

Amyloid-beta Interactions with ABC Transporters and Resistance Modifiers

JOSEPH MOLNAR¹, IMRE OCSOVSZKI² and ROZALIA PUSZTAI¹

¹Department of Medical Microbiology and Immunobiology,

²Department of Biochemistry, University of Szeged, Szeged, Hungary

Abstract. *Background/Aim:* Failure of cancer chemotherapy caused by multidrug resistance (MDR) of tumor cells is mediated by ABC transporters that reduce the uptake of cytotoxic agents. Similar transporters are responsible for amyloid clearance in nerve cells in Alzheimer's disease (AD). The aim of this study was to compare the biological effects of amyloid complexes of some known ABC transporter inhibitors e.g. disiloxanes. One of the most active fragments of the pathological "endogen" substrate responsible for AD was investigated in the presence of amyloid-beta fragment on the reversal of multidrug resistance and apoptosis induction on multidrug-resistant tumor cells in model experiments. *Materials and Methods:* The efflux pump activity of the cells treated with amyloid-beta complexes was studied by Rhodamin-123 accumulation. Apoptosis induction was measured by staining of treated cells by Annexin-V and propidium iodine. The fluorescent activity FL-1 and FL-2 of the cells was measured and analyzed on a PARTEC FACScan instrument. *Results:* The resistance modifiers: disiloxanes and memantine complexed with amyloid-beta 1-42 reduced the activity of ABC transporter in MDR tumor cells. Early apoptosis was moderately increased by amyloid-beta complexes. Late apoptosis and the number of total viable cells were not changed. *Conclusion:* Amyloid-beta and its complexes inactivate the efflux pump of tumor cells resulting in accumulation of amyloid. It is supposed that reduced membrane transport can explain the lower incidence of cancer in AD.

This article is freely accessible online.

Correspondence to: Joseph Molnar, Department of Medical Microbiology and Immunobiology, University of Szeged, Szeged, Hungary. Tel: +36 62545114, Fax: +36 62545113, e-mail: molnar.jozsef@med.u-szeged.hu

Key Words: ABC transporter, human MDR1 gene transfected mouse lymphoma cell, amyloid-beta peptide, amyloid-memantine and amyloid-disiloxane complexes, Alzheimer's disease.

The ABC transporters belong to a functionally heterogeneous group of transporters containing two different groups of ATP-dependent enzymes. The first group is responsible for transport of endogenous substrates e.g. in amyloid-beta transport responsible for the development of AD. The second one participates in the blood brain barrier (BBB) and transport of external substances e.g. by exporting toxic chemicals, anticancer drugs from MDR tumor cells. The efflux pump activity of the P-glycoprotein (pgp) in brain capillary endothelial cells and mouse lymphoma cells responsible for MDR were compared in sensitivity to different multidrug resistance modifiers (1, 2). In the last few years amyloid-beta "secretion" from the brain cells of patients with AD was systematically investigated in the presence of various compounds on cultured cells. In a prospective longitudinal study Behrens *et al.* demonstrated an inverse association between AD and cancer (3). Recently, Dao *et al.* demonstrated that phenothiazines act as inhibitors of β -amyloid aggregation and are applied as imaging probes for amyloid plaques in AD (4).

Our hypothesis was that inhibition of these membrane transporters can be exploited in drug design for treatment of different diseases. The efflux activity of the pgp in MDR tumor cells as a model of the brain capillary endothelium was measured to define interaction between MDR modifiers and amyloid-beta.

In this study the effects of amyloid-beta 1-42 alone and its complexes with resistance modifiers, disiloxanes and memantine, were investigated on the activity of MDR efflux pump and on apoptosis induction in mouse lymphoma cells.

Materials and Methods

Materials. Amyloid-beta 1-42 and memantine were purchased from Sigma (St. Louis, MO, USA) and verapamil from EGIS (Hungarian Pharmaceutical Company, Budapest, Hungary). 12Hbenzo(a)phenothiazine was provided by Motohashi (Tokyo, Japan). Disiloxanes as sila-409 and sila-421 were prepared as previously described (5, 6).

Cell culture. L5178 mouse T-cell lymphoma cells were transfected with a retrovirus carrying the MDR gene, that codes for pgp, as previously described (6).

Assay for reversal of MDR in mouse lymphoma cells in the presence of amyloid complexes. The L5178 MDR and L5178Y parent cell lines were grown in McCoy's 5A medium containing 10% heat-inactivated horse serum, L-glutamine and antibiotics. The fluorescence of the cell population was measured with a PARTEC FACScan flow cytometer.

Apoptosis assay. The assay was carried out according to the Protocol of Alexis Biochemicals (Darmstadt, Germany) with minor modifications. The cells were transferred to Eppendorf tubes, centrifuged and resuspended in 1.0 ml. Annexin V-FITC was added to the samples. The fluorescent activity (FL-1 and FL-2) of the cells was measured and analyzed on a PARTEC FACScan instrument. The anti-Alzheimer drug memantine was used as a control (7-9).

Results and Discussion

Membrane transporters in various barriers *e.g.* BBB, blood placenta barrier, blood retinal barrier, etc. have important roles in maintaining the normal function of cells in the organism. The effectiveness of cancer chemotherapy as in the case for AD therapy, lies in its ability to reach its target(s). This ability is challenged by membrane transporters called pgp or ABC transporters. These transporters extrude anticancer agents and when over-expressed by suboptimal therapy, render the cancer cell resistant not only to the specific agent, initially employed, but to other anticancer agents as well. Amyloid-beta peptides are implicated in the development of AD inasmuch as it is the main component of non-soluble amyloid plaques that accumulate intracerebrally (10). Amyloid-beta peptide is chemically "sticky" and gradually builds up into plaques and interferes with nerve transmission signals. As mentioned before, pgp, also known as ABCB1 protein, plays an important and substantial role in the elimination of amyloid from the brain via its activity at the BBB. Relevant to this fact, is the proven ability of this ABC transporter to extrude amyloid from MDR1 cancer cells (9).

The reversal of the MDR phenotype of cancer cells takes place by inhibition of the ABC transporter that is over-expressed in our model experiments. The effects of MDR modifiers such as disiloxanes sila-409 and sila-421 alone and memantine complexed with amyloid-beta peptides reduced rhodamine accumulation in the tumor cells. These findings may indicate some new directions for possible drug design to modify amyloid efflux (Table I). Amyloid-beta peptides 1-42 have toxic effects on cells. The complexes formed between amyloid-beta and resistance modifier disiloxanes were not able to increase Rhodamine123 accumulation in the treated cells despite the fact that the disiloxanes and the positive verapamil control alone significantly inhibited Rhodamine123 efflux from the treated tumor cells resulting in increased intracellular accumulation of rhodamine.

These results demonstrate that amyloid-beta peptides and memantine alone have opposite effects on the ABC transporter of human MDR1 transfected into mouse cells. In

the case of the amyloid-beta peptides 1-42 complexed with the two disiloxanes, the sila-409-amyloid complex reduced rhodamine accumulation, possibly due to direct complex formation with amyloid. In the case of sila-421-amyloid complex, the amyloid was less effective.

The effect of the resistance modifiers alone and their amyloid complexes on apoptosis induction was examined using MDR mouse lymphoma cells (Table II). Small differences were found in the frequency of early apoptosis induction which indicates some non-specific membrane effects. The rate of viable cells was found above 90-95% percent in the presence of the tested compounds and their complexes with amyloid-beta 1-42. The MDR modifiers verapamil, sila-421, sila-409, and amyloid alone resulted in the reversal of resistance to doxorubicin. Whereas amyloid-beta 1-42 prevented the MDR reversal effect of the resistance modifiers including memantine, sila-409 and sila-421. The compounds studied showed an early apoptosis induction. The memantine and sila-421-complexes of amyloid increased apoptosis. Late apoptosis and viability of cells were not changed significantly by memantine and disiloxane-complexes.

The authors suggest that resistance modifiers form complexes with amyloid. The ABC transporters may have theoretical interest since they are responsible for the functions of barriers in different tissues *e.g.* BBB, gut, kidney, placenta, testis, liver, adrenal cortex and breast by regulating efflux of endogenous and exogenous substrates including amyloids, hormones and chemotherapeutics. One strategy for reversal of the resistance in tumor cells expressing ABC transporters is the combination of antitumor chemotherapeutics with resistance modifiers. The clearance of amyloid-beta from the brain represents a therapeutic target for a human brain endothelial cell line hCEMC/D (3). Tai *et al.* suggested that pgp and BCRP might act to prevent blood borne amyloid-beta from entering the brain (8). Brenn and coworkers had shown that amyloid beta 1-42 itself down regulates the expression of pgp and amyloid beta transporters could enhance the intracerebral accumulation of amyloid-beta. As a consequence, accelerate neurodegeneration in AD and cerebral beta-amyloid angiopathy *in vivo* (10).

The two main problems related to chemotherapy of cancer and AD are theoretically similar. Chemotherapy inefficiency due to drug resistance can be overcome by inhibition of ABC transporter. In case of cancer, the reversal of drug resistance of tumor cells expressing ABC transporters by the combination of anticancer drugs and chemosensitizers holds a great promise for improved chemotherapy. However, in AD the reduced clearance across BBB and accumulation of beta-amyloid –as a pgp substrate– are responsible for the neurologic dysfunction, that has a key role in the development of beta-amyloid mediated angiopathy. In this case beta-amyloid is considered as endogenous substrate, however in

Table I. MDR reversal in the presence of amyloid complexes.

	Concentration µg/ml	FSC Forward scatter count	SSC Side scatter count	Mean fluorescence	FAR fluorescence activity ratio	Peak channel	
1	PAR cells treated	-	2339	790	111	-	96.5
2	PAR cells Non-treated	-	2297	693	105	-	93.1
3	MDR MDR MEAN	-	2435 2318	965 942	1.48 1.13	-	1.29 -
4	Verapamil	10	2440	956	3.91	3.46	2.46
5	Memantine hydrochloride	4	2457	951	1.49	1.31	1.15
6	40	2405	950	1.84	1.62	1.43	
7	Sila-409	4	2383	999	137	121.23	133
8	40	1401	1264	1.82	1.61	1.72	
9	Sila-421	4	2324	964	84.2	74.51	80.6
10	40	1395	1320	23.8	21.06	75.0	
11	Amyloid β-protein	2	2388	1002	1.16	1.02	1.00
12	20	2251	967	0.846	0.74	0.806	
13	Memantine hydrochloride + Amyloid β-protein	40+2	2358	949	1.31	1.15	0.965
14	40+20	2159	948	1.15	1.01	0.866	
15	Sila-409 + Amyloid β-protein	40+2	1488	1280	1.33	1.17	1.29
16	40+20	1392	1164	1.20	1.06	1.04	
17	Sila-421 + Amyloid β-protein	40+2	1392	1289	1.99	1.76	1.15
18	40+20	1449	1291	1.87	1.65	0.931	
19	DMSO	20 µL	2065	944	0.810	0.71	0.698
20	MDR	-	2201	919	0.787	-	0.649

Table II. Apoptosis induction in the presence of amyloid complexes.

MDR	ml	Q1 necrosis	Q2 late apoptosis	Q3 early apoptosis	Q4 viable non-apoptotic cells		
1	Annexin ⁻ Propidium iodide-	0.56	0.005	0.030	99.4		
2	Annexin ⁻ Propidium iodide ⁺	1	0.005	0.025	99.0		
3	Annexin ⁺ Propidium iodide-	0.005	0.00	2.92	97.1		
4	Annexin ⁺ Propidium iodide ⁺	0.716	0.686	1.78	96.8		
5	DMSO control	1%	0.428	0.732	2.46	96.4	
6	M627 (as positive control in apoptosis)	50 µg/ml	11.6	6.76	7.83	73.8	
7	Memantine	4 µg/ml	0.648	0.663	1.92	96.8	
8	Sila-409	4 µg/ml	0.667	0.717	2.82	95.8	
9	Sila-421	4 µg/ml	1.34	0.762	3.91	94.0	
10	Amyloid β	4 µg/ml	0.588	0.658	2.60	96.2	
11	Memantine	Amyloid β 4 µg/ml	4 µg/ml	1.21	0.935	2.60	95.3
12	Sila-409	Amyloid β 4 µg/ml	4 µg/ml	0.952	0.768	2.60	95.7
13	Sila-421	Amyloid β 4 µg/ml	4 µg/ml	2.75	0.987	5.58	90.7

cancer the anthracyclines are exogenous substrates. Consequently, simultaneous administration of resistance modifiers to block the membrane transporter can improve the effectiveness chemotherapy both in cancer and in AD. The reversal of MDR and induction of apoptosis might offer a potential benefit in cancer. We suppose that a synergistic

combination between some resistance modifiers *e.g.* memantine or sila compounds with amyloid-beta may offer further opportunities for chemotherapy of cancer and AD.

Behrens (3) had shown an inverse association between AD and cancer. The question arises: what is the possible common factor in AD and cancer. Nevertheless, a

considerable attention has been given to the mechanisms by which amyloid-beta might be transported between the brain and blood and evidence suggests that MDR pgp plays a role in amyloid-beta transport (11). The conclusion of this publication emphasizes that treatments currently under investigation to prevent or to treat AD might lead to a greater risk of cancer development, and inversely, treatments to halt cancer, could predispose to development of AD (6, 7). In cancer, cell regulation mechanisms are disrupted with augmentation of cell survival and proliferation, whereas conversely AD is associated with increased neuronal death, either caused by concomitant beta-amyloid complexes and tau deposition. Association between cancer and AD is made more imperative considering that treatments currently under investigation to prevent, and treat AD, might lead to a greater risk of cancer development (7, 8).

Significantly lower expression of ABC transporters was found following expression of amyloid-beta 1-42, but not its scrambled or complexed equivalent (12). In our experiments the two studied disiloxanes had shown some chemopreventive effects *in vivo* (13). Similar effect was found also *in vivo* in human pancreatic cancer xenograft-inoculated mice (14). In addition to the beneficial effects of the sila-409 and sila-421 on chemoprevention, the identified vascular activity of the two compounds can be promising for further *in vivo* studies (model experiments) in rational drug design (15).

Acknowledgements

The Authors would like to express sincere gratitude for the assistance to Aniko Vigyikán. This study was supported by Szeged Foundation for Cancer Research and GINOP 2.3.2-15-2016-00038. University of Szeged, Hungary.

References

- Molnar J, Szabo D, Mándi Y, Mucsi I, Fisher J, Varga A, Köning S and Motohashi N: Multidrug resistance reversal in mouse lymphoma cells by heterocyclic compounds. *Anticancer Res 18*: 3033-3038, 1998.
- Fakla A, Hevér A, Molnár J and Fisher J: Tomato lectin labels the 180 kD glycoform of p-glycoprotein in brain capillary endothelia and mdr tumor cells. *Anticancer Res 18*: 3107-3112, 1998.
- Behrens MI, Lendon C and Roe CM: A common biological mechanism in cancer and Alzheimer's Disease. *Curr Alzheimer Res 6(3)*: 196-204, 2009.
- Dao P, Ye F, Liu Y, Du ZY, Zhang K, Dong CZ, Meunier B and Chen H: Development of phenothiazine-based theranostic compounds that act both as inhibitors of β -amyloid aggregation and as imaging probes for amyloid plaques in Alzheimer's disease. *ACS Chem Neurosci 8(4)*: 798-806, 2017.
- Hegyés P, Molnar J, Mucsi I, Hevér A, Szabo D, Kiessig ST, Lage H, Gaál D and Nacsá J: Substituted disiloxanes method for production thereof and the use thereof for reversal of multidrug resistance (MDR) (PCT/DE00/04110,2000., and Molnar J: European Patent No, Patent haber 1432717, 01.03.06, München.
- Molnar J, Mucsi I, Nacsá J, Hevér A, Gyémánt N, Ugoicsai K, Hegyés P, Kiessig ST, Gaál D, Lage H and Varga A: New silicon compounds as resistance modifiers against multidrug resistant cancer cells. *Anticancer Res 24(4C)*: 665-872, 2004.
- Kimura M, Komatsu H, Ogura H and Sawada K: Comparison of donepezil and memantine for protective effect against amyloid-beta (1-42) toxicity in rat septal neurons. *Neurosci Lett 391(1-2)*: 17-21, 2005.
- Tai L, Loughlin AJ, Male DK and Romero IA: P-glycoprotein and breast cancer resistance protein restrict apical-to-basolateral permeability of human brain endothelium to amyloid beta. *J Cerebral Blood Flow Metab 29(6)*: 1079-1083, 2009.
- Kuhnke D, Jedlitschky G, Grube M, Krohn M, Jucker M, Mosyagin I, Cascorbi, Walker LC, Kroemer HK, Warzok RW and Vogelgesang SL: MDR1-P-Glycoprotein (ABC1) mediates transport of Alzheimer's amyloid beta peptides – implications for the mechanisms of A-beta amyloid clearance at the blood brain barrier. *Brain Pathol 17(4)*: 347-353, 2007.
- Brenn A, Grube M, Peters M, Fischer A, Jedlitschky G, Kroemer HK, Warzok RW and Vogelgesang S: Beta-amyloid down regulates MDR1-P-glycoprotein (Abcb1) expression at the blood brain barrier in mice. *Int J Alzheimer's Dis 2011*: 690121, 2011.
- Wei W, Bodles-Brakhop AM and Barger SW: A role for p-glycoprotein in clearance of Alzheimer amyloid beta peptide from the brain. *Curr Alzheimer Res 13(6)*: 615-630, 2016.
- Kania KD, Wijesuriya HC, Hladky TB and Barrand MB: Beta amyloid effects on expression of multidrug efflux transporters in brain endothelial cells. *Brain Res 1418*: 1-11, 2011.
- Tokuda H, Maoka T, Suzuiki N, Hohmann J, Vasas A, Engi H, Mucsi I, Olszewski U, Hamilton G, Amaral L and Molnar J: Effects of two disiloxanes ALIS-409 and ALIS-421 on chemoprevention in model experiments. *Anticancer Res 33(5)*: 2021-2028, 2013.
- Zalatnai A and Molnár J: Effect of SILA-409, a new organosilicon multidrug resistance modifier on human pancreatic cancer xenografts. *In Vivo 20(1)*: 137-140, 2006.
- Fusi F, Ferrara A, Zalatnai A, Molnar J, Sgaragli G and Saponara S: Vascular activity of two silicon compounds, alis 409 and alis 421, novel multidrug-resistance reverting agents in cancer cells. *Cancer Chemother Pharmacol 61(3)*: 443-451, 2008.

Received March 29, 2018

Revised April 26, 2018

Accepted May 2, 2018