The native enzyme’s function. The pGL3 reporter vectors used in this region code for a peroxisome signal peptide of importance for pF2-GALU is pTN23. A good correlation between splicing and protein function. A different study using lacZ-luc enzymatic activity of the fusion protein is the same as for the plasmid derived from pBgalluc. For the latter plasmid it was shown that the C-terminal amino acids of firefly luciferase are functional. In fact, this region codes for a peroxisome signal peptide of importance for the native enzyme’s function. The pGL3 reporter vectors used in our study do not carry this peroxisomal targeting sequence and express a cytosolic form of luciferase termed lacZ. However, we found comparable results for the pF2 stopmut-type plasmids using the prothrombin stop codon and those using the luciferase stop codon. This argues against an appreciable effect of the fusion on protein function.

The parental plasmid for the double-reporter assays with pF2-GALU is pTN23. A good correlation between splicing efficiency by reverse transcription–polymerase chain reaction (RT-PCR) and the resulting luciferase–β-galactosidase ratio was shown for this plasmid including the faithful reproduction of effects of known mutations on splicing. The pTN23 plasmid is derived from pBgalluc. For the latter plasmid it was shown that the enzymatic activity of the fusion protein is the same as for the nonfused luciferase and β-galactosidase. This is in line with a different study using lacZ-luc fusion proteins that also found no evidence for an altered specific activity as well as another study using renilla and firefly luciferase fusion proteins.

In conclusion, we believe that our experiments substantiate that the prothrombin 19911 polymorphism is functional. The 19911G mutation creates an intronic splice enhancer, which is the best explanation for the prothrombotic phenotype observed in case control studies so far. Further studies are needed to prove or disprove whether a further stratification according to additional 19911 carrier status can help to distinguish a higher risk from a lower risk group of 20210G>

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References


To the editor:

Treatment with cyclosporin A cream for the cutaneous reactions associated with imatinib therapy

As more patients are being treated with imatinib (formerly STI 571), cutaneous reactions are being recognized as very common among the side effects of the drug, ranging from mild macular erythema to widespread papulous lesions, that sometimes are pruriginous and cause discomfort to the patients. Some rare cases of Stevens-Johnson syndrome have also been described. In some patients, the cutaneous side effects of imatinib led to the discontinuation of the treatment, while in others the drug could be reinitiated progressively after a course of corticosteroids.

Two of our patients treated with imatinib developed widespread cutaneous reactions characterized as symmetric pruriginous maculopapular exanthema that was confluent in some areas. The skin biopsy showed in both cases an inflammatory infiltrate in the superficial dermis, mainly of perivascular lymphocytes. The physiopathology of this type of cutaneous lesion is unknown, as it is not known whether it is immunologically mediated. We tried not to give corticosteroids, because high doses of prednisone are sometimes required and could be harmful, but we continued to give imatinib while looking for an alternative solution.

We searched for a drug that could inhibit the cellular mechanisms presumably implicated in this type of skin lesion, and as a result we decided to try cyclosporin A because of its known immunosuppressive action. However, the systemic action of cyclosporin A given orally seemed to us an excessive treatment, so we decided to try an ointment of cyclosporin A cream, with the aim of determining its efficacy and its absorption through the skin. The cream was prepared in the British Hospital Pharmacy Department because it is not commercially available. Briefly, a 1% cyclosporin A cream was prepared as follows: 1000 mg cyclosporin A (10 mL of Sandimmune neoral solution; Novartis, Basel, Switzerland) was mixed with urea 1%, 100 000 IU vitamin A palmitate, vitamin E (as tocoferol acetate 2%), 10 drops lemon essence, and hydrophilic cream in an amount to equal 100 grams for the entire solution.

Patient 1 was a 50-year-old female with chronic myeloid leukemia (CML), with an interval of 16 months since diagnosis.

Patient 2 was a 50-year-old male with chronic myeloid leukemia (CML), with an interval of 16 months since diagnosis.

To the editor:

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Patient 1 was a 50-year-old female with chronic myeloid leukemia (CML), with an interval of 16 months since diagnosis.
She started imatinib (400 mg daily) because of interferon resistance and intolerance. After 3 weeks on imatinib, she presented with a pruriginous maculopapular exanthema in the abdomen and both legs. She was treated with the 1% cyclosporin A cream, 3 to 4 times per day. After 48 hours, an initial response was seen, with less erythema and pruritus. After continuous treatment in the way described, the cutaneous lesions completely disappeared in 30 days.

Patient 2, a 63-year-old male with chronic-phase CML diagnosed 48 months earlier, was switched to imatinib therapy (400 mg daily) because of cytogenetic resistance to interferon. After the first week, he presented with a pruriginous maculopapular exanthema that affected arms, legs, and abdomen. He was treated with cyclosporin A cream in the same schedule as the previous patient, with complete resolution of the cutaneous lesions after 2 months. As mentioned, none of the patients required corticosteroids, and the imatinib administration was maintained at the same dosage during the time of the skin treatment. Serial blood determinations of cyclosporin A disclosed undetectable levels, so we conclude that the absorption of cyclosporin A through the skin was minimal or null while it exerts its pharmacologic action locally.

After the complete disappearance of the cutaneous reactions induced by imatinib, both patients stopped the treatment with the cyclosporin A cream while maintaining the imatinib administration. No new cutaneous reactions have been observed in either patient since that time, with a follow-up period of 24 months.

The exact mechanism of action of cyclosporin A in the skin is controversial. Some report a direct effect on Langerhans cells, while other experimental papers report an inhibition on T cells as measured in the in vitro mixed skin–cell lymphocyte reaction. We think that this small experience could be expanded in the future, because more patients are being treated with imatinib in different indications (such as first-line treatment in CML) and more cutaneous reactions to the drug are expected. In this way, perhaps this cyclosporin A–based local treatment could be compared with other different type of creams, like the commercially available corticosteroid-containing creams. This approach could also be tested for the treatment of cutaneous reactions associated with other drugs (such as antibiotics) or of the localized lichenoid cutaneous lesions in patients with chronic graft-versus-host disease.

To the editor:

Quantitative RT-PCR analysis of activation-induced cytidine deaminase expression in tissue samples from mantle cell lymphoma and B-cell chronic lymphocytic leukemia patients

Expression of activation-induced cytidine deaminase (AID) is crucial for immunoglobulin V gene somatic hypermutations (SHMs) and immunoglobulin class switch recombinations (CSRs). Expression of AID is associated with the germinal center (GC) reaction and is not expressed in naïve B cells. AID expression requires CD40 signaling and interleukin 4, likely to be encountered in the lymphoid tissues. Previously, we and others have shown that AID mRNA in B-cell non-Hodgkin lymphomas was confined to GC-derived lymphomas. Recently, Babbage et al reported AID mRNA by reverse transcriptase–polymerase chain reaction (RT-PCR) in circulating tumor cells from 17 of 18 mantle cell lymphoma (MCL) patients. Because no AID mRNA was detected in blood cells of healthy donors, they concluded that AID expression in MCL is a tumor-related activation phenomenon.

By use of Taqman quantitative RT-PCR, we detected AID expression in tissue samples from 14 of 17 MCL patients, but with the exception of 2 cases, the level was very low (Figure 1). In reactive tonsils, lymph nodes, and tonsillar GC cells expression was greater than 100-fold higher. Compared with circulating naïve B cells, AID expression was on average 2-fold higher in MCL tissue samples. The AID expression levels were comparable in 5 IGVH-mutated MCL cases versus 5 IGVH-unmutated MCL cases (SHM cutoff 2%), concordant with the study by Babbage et al. A salient discordance between IGVH mutational status and AID expression has been reported for B-cell chronic lymphocytic leukemia (B-CLL), although AID expression levels in B-CLL cells remained well below that of GC cells. In that study, AID was determined in circulating B-CLL cells. In the tissue compartment, B-CLL cells are organized into “pseudo-follicles,” in which scattered CD4+ T cells capable of delivering signals involved in AID expression are present. We therefore also quantified AID mRNA expression in tissue samples from B-CLL patients (n = 12). Four of 6 cases expressing zeta-associated protein 70 (ZAP-70), thus presumably containing a low number of mutations, showed high expression of AID comparable to reactive tonsils and lymph nodes. This expression was much higher than in MCL tissues. In 6 cases without ZAP-70 expression, AID expression was on average 300-fold lower compared with tonsils and lymph nodes. These results underscore the inverse correlation between IGVH mutational status and AID expression in B-CLL. Whether AID expression in unmutated/ZAP-70–expressing B-CLL is related to CSR activity remains to be established.

It is conceivable that AID expression is induced in the tissue compartment of ZAP-70–expressing B-CLL and wanes in the circulating tumor cells. We conclude that with the exception of a very few cases, AID expression is very low in MCL and is not

References

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