX1 fusion. In summary, the bone marrow biopsy was mildly hypercellular with striking megakaryocytosis with marked atypia, marrow fibrosis, and dyserythropoiesis.

Treatment and outcome

Tacrolimus was discontinued on admission. The patient received red blood cells and platelets transfusions and was treated with steroids for newly diagnosed systemic lupus erythematosus (SLE). The symptoms were completely resolved 2 months later and the CBC slowly recovered,

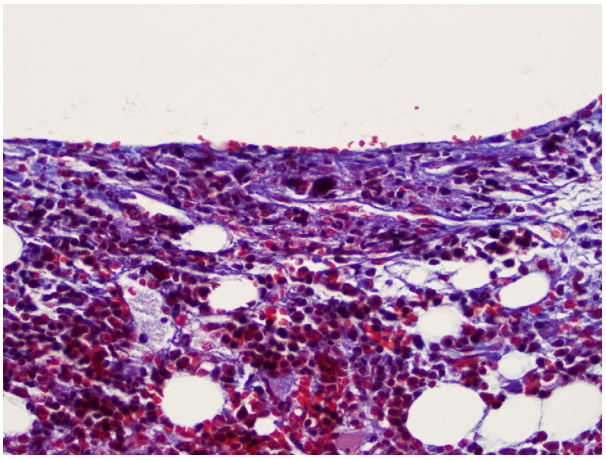

Figure 6. Focal dense collagen fibrosis in the bone marrow (trichrome stain, bone marrow biopsy).

with leukocytes recovering fastest (4×10^3/mcL at 3 weeks) and red blood cells and platelets recovered more slowly (16.1 g/dL and 233×10^3/mcL at 2 months), and remained normal 6 months later (WBC 9.0×10^3/mcL, Hb 15.3 g/dL, Plt 291×10^3/mcL).

Another bone marrow biopsy was not performed because of the excellent recovery.

Discussion

Tacrolimus is a macrolide immunosuppressant used in solid organ transplantation and steroid- resistant nephrotic syndrome [1–3]. Tacrolimus can bind to FKBP in T lymphocytes and the final outcome is inhibition of the gene transcription for IL-2 and other transcription factors essential to early T cell activation [4]. Hematopoietic abnormalities associated with tacrolimus are rare with reversible pure red cell aplasia the most common [5–8]. The mechanism by which tacrolimus causes bone marrow hypoplasia is unclear.

We report here a 17-year-old male with SLE treated several years with tacrolimus who had abnormal bone marrow findings including (1) mild hypercellularity; (2) a predominance of myelocytes; (3) megaloblastoid changes with nuclear irregularity and nuclear budding in erythroid precursors; (4) striking megakaryocytosis with clustering and marked abnormal morphology; and (5) marrow fibrosis. Flow cytometry, cytogenetic, and molecular genetic studies including the ALL panel, BCR/ABL1, MLL rearrangement, and ETV6/RUNX1 fusion were normal. Tacrolimus was discontinued and complete resolution of symptoms occurred 2 months later. Therefore, we conclude that the abnormal bone marrow findings are associated with the use of tacrolimus. To our best knowledge no such adverse effect of tacrolimus has been reported.

This patient developed pancytopenia 4 months following increased dose of tacrolimus, suggesting that the adverse hematopoietic effect of tacroliums is dose-dependent. The fact that the patient's symptoms and CBC counts slowly recovered upon the discontinuation of tacrolimus indicates that the hematopoietic effect of tacrolimus is reversible.

It is well known that myelodysplastic syndrome (MDS) can result from treatment with certain medications. Therapy-related myeloid neoplasms include therapy-related MDS, therapy-related AML, and therapy-related myelodysplastic/myeloproliferative neoplasms [9, 10]. Therapy-related AML is further subdivided in the WHO classification into alkylating agent-related and topoisomerase II inhibitor-related types. These therapy-related myeloid neoplasms occur a few months to years following the therapy and are commonly associated with various cytogenetic changes [9–11].

The bone marrow in therapy-related MDS cases is usually less cellular than in primary cases and significant reticulin fibrosis is more common in therapy-related MDS cases. MDS with fibrosis (MDS-f), a MDS variant, is characterized by a marked increase in bone marrow reticulin fibers with prominent dysmegakaryopoieis. MDS-f accounts for 10–15% of primary MDS cases and >50% of therapy-related MDS. The megakaryocytes can show a marked degree of pleomorphism with both small dwarf forms and large abnormal cells [11].

Myelodysplastic morphology can also result from benign causes including vitamin B12, folate, and copper deficiencies, parvovirus B19 infection, and after exposure to certain drugs and toxins. These conditions are neither neoplastic nor preleukemic. Treating or removing underlying causes reverse the morphological changes [11, 12]. In the current case, the patient presented with pancytopenia clinically and the bone marrow showed myelodysplastic changes including dyserythropoiesis and marked dysmegakaryopoiesis in addition to marrow fibrosis. However, there was no increased blast count in the bone marrow and no cytogenetic abnormalities were identified. The patient's pancytopenia improved upon discontinuation of the tacrolimus therapy and returned to normal. All these features suggest that the patient did not have true therapy-related MDS. However, it cannot be ruled out that the patient will eventually develop true MDS or even AML if the treatment with tacrolimus was continued.

One confounding factor in this case is that the patient was diagnosed with SLE. Myelodysplastic changes and autoimmune myelofibrosis (AM) has been associated with SLE. One study shows that the features of AM include hypercellularity, left-shifted erythroid hyperplasia, increased numbers of megakaryocytes, absence of megakaryocyte clusters and bizarre-shaped megakaryocytes, and marked reticulin fibrosis with large zones of collagen fibrosis [13]. The abnormal bone marrow findings while superficially similar in this case included megakaryocytosis with clustering and abnormal morphologies of megakaryocytes and do not support the diagnosis of AM. In addition, the resolution of symptoms immediately correlate with discontinuation of tacrolimus therapy, suggestive of a causal role.

Tacromlimus has been shown to be involved in the calcineurin pathway and is considered an inhibitor of calcineurin. Recent studies have shown that overexpression of FKBP51 in megakaryocytic progenitors leads to megakaryocyte accumulation by inducing the resistance to apoptosis mediated by deregulation of JAK2/STAT5 activation, presumably through inhibition of calcineurin, in idiopathic myelofibrosis (IMF), indicating overexpression of FKBP51 is responsible for megakaryocytosis in IMF through the calcineurin-dependent anti-apoptotic pathway [14, 15]. Another study showed that overexpression of FKBP51 induced sustained NF-κB activation that is involved in TGF-β secretion, which is responsible in part for marrow fibrosis in IMF [15]. We hypothesize that tacrolimus effects on the calcineurin pathway by binding to FKBP resulted in megakaryocytosis and marrow fibrosis in this patient.

One limitation of this study is the lack of morphologic follow-up with a repeat bone marrow examination. Discontinuation of tacrolimus resulted in a quick and steady recovery of hematologic parameters without need for further invasive procedures. However, it would be interesting to compare pre- and post-therapy samples to ascertain whether there was resolution of the morphologic findings. This certainly would be suitable for future study.

Conclusion

In summary, we demonstrate for the first time that long-term tacrolimus can cause bone marrow changes which are morphologically indistinguishable from a myelodysplastic syndrome. As has been reported with other disorders, such as copper deficiency, these effects appear to be reversible upon correction or, in this case, discontinuation of the offending agent. It is also noteworthy that symptoms appeared to follow an increase in dose, suggesting a dose-dependent effect. Awareness of the possibility of these changes should help prevent the misdiagnosis of a true myelodysplastic syndrome in patients on long-term tacrolimus therapy.

Conflict of Interest

None declared.

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