BORON UPTAKE IN HUMAN CELLS *IN VITRO:* A STUDY OF THE PREREQUISITES FOR BNCS OF THE INFLAMED SYNOVIA IN THE RA-JOINT

Maria Dahlström[1] and Annelie Lindström[1]

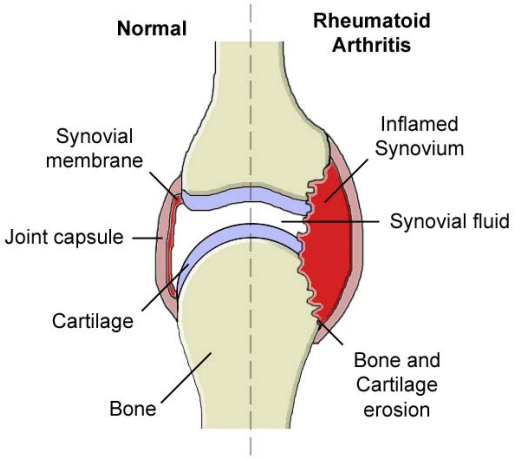
[1]Division of Cell Biology, Department of Biomedicine and Surgery, Faculty of Health Sciences, Linköping University, S-581 85 Linköping, Sweden

ABSTRACT

The auto-immune disease rheumatoid arthritis (RA) is characterised by inflamed and painful joints. Treatment of RA is dedicated to inflammatory control for prevention of disease development and degradation of bone and cartilage. Not all patients respond to drug treatment and therefore need synovectomy i.e. the inflamed synovium is removed surgically or otherwise. However, it is desirable to avoid surgical encroachment of the joint due to unwanted minor damage to healthy tissue. Boron neutron capture synovectomy (BNCS), a radiotherapy based on the activation of the stable isotope ^{10}B, could be an interesting alternative for treatment of RA-patients. The aim of this study is to use *in vitro* models for investigation of the prerequisites for BNCS. The accumulation of the boron compound boronophenylalanine, BPA, is investigated in a synovial sarcoma cell line, a monocytoid cell line and a fibroblast cell line, all of human origin. The preliminary results indicate differences in accumulation ratio (ratio between cell-associated and extracellular boron). The cell lines might be of interest in constructing a three dimensional *in vitro* model of the inflamed synovium of the RA-joint.

Introduction

Rheumatoid arthritis (RA) is an auto-immune disease characterised by inflamed and painful joints (figure 1). The primary reason for the inflammation is still unknown, but an invasion of inflammatory cells from the circulation can be seen in synovial tissue and fluid at an early stage of the disease. With time, the inflammation develops to a chronic state that causes structural changes due to the extensive growth of cells in the synovial tissue. The highly proliferating cells, the synoviocytes, do not respect the normal limits and structure of the joint tissue (Edwards 1994). They grow fast and invade the joint cavity. The reason for hyper proliferation of the synovial joint tissues is still unknown but it is likely to be caused by growth stimulating cytokines, based on the fact that cytokines are often highly expressed in areas with abnormal cell growth and fibrosis (Kapp 1993, Babyatsky et al. 1996). The synoviocytes, together with the inflammatory cells, excrete high amounts of cytokines and enzymes into the synovial fluid. These cytokines and enzymes play an important role in the destruction of bone and cartilage that is seen in the inflamed joint (Yanch et al. 1999). The synovial cells are of two types, the type A cells are macrophage-like while the type B cells are fibroblast-like (Edwards 1994).



Figur 1 The normal synovium and the inflamed synovium (rheumatoid arthritis) in a joint.

Treatment of RA is dedicated to inflammatory control for prevention of disease development and degradation of bone and cartilage. For a majority of the patients, RA can be controlled by the use of drugs. Patients that do not show positive response to the drugs need synovectomy which means that the inflamed synovium is removed; surgically or otherwise. However, surgical encroachment of the joint can lead to unwanted minor damage to healthy joint tissue and the procedure demands a rehabilitation period for the joint to recover. To avoid the negative effects of surgery, efforts have been made to achieve synovectomy by local irradiation of the synovium tissue using a β-emitting isotope. A major drawback of this method was leakage of radioactive material into the circulation. Healthy tissue was exposed to unacceptable doses of ionising radiation. A symptom-free period of 2-5 years is achieved for the patient with surgical or radioisotope based synovectomy. (Yanch et al. 1999.)

Boron neutron capture synovectomy (BNCS) is a highly interesting radiotherapy, it takes advantage of the ability of the stable isotope boron-10 to capture thermal neutrons according to $^{10}B(\alpha, n)^{7}Li$. The α and Li particles have high energy and short path length, approximately one cell diameter, which results in local irradiation of target tissue. It is highly relevant to look for specific targets in pathologically proliferating joint synovium. If RA is initiated and regulated by cytokines excreted by the cells in the joint tissue it is potentially possible to construct boron compounds with specificity against structures on these cells. The consequence would hopefully be specific elimination of cells responsible for the inflammation which may end up in a long lasting effect.

We will investigate the prerequisites for BNCS of the RA-joint using *in vitro* models. The aim is to construct and evaluate boron compounds suitable for targeting the inflamed synovia. Specific inhibition of proliferating and inflammatory cells in the inflamed synovium, without the drawbacks of surgery and radioisotope based synovectomy, could provide an effective alternative treatment for aggressive and/ or therapy-resistant RA.

Material and methods

The human cell lines used are; a synovial sarcoma cell line, 4SS, a monocytoid cell line, U937-1, and a fibroblast cell line, Ag1523. The 4SS cell line is used for experiments in two different states: confluent ($1.12 \cdot 10^5$ cells/cm^2) and nonconfluent ($2.1\text{-}8.9 \cdot 10^4$ cells/cm^2). The fibroblast cell line is used as a control of normal cells and is used in different cellular states: proliferating ($2.7 \cdot 10^4$ cells/cm^2) and non-proliferating ($3.0\text{-}3.6 \cdot 10^4$ cells/cm^2). The cells are incubated with boronophenylalanine (BPA) or boric acid for 18 h before harvesting by trypsination, concentration and resuspension. Cell-associated boron is separated from extracellular by rapid oil filtration (Capala et al. 1996, Dahlström et al. 2003). Boron measurements are accomplished by means of inductively coupled plasma atomic emission spectroscopy (ICP-AES). Flow cytometry is used for characterisation of expression of cytokines and receptors on the cells.

Preliminary results

Figure 2 shows cell-associated amounts of boron after 18 h incubation with BPA for the 4SS cell line, confluent and nonconfluent, and the U937-1 cell line. A difference can be seen between the cell lines and also between confluent and nonconfluent 4SS. The confluent 4SS shows an accumulation ratio of 1.99 while the ratio for the nonconfluent is 3.66. The accumulation ratio for U937-1 cell line is 1.60 (Table 1).

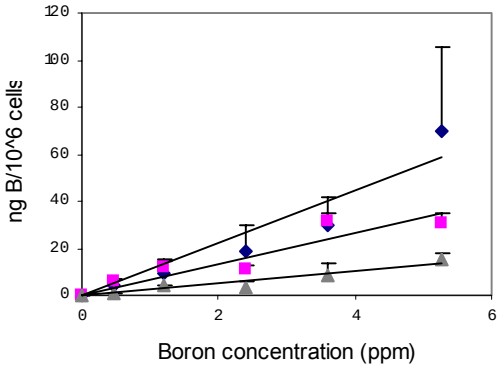


Figure 1 Cell-associated boron (ng B/10^6 cells) for 4SS confluent (■), nonconfluent (♦) and U937-1 (▲) as a function of boron concentration in cell culture medium at 18 h incubation time. Data points represent the mean± SD of three independent measurements.

Table 1 shows the calculated accumulation ratios for the cell lines. The accumulation ratio is defined as the ratio between cell-associated boron and extracellular boron (Dahlström et al. 2003). The proliferating fibroblasts show an accumulation ratio of 1.54 and the non-proliferating 0.70.

Table 1. Obtained accumulation rates after 18 h incubation with BPA or boric acid and calculated accumulation ratios for 4SS, U937-1 and Ag1523.

	Accumulation rate		Accumulation ratio
Cell type	BPA	Boric acid	
4SS			
confluent	6.57 (R^2=0.855)	3.30 (R^2=0.988)	1.99
nonconfluent	11.13 (R^2=0.910)	3.04 (R^2=0.956)	3.66
U937-1	2.53 (R^2=0.925)	1.57 (R^2=0.929)	1.60
Ag1523			
proliferating	10.58 (R^2=0.962)	6.89 (R^2=0.999)	1.54
non-proliferating	4.89 (R^2=0.325)	6.97 (R^2=0.928)	0.70

Results for flow cytometry of 4SS cells showed expression of transforming growth factor-alpha, TGF-α, and its receptor epidermal growth factor receptor, EGFR. Both were localised on the cellular membrane.

Discussion

The 4SS cell line represents, in this study, the fibroblast-like type B synovial cells while the U937-1 cell line represents macrophage-like type A synovial cells. The preliminary results for these cell lines indicate a difference in accumulation ratio between 4SS cells, confluent and nonconfluent, and U937-1 cells. The confluent 4SS shows an accumulation ratio slightly greater than U937-1 cells (1.99 and 1.60, respectively) while the nonconfluent shows a ratio of approximately 3.7. The fibroblast cell line, here used as a control of normal cells, shows a growth dependent uptake of BPA. The proliferating cells showing an accumulation ratio twice that of non-proliferating (1.54 and 0.70, respectively). The 4SS and U937-1 cell lines are thought to be used in a three-dimensional *in vitro* model of RA. Set up of such a model, but based on human primary synovial cultures, has previously been reported (Taguchi et al. 1997). A controlled *in vitro* system can be of advantage when evaluating the targeting

properties of boron compounds. The model will be used for screening purposes and promising boron compounds will be further evaluated *in vivo*.

For a majority of RA-patients, the disease development can be hampered and the symptoms controlled by the use of systemic drugs. However, not all patients show a positive response to the treatment. Treatment with systemic drugs is also associated with side effects and the fact that the effects of the drugs diminish during the treatment time. Patients can undergo synovectomy when not responding to treatment with drugs. Unfortunately, surgical synovectomy can lead to unwanted minor damage of healthy tissue and a rehabilitation period is necessary. The use of radioactive isotopes for synovectomy is associated with leakage and irradiation of healthy tissue. BNCS is therefore an interesting alternative to the treatment regimens used today for treatment of patients with RA. If BNCS is used for therapy it will most likely give the same effects as surgical and radioisotope based synovectomy i.e. a symptom-free period of 2-5 years. For the method to be highly efficient the boron compounds must be specific to joints. It is therefore highly relevant to look for specific targets in pathologically proliferating joint synovium. If RA is initiated and regulated by cytokines excreted by the cells in the joint tissue it is potentially possible to construct boron compounds with specificity against structures in these cells. The consequence would hopefully be specific elimination of cells responsible for the inflammation and a long lasting effect. It has been shown that synovial fluid from patients with RA contains higher amounts of the growth factor TGF-α compared to joint fluid from healthy controls (Hallbeck et al. 1999). This work is continued by our research group with the ambition to map the presence of TGF-α and it's receptor, EGFR, in normal and inflamed joint. The roles that TGF-α and EGFR play in the origin and development of RA will also be elucidated. This work might give important insights to the abnormal growth of joint tissue that occurs in RA-joints.

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