

The breast cancer resistance protein: gatekeeper to the synovium?

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KEYWORDS: antirheumatic drugs ■ BCRP ■ breast cancer resistance protein
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Disease-modifying antirheumatic drugs & drug resistance

Disease-modifying antirheumatic drugs (DMARDs) are still the first choice treatment and mainstay in the management of rheumatoid arthritis (RA). However, clinicians are aware that DMARD resistance is a reality in rheumatology practice. In general, there are two different types of drug resistance at the cellular level for all drugs. First, cells may already be resistant before receiving therapy, this is called ‘primary’ (inherited) resistance. The other resistance mechanism is ‘secondary’ resistance in which cells that were initially sensitive to drugs develop resistance during the course of treatment [1]. Morgan *et al.* have assessed the multi-drug resistance (MDR) phenomenon in their retrospective analysis of 265 RA patients. They found one, two and three or more DMARDs were effective in 40, 11 and 5% of patients, respectively [2]. Overall, MDR occurred in 5% of RA patients.

MDR proteins & breast cancer resistance protein overview

Human cells interact with environmental toxins several times everyday and cells develop protection mechanisms against these toxins during the course of evolution. The efflux of drugs from inside the cell to outside is a special and unique function for cleaning toxins in human cells. MDR proteins are key components in this pathway. The MDR concept is well recognized, particularly in oncology and infectious diseases. In contrast with the oncology literature, limited evidence exists regarding DMARD resistance in rheumatology. Overexpression of the ATP-binding cassette (ABC) transporter system is

responsible for this MDR. ABC transporters utilize the energy derived from ATP to drive substrate (e.g., amino acids, lipids, ions, peptides, proteins and drugs) translocation across the plasma membrane [1]. ABC transporters can be grouped into subfamilies based on the amino acid sequence and the seven ABC transporters are named from ABC-A to ABC-G [1]. ABCB1 or P-glycoprotein is the most popular protein in oncology literature and was discovered in 1976 [1]. The first manuscript regarding MDR in rheumatology was published in 1995 [3] and almost 20 articles have been published to date. ABCG2 or breast cancer resistance protein (BCRP) is a relatively new ABC transporter protein that was discovered a decade ago [4]. BCRP is a 72-kDa protein composed of 655 amino acids. It has an N-terminal ATP-binding domain and a C-terminal transmembrane domain [4]. BCRP is highly expressed in the maternal–fetal barrier of the placenta and also expressed in lower levels in the brain, prostate, small intestine, adrenal gland, testis, ovary, breast, venous endothelium, capillaries and liver. However, BCRP is not expressed in the heart, lung, skeletal muscle, kidney, pancreas, spleen, thymus or peripheral blood leukocytes [4]. Tissue localization of BCRP in human cells suggests that BCRP may have a role against xenobiotics in these cells, and BCRP eliminates a variety of compounds such as antibiotic (e.g., kinolons and erythromycin), antiviral (e.g., lamivudine and zidovudine) and anticancer drugs (e.g., imatinib, daunorubicin, topotecan and etoposide) [4]. Interestingly, frequently used DMARDs such as methotrexate, leflunomide and sulfasalazine are all substrates for BCRP [5]. BCRP may have a role in chemotherapy resistance in leukemia, but data are unclear in solid tumors [4].



U Kalyoncu

Author for correspondence:
Hacettepe University, Department of Internal Medicine, Division of Rheumatology, Ankara, Turkey
Tel.: +90 312 310 0194
Fax: +90 312 310 0194
umutkalyoncu@yahoo.com.tr



I Ertenli

Hacettepe University, Department of Internal Medicine, Division of Rheumatology, Ankara, Turkey

BCRP & RA

Until now, there have been five published articles regarding BCRP and RA/DMARDs [5–9]. Two articles are related to sulfasalazine efficacy *in vitro* and an experimental mouse study. In one study, van der Heijden *et al.* demonstrated that *in vitro* long-term exposure of human T cells to sulfasalazine may result in upregulated expression of BCRP, reduced intracellular drug levels and increased production of TNF- α , a key cytokine of RA [5]. In a mouse model, after oral administration of 20 mg/kg sulfasalazine, the area under the plasma concentration time profile in BCRP^{-/-} knockout mice was approximately 111-fold higher than that in wild-type mice [6]. This study demonstrated that bioavailability of orally administered drugs may be restricted by BCRP owing to its apical localization in the intestine.

In another study, BCRP levels were not found to be different between RA patients and healthy controls in peripheral blood lymphocytes [7]. However, synovium is a target tissue in RA and blood levels of MDR proteins may not reflect tissue levels very well; synovial pathological investigations might reflect its presence better than blood levels. To date, there have been two studies published regarding BCRP and synovial biopsy. In 2009, van der Heijden *et al.* assessed the expression of ABC transporters in RA synovial tissue biopsy samples that were obtained prior to treatment and after 4 months of therapy with methotrexate (17 patients) and leflunomide (13 patients), both of which are substrates of BCRP [8]. Immunohistochemical and immunofluorescence analysis of P-glycoprotein, multidrug resistance protein (MRP)1–9 and BCRP were obtained from biopsy specimens. The most abundantly expressed protein in synovial intimal lining layer and the synovial sublining appeared to be BCRP. Only a few BCRP-positive cells were detected in noninflammatory synovial tissue from orthopedic patients. Interestingly, detailed fluorescence microscopy investigations have shown that BCRP was expressed mainly on RA macrophages and also on endothelial cells and perivascular CD3⁺ T cells. They also found that MRP-1 was detected in a third of the RA patients but P-glycoprotein and MRP-2–9 were not detectable in the synovium. The median number of BCRP-positive cells were higher in nonresponders (both methotrexate and leflunomide groups) than responders.

We also assessed synovial pathological involvement of MDR proteins [9]. Our patient group was selected from 1905 pathological

specimens with knee or hip prosthesis. Synovial specimens of 17 RA patients were used as the study group and specimens from patients with ankylosing spondylitis (nine patients), juvenile chronic arthritis (six patients) and osteoarthritis (nine patients), and patients with fracture after trauma (nine patients) were used as control groups. The specimens were evaluated via immunohistochemical methods for P-glycoprotein, BCRP and MRP-1. BCRP staining was detected in 41% of RA patients in macrophages and endothelial cells but none of the control group patients had BCRP staining. Three patients had mild BCRP staining, three patients had moderate and one patient had severe staining. Although patients with BCRP staining had a higher Disease Activity Score (DAS)-28 than patients without BCRP staining, it was not significant [10].

Conclusion & future perspective

Over the past few decades, clinicians and researchers have tried to answer the following important questions: “which patients will be treated effectively with DMARDs therapy?”; and “when should patients be treated with combined/aggressive DMARDs and/or biological therapy?” Currently, it is impossible to answer these questions fully. In order to answer these questions, rheumatologists can learn from certain data from oncology literature that drug resistance is a life-threatening problem for the management of cancer patients. In 1976, they discovered that P-glycoprotein is a MDR protein; however, it appears that BCRP will be the important MDR protein for rheumatologists. We and others have demonstrated that BCRP is present in RA synovial lining and sublining layer macrophages and endothelial cells. Drugs have to enter the synovium from the circulation via endothelial cells. However, the presence of BCRP in endothelial cells may prevent DMARDs such as methotrexate, sulfasalazine and leflunomide from entering these cells, thus acting as a barrier. It is well known that macrophages are one of the key cells in RA synovium but the pathophysiological role of BCRP expression on synovial macrophages is not yet known. Future research should investigate this unexpected association.

Breast cancer resistance protein absence on macrophages was found to be more prominent in the DMARDs and, as expected, the BCRP-positive macrophage count decreased after methotrexate or leflunomide therapy [9]. These results have shown that the ‘therapy-induced phenomenon’, which was first described in

oncology literature where MDR proteins were observed after certain chemotherapy, was not found in RA. This result is important for the future direction of RA research. Perhaps, clinicians will decide to start different DMARDs and/or biological therapies depending on the concentration of BCRP on macrophages in at least a subgroup of RA patients. For this to occur, a simple method of determining the BCRP status of RA patients is needed as synovial biopsy is difficult and may not be possible every time.

Several strategies to overcome DMARD resistance may be proposed. Blocking BCRP could be one of these strategies, and this may result in an increase in the plasma and intracellular drug concentrations. As discussed earlier, the concentration of sulfasalazine is approximately 111-fold higher in BCRP knockout mice than control mice and can be returned to normal levels using gefitinib, a known inhibitor of BCRP [6]. However, increased intracellular drug concentration may also lead to frequent and severe side effects and this has to be evaluated

carefully in future investigations. There are many known inhibitors of BCRP, and interestingly one of these is also a DMARD, cyclosporine [4]. It is well known that cyclosporine is an effective alternative drug for management of RA treatment. The combination of cyclosporine with either leflunomide or methotrexate has produced better therapeutic results than monotherapy [10,11]. It can be speculated that cyclosporine may increase intracellular methotrexate and/or leflunomide concentration by blocking BCRP. Researchers should also investigate this existing relationship in the future.

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