The polymorphism of Multi-Drug Resistance 1 gene (MDR1) does not influence the pharmacokinetics of Dexamethasone loaded into autologous erythrocytes of patients with inflammatory bowel disease

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Abstract. – Background: We have recently demonstrated that low doses of Dexamethasone 21-P (Dex 21-P), loaded in autologous erythrocytes and administered at monthly intervals, have been able to maintain steroid-dependent patients with Crohn’s disease (CD) and ulcerative colitis (UC) in clinical remission with a progressive and complete tapering of systemic steroids.

Aim: Since multi-drug resistance 1 gene (MDR1) has a potential influence on Dexamethasone (Dex) bioavailability, we designed this study to investigate the correlation between MDR1 genotype and Dex pharmacokinetic after its delivery in patients with inflammatory bowel disease (IBD).

Materials and Methods: Seventeen steroid-dependent consecutive patients with IBD (10 UC mean age 36 ± 12, and 7 Crohn’s disease mean age 31 ± 5) were consecutively recruited. The C3435T polymorphism of MDR1 gene was studied by Denaturing High Performance Liquid Chromatography (DHPLC). Serum level of Dex were determined at the end of the infusion and after 15 days by high performance liquid chromatography electrospray mass spectrometry.

Results: The mean dose of Dex 21-P administered was 9.9 mg ± 4 (range 2.7-20.3), while the mean levels of Dex at the end of the infusion and after 15 days were 0.66 ± 0.23 mM and 0.06 ± 0.06 mM, respectively. Concerning the C3435T genotype, two patients were wild-type, eleven heterozygotes, and four homozygotes. No correlation between basal or 15-days plasma level of Dex and MDR1 genotype was found (r = 0.19 and r = 0.21, respectively).

Conclusion: Our findings demonstrated that Dex plasma level, after infusion of autologous erythrocytes loaded with Dex 21-P are completely independent by the MDR 1 gene polymorphism. This could be another potential advantage of this modality of drug delivering.

Key Words: Crohn’s disease, Infliximab, SonoVue, Ultrasonography.

Introduction

Crohn’s disease (CD) and Ulcerative Colitis (UC) are the two main clinical subtypes of inflammatory bowel disease (IBD); they are chronic relapsing disorders with no definitive cure. The steroids are still the mainstay of the therapy in the moderate to severe flares of the disease¹. However, their use carries a considerable number of adverse events² and up to 30% of patients may become steroid-dependent³,⁴. Furthermore up to one quarter of patients may become steroid-refractory during the disease course³,⁴.

It is well known that human erythrocytes, due to the noteworthy ability to open and reseal their membrane, may provide a potential vehicle for drugs delivering in the blood stream. Previous experiences in our laboratory have demonstrated that the non-diffusible pro-drug Dexamethasone-21-Phosphate (Dex 21-P) can be entrapped into human erythrocytes, where is subsequently slowly dephos-
phorylated to the active and diffusible Dexamethasone (Dex), by erythrocyte enzymes. More recently a new equipment called “Red Cell Loader” (Compact A, Sorin Group Italy s.r.l., Mirandola, Italy) that allows the automatic encapsulation of drugs in human erythrocytes has been developed for clinical use.

We have recently demonstrated, in a pilot study, that low dose of Dex 21-P loaded in autologous erythrocytes (RBC) and administered at monthly intervals, have been able to maintain steroid-dependent patients with CD and UC in clinical remission with a progressive and complete tapering of systemic steroids.

The great interest in clinical use of Dex is that very low dose of drug are employed with a constant and prolonged delivery by autologous erythrocytes. This delivery might enhance the clinical efficacy with no interference with the pituitary-adrenal axis, due to the low dosage administered.

One potential modifier of the bio-availability of Dex after erythrocyte delivery is the activity of P-glycoprotein 170 (PGP). This protein is found in high concentrations on the apical surfaces of superficial columnar epithelial cells of the colon and the distal small bowel, and seems to play a critical role in the host-bacterial interactions and maintenance of intestinal homeostasis. Moreover, PGP acts as an efflux pump, moving from the inner to the outer leaflet of the cell membrane a number of substrates and drugs, including corticosteroids like the Dexamethasone. The PGP is coded by the multidrug resistance 1 gene (MDR1), which maps at the level of chromosome 7q21.1. The gene is highly polymorphic, with 50 single nucleotide polymorphisms (SNPs) and 3 insertion/deletion polymorphisms reported thus far. The exon 26 SNP C3435T is of special interest; although it is a silent mutation, located in the wobble position of a codon, the TT genotype is associated with a 2-fold reduction of PGP expression in the intestine and lymphocytes, with functional consequence on drugs uptake.

We hypothesized that because of the slow and constant delivery, the Dexamethasone pharmacokinetics should not be influenced by different genotypes of the MDR1 gene, and namely the C3435T polymorphism. Therefore we designed this study with the aim to investigate the potential correlation between MDR1 genotype and the pharmacokinetic of Dex after its delivery from autologous erythrocytes in patients with IBD.

**Materials and Methods**

**Study Population**

Seventeen steroid-dependent consecutive patients with IBD were consecutively recruited at the Casa Sollievo della Sofferenza Hospital in San Giovanni Rotondo, Italy. Diagnosis of CD and UC was established according to accepted clinical, endoscopic, radiologic, and histologic criteria. Ten patients had an Ulcerative Colitis (6 males, mean age 36 ± 12), while 7 patients had a Crohn’s disease (4 males, mean age 31 ± 5). Duration of disease ranged from 2 to 10 years; seven patients were in clinical remission (DAI = Disease activity index for Ulcerative colitis) (CDAI = Crohn’s disease activity index), while ten had a mild activity. Mean dose of steroids used was 20 mg of Prednisolone (range 8-30 mg).

**Study Protocol**

After obtaining the informed consent, the infusion of Dex-P was administered (see below). Before the infusion, at the end of the infusion, and after 1, 7, and 15 days, a blood sample was obtained to investigate the pharmacokinetic of Dex (see below) and DNA extraction. The study protocol was approved by the local Ethics Committee.

**Erythrocyte Loading With Dex 21-P**

The entire process was carried out by using a new equipment, the “Red Cell Loader” (Compact A, Sorin Group Italy s.r.l., Mirandola, Italy), which allows automatic encapsulation under blood-banking condition. An assistant nurse easily performed the procedure within two hours at room temperature. Essential steps of the process involve lyses of red blood cell in a hypotonic solution, entrapping the Dex 21-P, resealing of erythrocytes, and their infusion into original donors. Briefly, 50 ml of blood are collected from each patient into heparinized syringes and mixed with normal saline. Red blood cells are separated by centrifugation and dialyzed against a hypotonic solution to allow opening of pores within their cell membranes, and diffusion of the
cellular content into the dialyzing solution. Subsequently, lysed erythrocytes are concentrated in a haemofilter, and incubated with Dex 21-P (disodium salt) to a 10 mM final concentration (corresponding to 0.5 g of drug). After equilibrium has been reached, osmolarity is restored to permit rescaling of membrane pores. The procedure is followed by washing out non-trapped drug. Loaded erythrocytes are finally collected and re-infused into the original donor, at the infusion rate of 20 ml/min.

**Determination of the Amount of Dex 21-P Administered**

One hundred ml of the final red blood cells suspension were diluted with 0.9 ml of double-distilled water, boiled for 5 min and filtered through 0.22 mm filters. Dex 21-P was determined by HPLC, as previously described5.

**Pharmacokinetic Study**

Ten ml of blood were drawn at time 0 (before infusion), at the end of the infusion, 7 and 15 days after infusion. After centrifugation at 2,000 g, plasma was separated, and Dex extracted using diethyl ether. After centrifugation at 2,000 g, the diethyl ether was separated, concentrated with a Speed Vac concentrator (Savan Instruments) and resuspended in acetonitrile. The amount of Dex was determined by high performance liquid chromatography electrospray mass spectrometry (HPLC-ESPO-MS).

**Genotyping**

Genomic DNA samples were extracted from peripheral blood leukocytes, according to standard protocols.

The sequences of the primers used for MDR1 genotyping, designed using Primer Input program, were as follows:

- MDR1_3435-F (5’-TGT TTTCAGCT-GCTTGATGG-3’)
- MDR1_3435-R (5’-AGGCCAGTGACTCGATGAAGG-3’);

PCR reaction (50 ml) was done in 1X GeneAmp Buffer II, 2.5 mM MgCl2, 3U AmpliTaq Gold DNA polymerase, 0.2 mM for each primer, 0.5 mM dNTPs and 50 ng of genomic DNA. After initial denaturation at 95°C for 10 min, reaction was subjected to amplification for 35 cycles: 1 min at 95°C, 1 min at 60°C, 1 min at 72°C. Final extension was 72°C for 10 min. The C3435T polymorphism was genotyped using DHPLC (Denaturing High Performance Liquid Chromatography, Wave System, Transgenomic Ltd., UK) as previously described22.

**Statistical Analysis**

Comparison of continuous variables of pharmacokinetics was performed with linear and logarithmic regression analysis. Data of C3435T genotype were expressed as wild-type, heterozygote and homozygote for the mutation known to modify the PGP protein function.

The level of accepted statistical significance was \( p < 0.05 \).

**Results**

All subjects completed the infusion of Dex 21-P without adverse events.

At the end of encapsulation procedure 110 ± 21 ml (range 40-126) of erythrocyte suspension at 10.2 ± 3 % (range 8.5-15.8) hematocrit was obtained. The amounts of Dex 21-P administered and the value of serum Dex at the end of the infusion and after 15 days are given in the Table I. The mean dose of Dex 21-P administered was 9.9 mg ± 4 (range 2.7-20.3), while the mean plasma levels of Dex at the of the infusion and after 15 days were 0.66 ± 0.23 µM and 0.06 ± 0.06 µM, respectively.

Stable plasma levels of the drug were found during the two weeks following the infusion.

Concerning the C3435T genotype, two patients were wild-type, eleven heterozygotes, and four homozygotes.

When the basal (at the end of RBC infusion) and final (15-days) pharmacokinetic values were correlated with the MDR1 genotype, no significant difference was found with a very low correlation between genotype and plasma level (\( r=0.09 \) basal, \( r=0.21 \) at 15 days, respectively).

**Discussion**

The therapy of IBD is still a challenge. More than half of patients need steroids during their life and about one-third may be-
came steroid-dependent. Furthermore, although azathioprine or 6-mercaptopurine are used as steroid-sparing agents, still one-third of patients (approximately 3-5% of all IBD patients) are intolerant or non-responders to immunosuppressive drugs.

In a pilot study we have demonstrated the efficacy of very low doses of Dex released by autologous erythrocytes to stop the administration of systemic steroids in steroid-dependent IBD patients. The very low dose of Dex released in blood stream was able to maintain steroid-dependent IBD patients in clinical remission, with progressive tapering and suspension of steroids within two months.

Multidrug resistance is one of the most serious causes of the failure of chemotherapy and the down-regulation of the uptake or induction of efflux systems (e.g. MDR1) is one of the best characterized mechanisms.

In this study we investigated the potential influence of MDR1 gene on pharmacokinetics of Dex delivery by autologous erythrocytes, since Dex is one of the MDR1 substrate. More specifically we investigated whether the TT genotype of the C3435T polymorphism of MRD1, which determines a 2-fold reduction of PGP expression, was correlated with a different bioavailability of Dex delivered by erythrocytes in the blood stream. Our findings demonstrated, however, that the Dex detection was completely independent by the MDR 1 gene polymorphism. This finding, if demonstrated in larger sample size, could be another potential advantage of this drug delivery methodology.

**References**


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