Arch. Immunol. Ther. Exp. (2016) 64:515–522
DOI 10.1007/s00005-016-0408-8

SHORT COMMUNICATION

The Hidden Side of Disodium Cromolyn: from Mast Cell Stabilizer to an Angiogenic Factor and Antitumor Agent

Anca Maria Cimpean[1] · **Marius Raica**[1]

Received: 29 November 2015 / Accepted: 9 March 2016 / Published online: 11 June 2016

Abstract Scattered data suggested that disodium cromolyn, well known as a mast cell stabilizer shows some effects on tumor cells and tumor-associated newly formed vascular networks. Most of these studies used tumor cell lines assessed by in vitro studies. Nor disodium cromolyn effects on melanoma cell lines were studied yet, neither its influence on recruited tumor blood vessels or angiogenic growth factors expression. We designed here a study regarding disodium cromolyn effects on A375 melanoma tumor cells implanted on chick embryo chorioallantoic membrane (CAM) and on blood vessels recruited by the experimental melanoma in the absence of mast cells, knowing that within CAM, the existence of mast cells are not certified yet. We also assessed the role of disodium cromolyn on the expression of several angiogenic growth factors. Disodium cromoglycate differentially acts on tumor cells and blood vessels. Extensive necrotic areas of experimental melanoma together with an increased number of peritumor blood vessels were observed in treated specimens as compared with untreated tumors. Disodium cromolyn inhibited VEGF and PDGF-BB expression, and had no effects on EG VEGF expression between treated and non treated specimens in a mast cells free microenvironment. Our results sustain the direct antitumor effects of sodium cromolyn and suggest the involvement of several growth factors in the recruitment of tumor vessels by A375 melanoma tumor cells. The expression of growth factors is differentially influenced by sodium cromolyn treatment.

Keywords Sodium cromolyn · Blood vessels · Tumor cells · Chorioallantoic membrane

✉ Anca Maria Cimpean
ancacimpean1972@yahoo.com

Marius Raica
raica@umft.ro

[1] Department of Microscopic Morphology/Histology, Angiogenesis Research Center, "Victor Babes" University of Medicine and Pharmacy, Pta Eftimie Murgu 2, Timisoara 300041, Romania

Introduction

Mast cells degranulation initiates a cascade of events through their mediators which act in a complex manner on blood vessels mainly. Mast cell mediators dramatic clinical effects are counteracted by the use of mast cells stabilizers which prevent the release of mediators that would normally attract inflammatory cells. Several mast cells stabilizers are known and used as treatment for allergic diseases (Platt 2014; Ridolo et al. 2014) and asthma (Netzer et al. 2012; Yang et al. 2010). Disodium cromolyn is one of the most extensively used mast cells degranulation inhibitors in allergy and asthma acting by stabilizing the granule membranes and preventing the release of mediators (Finn and Walsh 2013). Other cromolyn effects, not related to the inhibitory potential of mast cells are rarely reported in the literature. Sodium cromolyn has been tested in tumors as neoadjuvant therapy to inhibit lung cancer-associated cough (Moroni et al. 1996) but the mechanism of action has not been elucidated. Direct antitumor effects of cromolyn was reported for the first time by (Arumugam et al. 2006), who observed cromolyn ability to bind S100P, inhibits tumor growth, and increases the effectiveness of gemcitabine in three orthotopic models of pancreatic cancer. Recently, the same team designed a cromolyn analog, 5-methyl cromolyn (C_5OH), which reduced NF-κB activity to a greater

extent than cromolyn and using a ten times smaller dose. Treatment of mice-bearing syngeneic pancreatic ductal adenocarcinoma tumors showed that C_5OH treatment reduced both tumor growth and metastasis (Arumugam et al. 2013). Another independent team evaluated naproxen and cromolyn activities against cancer cells viability, proliferation, apoptosis, p53 and gene expression of survivin and caspase-3. Motawi et al. (2014) performed a detailed biochemical evaluation of the two drugs against colorectal adenocarcinoma (Caco2), hepatocellular carcinoma (HepG2), mammary gland carcinoma (MCF7), epitheloid cervix carcinoma (Hela), lung carcinoma (A5W9) and epidermoid larynx carcinoma (Hep2) cell lines, followed by viability tests using trypan blue, proliferation MTT assay, apoptosis, p53 and real-time polymerase chain reaction for gene expression of survivin and caspase-3. Except the two previous mentioned references no other in vitro or in vivo data are available regarding the effects of cromolyn on other malignant cells types. The possible influence of disodium cromolyn on blood vessels was usually correlated with the mast cells stabilization and, to a lesser extent, with the direct action of this therapeutic agent on endothelial cells or other components of tumor and non tumor blood vessels. Most of the data regarding the interaction between cromolyn and endothelial cells are indirect evidences derived from leucocytes, eosinophils and monocytes interaction with endothelial cells modulated by cromolyn (Ebihara et al. 2006; Horváthová et al. 1998; Yazid et al. 2010).

The role of mast cells stabilizers in the angiogenesis process is still a controversial topic. Most of the papers published in the field studied the influence of mast cells stabilizers on the variability of tumor mast cells number and degranulation, directly. Subsequently, mast cells variability under influence of mast cells stabilizers was correlated with tumor microvessel density and, to a lesser extent with angiogenic growth factors expression.

Mast cells stabilization followed by the decrease of blood vessels seems to be one of the most promising therapeutic option especially in tumor angiogenesis (Ribatti and Ranieri 2015) but also in other non tumor conditions as pulmonary arterial hypertension (Bartelds et al. 2012). Tryptase and chymase are the most common mast cell granules components studied in angiogenesis. Gabexate mesylate, nafamostat mesylate, and tranilast are mast cells inhibitors recently studied as new promising antiangiogenic agents (Ammendola et al. 2014). Disodium cromolyn, another mast cell stabilizer, has a widely accepted antitumor effects in several tumor types as cholangiocarcinoma (Johnson et al. 2016), pancreatic cancer (Kim et al. 2012) or breast malignancies (Samoszuk and Corwin 2003). All effects of mast cell stabilizers described above are related with mast cells inhibition. To a lesser extent, indirect evidences of direct effects of mast cell stabilizers on tumor cells and/or blood vessels were previously reported in older studies (Isaji et al. 1997; Yoon et al. 2004).

The Present paper proposed a different approach of mast cell stabilizer effects on tumor cells and blood vessels, in the absence of mast cells, using free mast cells chick embryo chorioallantoic membrane experimental model. The rationale of the study was to assess the direct effects of disodium cromolyn on both melanoma tumor cells and blood vessels without mast cells involvement and its influence on angiogenic growth factor expression. Moreover, disodium cromolyn effects on A375 melanoma cells has not previously reported.

We designed here a study regarding disodium cromolyn effects on A375 melanoma tumor cells implanted on chick embryo chorioallantoic membrane (CAM, where the presence of mast cells failed to be proved in normal conditions) and on blood vessels recruited by the experimental melanoma. We also assessed the role of sodium cromolyn on the immunohistochemical expression of vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF) and endocrine derived vascular endothelial growth factor (EG VEGF).

Materials and Methods

Cell Culture Technique

A375 human melanoma cell line is derived from a 54-year-old female with malignant melanoma (Giard et al. 1973). The cells were purchased from ECACC (European Collection of Cell Cultures, Wiltshire, UK). Human melanoma cell line A375 was cultured in Dulbecco's modified Eagle Medium (DMEM) with high glucose (4.5 g/l), 15 mM Hepes, and 2 mM L-glutamine, supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin, and 10 % fetal calf serum (FCS). Cells were kept in a humidified atmosphere with 5 % CO_2 at 37 °C and passages were done every 2 days. In the day of the experiment, A375 were prepared for inoculation according to the following steps: the old medium was removed; the cells were trypsin-treated for 5 min at 37 °C, suspended in medium and centrifuged. The pellet of cells was suspended in phosphate buffer saline and the cells were counted.

Chick Embryo Chorioallantoic Membrane Preparation and Design of the Experiment

Three groups of ten fertilized eggs each were organized for the experimental study. The chick embryo chorioallantoic membrane was prepared according to the method

previously described by Ribatti (2008). On day seven of incubation, a silicon ring was applied on the surface of chick CAM. First group was treated with 2 µl of disodium cromolyn applied inside the silicon ring daily, starting from the 7th day of incubation. For the second group, A375 human amelanotic melanoma cell line in medium (10^4 cells/2 µl) in volumes of 2 µl suspension were inoculated inside silicon rings on day 7 of incubation for the second experimental group. The eggs from the third group were treated with both A375 melanoma cells and 2 µl disodium cromolyn. The specimens were daily treated with disodium cromolyn for 7 days and evaluated by stereomicroscopy. On day 14 of incubation the experiment was stopped. We performed *in ovo* CAM fixation by using 10 % buffered formalin for 1 h. Then, the chick CAM specimens were collected and fixed in formalin for next 24 h.

Morphologic Primary Processing of the Tissues

After formalin fixation, chick embryo CAM biopsies were paraffin embedded and 3 µm thick serial sections were obtained. Hematoxylin and eosin stain was performed and specimen evaluation was followed by the selection of sections for immunohistochemistry.

Immunohistochemistry

Immunohistochemistry was performed in an automated manner by using BOND MAX workflow. We evaluated the presence of VEGF (mouse anti-human, monoclonal antibody, clone VG1, Dako, Carpinteria, USA), PDGF B (rabbit anti-human, ThermoScientific, Fremont, CA, USA), EG VEGF (goat anti-human, polyclonal antibody, Reliatech, Germany) in cromolyn treated and non treated specimens. Briefly, incubation with primary antibody was followed by the use of a free biotin working system (Novolink, Leica, Microsystems, UK). Nuclear stain and permanent mounting completed the immunohistochemical procedure.

Microscopic Evaluation and Data Acquisition

Stereomicroscopic assessment of chick embryo chorioallantoic membrane was performed with Zeiss AxioZoom V16 Stereomicroscope. Microscopic evaluation followed by images acquisition was done using Axio Imager A2Zeiss microscope. Presence, density and distribution of blood vessels and growth factors expression for specimens with and without disodium cromolyn treatment were assessed.

Results

CAM specimens treated with disodium cromolyn in the absence of A375 melanoma cells had a higher number of blood vessels compared with normal CAM. The blood vessels around the silicon ring treated with disodium cromolyn had a branched appearance, with tendance to develop vessels networks. Most of these vessels were sinuous, had lumen and were already perfused. Macroscopic view of these vascular networks was confirmed by microscopic assessment of blood vessels, showing small branched vessels convergent to the silicon ring filled with disodium cromolyn.

Inoculation of A375 melanoma cells on CAM surface was followed (in absence of disodium cromolyn) by the development of compact tumor mass, composed of closely packed viable tumor cells surrounded by a high number of blood vessels, also invaded by small perfused capillaries (Fig. 1a, c, e). Isolated tumor cells islands were observed far from the silicon ring where A375 tumor cells were initially implanted. These were also highly vascularized (Fig. 1g) tumor cells being strongly positive for VEGF and EG VEGF, while PDGF B was observed at vascular level and, to a lesser extent, in tumor cells (Fig. 2a, c, e).

A375 tumor implants treated with disodium cromolyn were completely different as compared with the previous untreated ones, regarding tumor cells morphology and development, vascular network distribution and growth factors expression. A375 melanoma cells derived tumors treated with disodium cromolyn failed to fill the silicon ring where they were implanted, only small tumor islands being found at the periphery of the silicon ring (Fig. 1b, d). These small tumor areas were characterized by extensive necrosis, most of the tumor cells were disintegrated, few viable cells and they were recognized by their specific morphology. Peritumor vascular network was still present after 7 days treatment with disodium cromolyn, their morphology and density being similar with those found in the normal CAM treated with the same agent. Differential expression of growth factors was assessed for this group of specimens. No positive reaction for VEGF was observed, meaning that VEGF expression was inhibited by disodium cromolyn in tumor cells and CAM chorion (Fig. 2b). Also, immunohistochemistry for PDGF B was slightly positive (Fig. 2d). A particular observation has been made regarding EG VEGF. Its expression was similar for treated and untreated tumor specimens (Fig. 2f). This means that disodium cromolyn has no effect on the expression of growth factors as EG VEGF and partially stimulated PDGF B expression in experimental melanoma cells and CAM blood vessels.

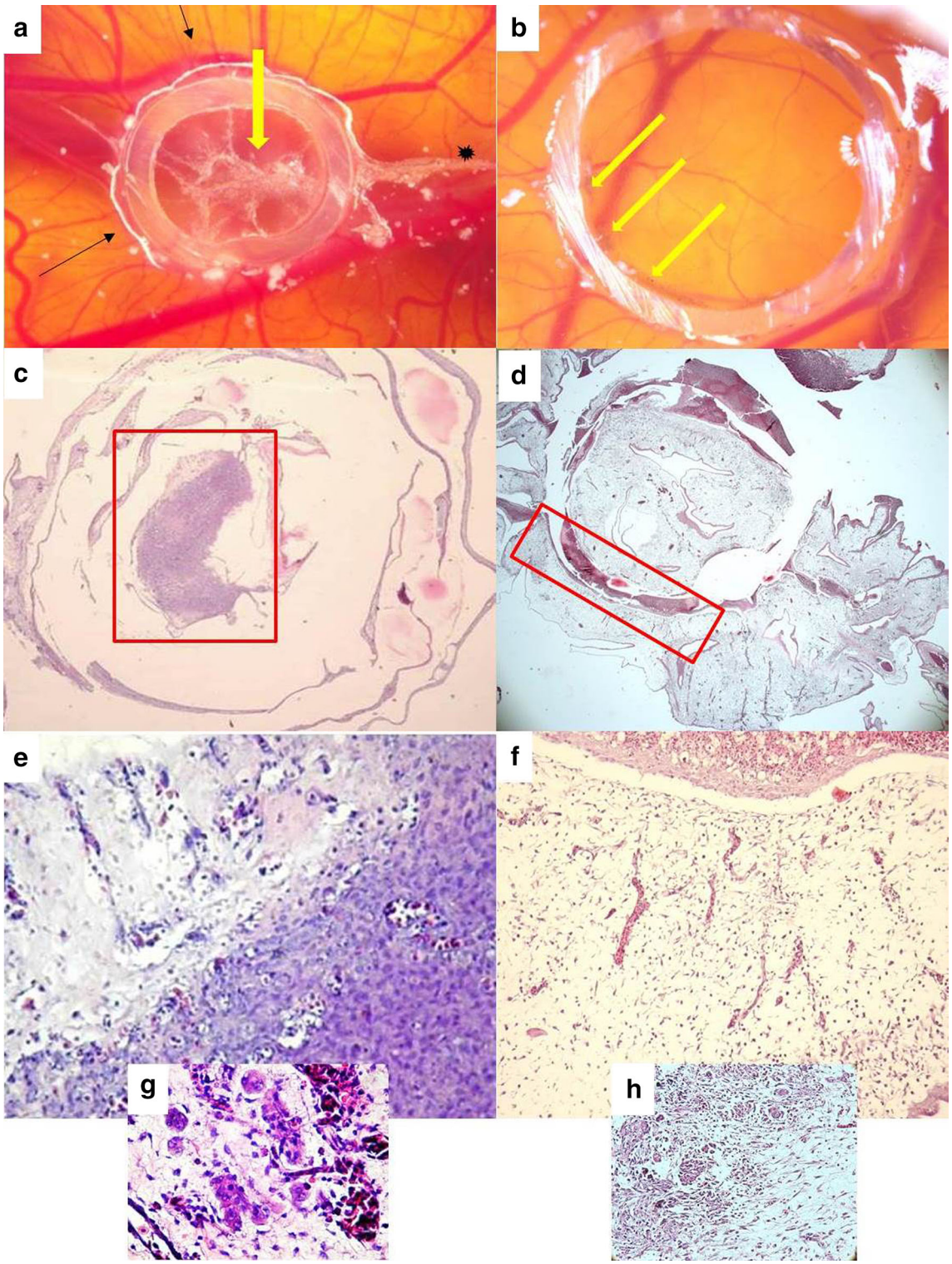

Fig. 1 Macroscopic (**a**, **b**) and microscopic (**c**–**h**) comparative assessment of A 375 melanoma cells behavior in disodium cromolyn treated and non treated specimens. Treated A375 melanoma cells were not able to form a well defined tumor (**b**, **d**) compared with non treated implanted cells which formed a melanoma tumor inside the silicon ring (**a**, **c**). Note that the number of blood vessels around the implants did not differ between treated (**f**) and non treated specimens (**e**). Also, distant metastases were not influenced by the disodium cromolyn (**g**, **h**) but induced fibroblast proliferation inside chick CAM chorion around metastatic islands (**h**)

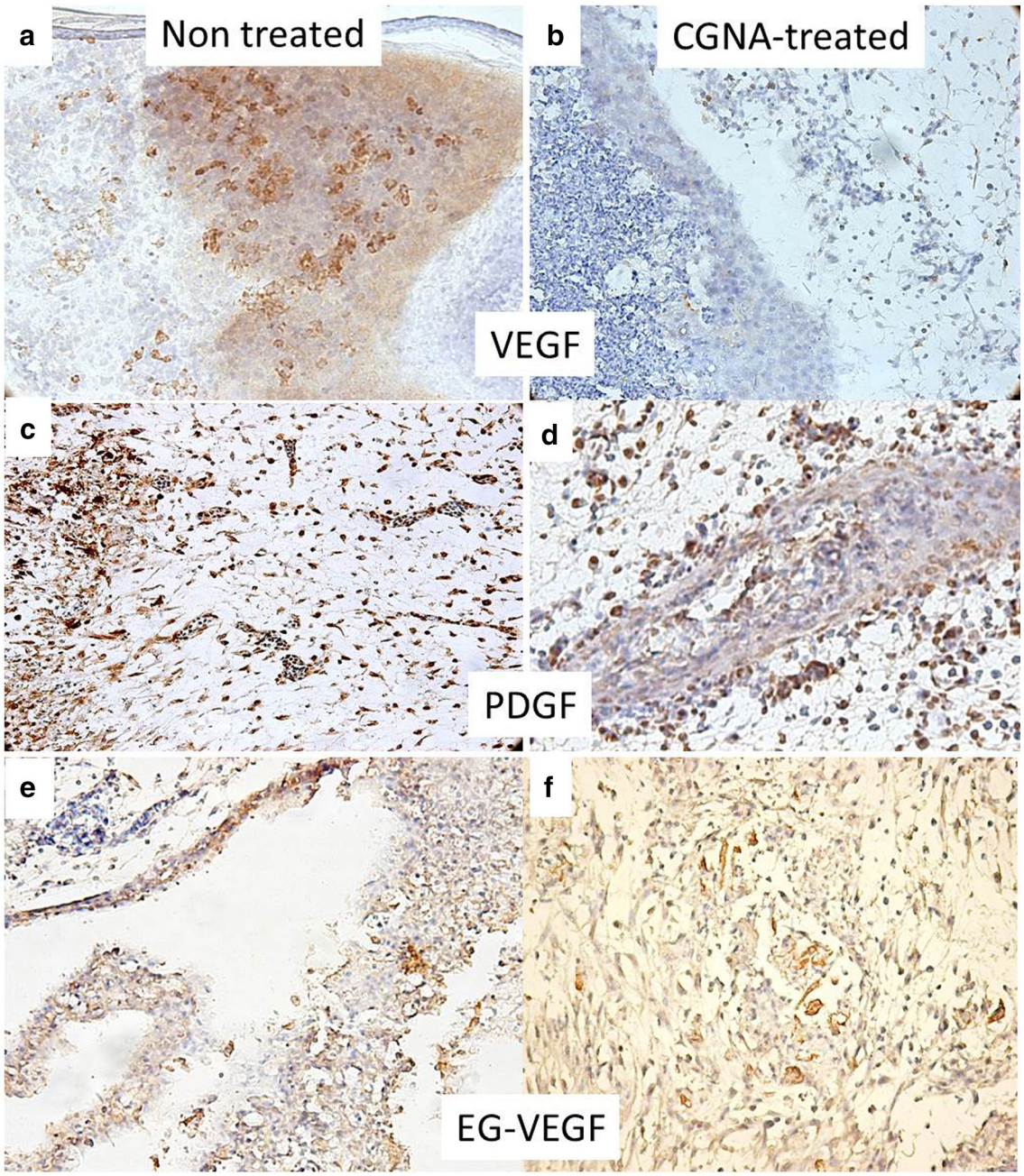

Fig. 2 Influence of disodium cromolyn treatment on the expression of growth factors secreted by melanoma cells, endothelial and perivascular cells. VEGF expression was highly inhibited in treated specimens (**b**) compared with non treated ones (**a**). PDGF B expression partially decreased in tumor cells after disodium cromolyn treatment but is still expressed in the chick CAM cellular components and vessels (**c**, **d**). Endocrine gland derived VEGF was not influenced by the treatment, remaining positive in both specimens (**e**, **f**)

Discussion

Disodium cromoglycate is a well known mast cells stabilizer used as main therapy in several disease involving mast cells in their pathogenesis (Castillo et al. 2015; Zhang et al. 2016). Its certified role as a drug which prevents the release of mediators that would normally attract inflammatory cells, recommends it as therapy for asthma (Küpper et al. 2012), allergic conjunctivitis (Pacharn and Vichyanond 2013), mastocytosis (Horan et al. 1990), dermographic uritcaria (Zuberbier et al. 2001) or ulcerative colitis (Raithel et al. 2007). Several recent papers described other less known functions of disodium cromolyn, not related to its main function. One

of the first evidence of cromolyn effects on tumor cells was reported by Samoszuk and Corwin (2003), when they observed that sodium cromolyn increases blood clotting and hypoxia in experimental model of subcutaneous implants of the 4T1 mammary adenocarcinoma cell line in mice. Their study was designed based on the high levels of chymase and tryptase observed in women with breast cancer and thus, they stated that mast cells inhibition may play an important role in regulating blood clotting and hypoxia in breast cancer. Compared to the murine model of tumor implants, for the chick embryo CAM the presence of mast cells failed to be proved until now. Thus, we considered that this model is an useful tool to study disodium cromolyn effects on tumor cells and tumor microenvironment in the absence of mast cells. Our observations about a higher density of blood vessels around silicon ring treated with disodium cromolyn in the absence of tumor cells and mast cells sustain the hypoxic effects of disodium cromolyn on normal tissues. Most probably, disodium cromolyn induced hypoxia is responsible for the recruitment of new blood vessels around the silicon ring treated with disodium cromolyn and, based on this, we may explain the higher density of blood vessels in disodium cromolyn treated specimens of CAM compared with non treated ones. To the best of our knowledge this is the first report regarding the effects of disodium cromolyn on the normal vasculature in the absence of mast cells. It seems that, hypoxic effects of disodium cromolyn are not restricted to normal tissues. Recently, few papers reported that the use of disodium cromolyn led to tumor hypoxia and tumor cells apoptosis in experimental models of pancreatic (Kim et al. 2012; Theoharides 2008) and thyroid cancer (Melillo et al. 2010) but for all of them, disodium cromolyn effects were related to tumor mast cells inhibition. Tumor cells necrosis was related to a decreased number of mast cells following therapy with disodium cromolyn. Our findings showed a direct effect of disodium cromolyn which induced tumor cells necrosis in the absence of mast cells. Several and controversial aspects regarding the relationship between disodium cromolyn and malignant melanoma have been reported. There is one report suggesting the development of malignant melanoma as a side effect of disodium cromolyn therapy, the percent of patients who developed such side effects being around 0.2387 % (http://factmed.com/study-CROMOLYN%20SODIUM-causing-MALIGNANT%20MELANOMA.php). These data are in conflict with our observations on implanted malignant melanoma treated with disodium cromolyn. Treated melanoma showed a high necrotic rate compared with untreated specimens, so we are not able to agree with previous hypothesis about disodium cromolyn induction of melanoma. A potential mechanism of malignant melanoma inhibition induced by disodium cromolyn could have as main target S100 protein family, already certified as a key player in the pathogenesis of malignant melanoma and, also as a target for disodium cromolyn (Okada et al. 2002; Shishibori et al. 1999). Moreover, disodium cromolyn has a weak effect as mast cell stabilizer by its limited inhibition of histamine and prostaglandin release to some extent, but not inflammatory cytokine release, through an unknown mechanism (Weng et al. 2012). The influence of disodium cromolyn on growth factors expression is still less discussed. Few indirect evidences from non-malignant conditions treated with cromolyn are now available. Sapmaz et al. (2015) reported that cromolyn treatment induced a significant reduction in VEGF-α in the lung parenchyma of the male Fischer 344 rats with aspiration-induced fibrosis (Chang et al. 2014). Bosquiazzo et al. (2007) used disodium cromoglycate to inhibit mast cells degranulation and, related to this, to study angiogenesis variability in rat uterine cervix during pregnancy. They reported an inhibition of VEGF mRNA by disodium cromolyn and also a decreased endothelial cell proliferation. It seems that stimulation of blood vessels development in our study despite disodium cromolyn addition, is sustained by the persistence of EG VEGF and incomplete inhibition of PDGF B.

The effects of disodium cromolyn on other growth factor expression were not proved, yet. Our results suggested that disodium cromolyn decreased PDGF B expression in tumor cells but does not influence EG VEGF expression.

Other mast cell stabilizers as gabexate mesilate and nafamostat mesilate, two inhibitors of trypsin-like serine proteases (Ribatti and Ranieri 2015) inhibit mast cell tryptase, a certified angiogenic factor in tumors. All mast cell stabilizers are studied and discussed regarding their effects on tumor cells and, indirectly as pro-angiogenic or anti-angiogenic factors. Similar to VEGF inhibition by disodium cromolyn found in the present study, Fujiwara et al. (2011) reported that in vitro, nafamostat mesilate inhibited activities of NF-κB, phosphorylated IκBα, ICAM-1, VEGF and MMP-9 in human pancreatic cancer cell lines (AsPC-1, BxPC-3 and PANC-1). As it is easily observed, all these factors are equally involved in different steps of tumor angiogenesis and the authors indirectly suggested an inhibition of tumor angiogenesis by nafamostat mesilate, covering a limited spectrum of angiogenic factors. Also, PDGF-BB inhibition by nafamostat mesilate was reported 15 years ago on a non tumor model of cerebral vasospasm model in mice, after subarachnoid hemorrhage (Zhang et al. 2001). The authors found that nafamostan mesilate suppressed the expression of PDGF-BB in the endothelial and medial smooth muscle cell layers. A generic overview of the presented data suggested

that mast cell stabilizers seems having an antiangiogenic effects on tumor blood vessels. But, one important issue is neglected: hypoxia induced by these agents on tumor and non tumor tissues. High rate of hypoxia can stimulate angiogenesis or can suppress a potential antiangiogenic effect of mast cell stabilizers sustained by angiogenic growth factor inhibition. This complex and dual role of mast cell stabilizers is sustained by a study published in 2010 (Doyle and Haas 2010), which reported that mast cell stabilization with cromolyn attenuated degranulation but did not inhibit the increased mast cell density, MMP-2 activity, VEGF protein levels or the increase in capillary number following muscle overload and mast cell activation is not critical to the angiogenic response following skeletal muscle overload.

In conclusion, disodium cromolyn induces melanoma cells necrosis by a direct mechanism most probably mediated through S100 pathway, in the absence of mast cells. Also, it induces blood vessels development following hypoxia in normal and peritumoral tissues.

Acknowledgments This work was supported in part by internal funds from Victor Babes University of Medicine and Pharmacy Timisoara, Romania. Special thanks to Stefana Avram and to Professor Cristina Adriana Dehelean both from Faculty of Pharmacy of Victor Babes University of Medicine and Pharmacy Timisoara, Romania for valuable technical support in experimental model and for providing us A375 melanoma cells.

References

Ammendola M, Leporini C, Marech I et al (2014) Targeting mast cells tryptase in tumor microenvironment: a potential antiangiogenetic strategy. Biomed Res Int 2014:154702

Arumugam T, Ramachandran V, Logsdon CD (2006) Effect of cromolyn on S100P interactions with RAGE and pancreatic cancer growth and invasion in mouse models. J Natl Cancer Inst 98:1806–1818

Arumugam T, Ramachandran V, Sun D et al (2013) Designing and developing S100P inhibitor 5methyl cromolyn for pancreatic cancer therapy. Mol Cancer Ther 12:654–662

Bartelds B, van Loon RL, Mohaupt S et al (2012) Mast cell inhibition improves pulmonary vascular remodeling in pulmonary hypertension. Chest 141:651–660

Bosquiazzo VL, Ramos JG, Varayoud J et al (2007) Mast cell degranulation in rat uterine cervix during pregnancy correlates with expression of vascular endothelial growth factor mRNA and angiogenesis. Reproduction 133:1045–1055

Castillo M, Scott NW, Mustafa MZ et al (2015) Topical antihistamines and mast cell stabilisers for treating seasonal and perennial allergic conjunctivitis. Cochrane Database Syst Rev 6:CD00 9566

Chang JC, Leung J, Tang T et al (2014) Cromolyn ameliorates acute and chronic injury in a rat lung transplant model. J Heart Lung Transplant 33:749–757

Doyle JL, Haas TL (2010) The angiogenic response to skeletal muscle overload is not dependent on mast cell activation. Microcirculation 17:548–556

Ebihara N, Nishikawa M, Murakami A (2006) Disodium cromoglycate inhibits capsaicin-induced eosinophil infiltration of conjunctiva independent of mast cells. Jpn J Ophthalmol 50:205–210

Finn DF, Walsh JJ (2013) Twenty-first century mast cell stabilizers. Br J Pharmacol 170:23–37

Fujiwara Y, Furukawa K, Haruki K et al (2011) Nafamostat mesilate can prevent adhesion, invasion and peritoneal dissemination of pancreatic cancer thorough nuclear factor kappa-B inhibition. J Hepatobiliary Pancreat Sci 18:731–739

Giard DJ, Aaronson SA, Todaro GJ et al (1973) In vitro cultivation of human tumors: establishment of cell lines derived from a series of solid tumors. J Natl Cancer Inst 51:1417–1423

Horan RF, Sheffer AL, Austen KF (1990) Cromolyn sodium in the management of systemic mastocytosis. J Allergy Clin Immunol 85:852–855

Horváthová M, Podivínsky F, Gazdík F et al (1998) Effect of disodium cromoglycate treatment on peripheral blood mononuclear cell adhesion to cultured endothelium in allergic asthmatics. Physiol Res 47:445–451

Isaji M, Miyata H, Ajisawa Y et al (1997) Tranilast inhibits the proliferation, chemotaxis and tube formation of human microvascular endothelial cells in vitro and angiogenesis in vivo. Br J Pharmacol 122:1061–1066

Johnson C, Huynh V, Hargrove L et al (2016) Inhibition of mast cell-derived histamine decreases human cholangiocarcinoma growth and differentiation via c-Kit/stem cell factor-dependent signaling. Am J Pathol 186:123–133

Kim CE, Lim SK, Kim JS (2012) In vivo antitumor effect of cromolyn in PEGylated liposomes for pancreatic cancer. J Control Release 157:190–195

Küpper T, Goebbels K, Kennes LN et al (2012) Cromoglycate, reproterol, or both–what's best for exercise-induced asthma? Sleep Breath 16:1229–1235

Melillo RM, Guarino V, Avilla E et al (2010) Mast cells have a protumorigenic role in human thyroid cancer. Oncogene 29:6203–6215

Moroni M, Porta C, Gualtieri G et al (1996) Inhaled sodium cromoglycate to treat cough in advanced lung cancer patients. Br J Cancer 74:309–311

Motawi TM, Bustanji Y, El-Maraghy S et al (2014) Evaluation of naproxen and cromolyn activities against cancer cells viability, proliferation, apoptosis, p53 and gene expression of survivin and caspase-3. J Enzyme Inhib Med Chem 29:153–161

Netzer NC, Küpper T, Voss HW et al (2012) The actual role of sodium cromoglycate in the treatment of asthma–a critical review. Sleep Breath 16:1027–1032

Okada M, Tokumitsu H, Kubota Y et al (2002) Interaction of S100 proteins with the antiallergic drugs, olopatadine, amlexanox, and cromolyn: identification of putative drug binding sites on S100A1 protein. Biochem Biophys Res Commun 292:1023–1030

Pacharn P, Vichyanond P (2013) Immunomodulators for conjunctivitis. Curr Opin Allergy Clin Immunol 13:550–557

Platt M (2014) Pharmacotherapy for allergic rhinitis. Int Forum Allergy Rhinol 4(Suppl 2):S35–S40

Raithel M, Winterkamp S, Weidenhiller M et al (2007) Combination therapy using fexofenadine, disodium cromoglycate, and a hypoallergenic amino acid-based formula induced remission in a patient with steroid-dependent, chronically active ulcerative colitis. Int J Colorectal Dis 22:833–839

Ribatti D (2008) Chick embryo chorioallantoic membrane as a useful tool to study angiogenesis. Int Rev Cell Mol Biol 270:181–224

Ribatti D, Ranieri G (2015) Tryptase, a novel angiogenic factor stored in mast cell granules. Exp Cell Res 332:157–162

Ridolo E, Montagni M, Caminati M et al (2014) Emerging drugs for allergic conjunctivitis. Expert Opin Emerg Drugs 19:291–302

Samoszuk M, Corwin MA (2003) Mast cell inhibitor cromolyn increases blood clotting and hypoxia in murine breast cancer. Int J Cancer 107:159–163

Sapmaz E, Sun Z, Chang JC et al (2015) Effects of mast cell stabilization on the development of aspiration-induced fibrosis in a rat model (abstract). Am J Transplant 15 (suppl 3). http://www.atcmeetingabstracts.com/abstract/effects-of-mast-cell-stabilization-on-the-development-of-aspiration-induced-fibrosis-in-a-rat-model/. Accessed 6 June 2016

Shishibori T, Oyama Y, Matsushita O et al (1999) Three distinct anti-allergic drugs, amlexanox, cromolyn and tranilast, bind to S100A12 and S100A13 of the S100 protein family. Biochem J 338(Pt 3):583–589

Theoharides TC (2008) Mast cells and pancreatic cancer. N Engl J Med 358:1860–1861

Weng Z, Zhang B, Asadi S et al (2012) Quercetin is more effective than cromolyn in blocking human mast cell cytokine release and inhibits contact dermatitis and photosensitivity in humans. PLoS One 7:e33805

Yang Y, Lu JY, Wu X et al (2010) G-protein-coupled receptor 35 is a target of the asthma drugs cromolyn disodium and nedocromil sodium. Pharmacology 86:1–5

Yazid S, Leoni G, Getting SJ et al (2010) Antiallergic cromones inhibit neutrophil recruitment onto vascular endothelium via annexin-A1 mobilization. Arterioscler Thromb Vasc Biol 30:1718–1724

Yoon WH, Jung YJ, Kim TD et al (2004) Gabexate mesilate inhibits colon cancer growth, invasion, and metastasis by reducing matrix metalloproteinases and angiogenesis. Clin Cancer Res 10:4517–4526

Zhang Z, Nagata I, Kikuchi H et al (2001) Broad-spectrum and selective serine protease inhibitors prevent expression of platelet-derived growth factor-BBand cerebral vasospasm after subarachnoid hemorrhage: vasospasm caused by cisternal injection of recombinant platelet-derived growth factor-BB. Stroke 32:1665–1672

Zhang T, Finn DF, Barlow JW et al (2016) Mast cell stabilisers. Eur J Pharmacol 778:158–168

Zuberbier T, Greaves MW, Juhlin L et al (2001) Management of urticaria: a consensus report. J Investig Dermatol Symp Proc 6:128–131