

CD94:NKG2A in NK and CIK in MM and nHL compared with normal donors. We have showed that the mean percentage of NK and CIK with CD94 expressing NKG2A is lower in MM patients compared with normal donors ($p < 0,05$). It means that there is an increased expression of non-functional CD94 on NK and CIK of myeloma patients.

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UNEXPECTED ANTAGONISTIC EFFECT OF RITUXIMAB WITH PROCARBAZINE DISCLOSED DURING AN IN VITRO TESTING OF RITUXIMAB-MEDIATED SENSITIZATION OF B-CELL LINES TO COMMONLY USED ANTICANCER DRUGS

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Backgrounds. Rituximab is a chimeric monoclonal antibody specific to the CD20-antigene expressed on mature B-lymphocytes. The antibody sensitizes lymphoma cells to differently acting cytotoxic drugs. Although some combinations of cytostatic agents with rituximab have already been tested, there are many others for which no information is available. **Aims.** To analyse some commonly used and some new combinations of rituximab with differently acting cytotoxic agents in vitro using permanently growing B-cell lines. **Methods.** The stable cell lines derived from a follicular lymphoma (WSU-NHL, DHL-4 and DOHH-2) and Burkitt lymphoma (RAMOS) were used for an *in vitro* viability assay. The cell lines were pretreated by 20 µg/mL of rituximab for 72 hours, followed by a subsequent incubation with the cytotoxic drugs (fludarabine, doxorubicin, vincristine, dexamethasone and procarbazine in four different concentrations) for 48 hours. A proliferation activity was estimated using a WST-1 assay. Obtained data were statistically evaluated using multi-way analysis of variance with interactions. The concentrations, presence or absence of pretreatment and plate variability were taken for the fixed effect. A cell cycle after the rituximab pre-treatment was analysed by flow-cytometry with propidium iodide. **Results.** Rituximab significantly decreased an S-phase of the DHL-4 cells, while no prominent effect on cell cycle was observed for the other cell lines. We observed a significantly different sensitivity of follicular lymphoma and Burkitt's lymphoma cells to vincristine and fludarabine (FL cells were highly sensitive to vincristine and rather poorly to fludarabine, while an opposite effect was seen for BL cells). The rituximab pretreatment sensitized all cell lines to vincristine, while none were sensitized to doxorubicin. Heterogenous results were obtained for the other combinations. A statistically significant influence of the rituximab pretreatment was proved for: dexamethasone at DOHH-2 and RAMOS, fludarabine at WSU-NHL and fludarabine and dexamethasone at DHL-4 cell lines. We obtained quite unexpected results for procarbazine in combination with rituximab. Although the drug strongly inhibited a metabolic activity in all tested cell lines, the effect was just opposite when the cells were pretreated with rituximab. A highly statistically significant antagonistic effect was proved for all the cell lines. **Summary.** The data confirm that rituximab might sensitize lymphoma B-cells to most of differently acting anti-cancer agents. There are, however, some drugs manifesting a strong antagonistic effect with respect to rituximab. Therefore, based on our experimental data, the combination of rituximab with chemotherapy regimens containing procarbazine (e.g. R-COPP) does not seem to be clinically warranted.

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ASPIRIN RESISTANCE IN PATIENTS AFTER ISCHAEMIC STROKE AND ISOPROSTANE (8-EPIPROSTAGLANDIN F2A) PLASMA CONCENTRATION

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Background. The limited efficacy of secondary prevention for ischaemic stroke may be partially related to aspirin resistance leading to continuous generation of intraplatelet thromboxane A2. Besides other underlying metabolic mechanisms, an oxidant stress along with nonenzymatic biosynthesis of isoeicosanoids supporting the platelet activation has been suggested. **Aims.** We analyzed the incidence of aspirin resistance in survivors of ischemic stroke and compared the usefulness of some platelet tests designed for its laboratory exploration. **Material and Methods.** Forty four patients, at least a month after acute onset of ischaemic stroke were included into the study. All of them have been receiving 75-150 mg aspirin daily at least for a month. The control group consisted

of 12 adequately matched healthy volunteers. The platelet function was investigated by platelet aggregation induced by either ADP (3.5 and 5.0 µM), collagen (2 µg/mL) or arachidonic acid (AA) (0.6 mM) and measurement of closure time on the collagen and epinephrine (Col/Epi) cartridge in PFA-100 analyzer. Thromboxane A2 metabolite - 11-dehydro Thromboxane B2 (11-dTxB2) and isoeicosanoid '8-epi Prostaglandin F2_α' (8-epiPgF2_α) plasma concentration by immunoenzymatic method (EIA Kits from Cayman Chemicals) were also determined. The aspirin ingestion was controlled by diminished intraplatelet concentration of malonyldialdehyde. Aspirin resistance has been determined by the following criteria: the intensity of platelet aggregation induced by ADP 60%, collagen 70%, AA 20%, PFA-100 closure time 165 s and as reference indicator; 11-dTxB2 concentration mean of the control group minus SD.

Table 1. Aspirin resistance in patients after stroke.

The frequency of aspirin resistance in patients after ischemic stroke					
ADP 3.5 µM	ADP 5.0 µM	Platelet aggregation		PFA-100	11-dTxB ₂
		Collagen	Arachidonic acid		
45%	52%	20%	7%	52%	43%

Statistically significant inverse correlations have been found between the plasma concentration of 11-dTxB2 and PFA-100 (Col/Epi) closure time ($r = -0.31$; $p = 0.039$) as well as between plasma concentration of 8-epiPgF2TM and PFA-100TM (Col/Epi) closure time ($r = -0.36$; $p = 0.019$). **Conclusions.** 1. Laboratory tests reveal aspirin resistance in almost half of patients after ischaemic stroke. 2. The most significant correlation has been found between plasma concentration of reference indicator 11-dehydro Thromboxane B2 and PFA-100TM closure time. 3. An important interrelationship observed between PFA-100TM closure time and plasma concentration of 8-epi Prostaglandin F2TM may support the hypothesis of nonenzymatic production of isoprostanoids with platelet proaggregatory activity, playing a role in aspirin resistance.

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INDUCTION OF APOPTOSIS IN NB4 CELL LINE TREATED WITH ARSENIC TRIOXIDE AND THE EFFECT OF VIT.D3 ASSESSED BY THE COMET ASSAY

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Backgrounds. Successful treatment of acute promyelocytic leukemia (APL) relies on the ability to kill or arrest the growth of the leukemic blasts. This can be accomplished by inducing maturation, as is the case in differentiation therapy and conventional chemotherapy by induction of Apoptosis. NB4 cells, a model of APL, have shown to undergo monocytic differentiation in response to 1α, 25 dihydroxy Vitamin D3 (1α, 25 D3) and apoptosis or partial differentiation in response to arsenic trioxide (As₂O₃). A change that usually happens during apoptosis is the severe fragmentation of cellular DNA, a characteristic that can be readily measured by single cell gel electrophoresis, known as the comet assay. **Aims.** Study of the effects of arsenic trioxide (ATO) and Vit.D3 on induction of apoptosis in NB4 cell line using the neutral comet assay. **Methods.** NB4 cells were treated with various doses of arsenic trioxide (0.1-3 µmol) and Vit.D3 (100-600 nM) alone or combined together. 24 hours later cells were mixed with low melting point agarose and placed onto a pre-coated slide. After lysis and electrophoresis in neutral condition, cells were stained with ethidium bromide and observed under a fluorescent microscope. The data were then analyzed and compared. **Results.** Results show that ATO induced apoptosis in NB4 cells at all doses used in this study. The effect was dose and time dependent and significantly different from controls ($p < 0.05$). In contrast, Vit.D3 at concentrations of 100-600 nM showed no effect on induction of Apoptosis. Treatment of the NB4 cells with arsenic trioxide in combination with Vit.D3, a monocytic inducer, resulted in reduction of apoptosis as compared to arsenic trioxide alone at the same concentration ($p < 0.05$) in all groups. **Conclusion:** Results show clearly that ATO is a potent inducer of apoptosis in NB4 cells and the effect is dose and time dependent. On the other hand, the results suggest that Vit.D3 decreases the sensitivity of cells to arsenic trioxide. A significant decrease in apoptosis in the various treatment groups, clearly gives evidence that Vit.D3 has a protective role (in this combination). Also neutral comet assay can be considered as a suitable method for detection of chemically induced apoptosis