Scientific Research Publishing

Optics and Photonics Journal, 2018, 8, 1-10
http://www.scirp.org/journal/opj
ISSN Online: 2160-889X
ISSN Print: 2160-8881

Granulocyte Colony—Stimulating Factor Multiplies Normal Blood ROS Generation at Less than 1 µg/l

Thomas Stief

Institute of Laboratory Medicine and Pathobiochemistry, University Hospital of Giessen & Marburg, Giessen & Marburg, Germany
Email: thomas.stief@uk-gm.de

How to cite this paper: Stief, T. (2018) Granulocyte Colony—Stimulating Factor Multiplies Normal Blood ROS Generation at Less than 1 µg/l. *Optics and Photonics Journal*, **8**, 1-10.
https://doi.org/10.4236/opj.2018.81001

Received: October 24, 2017
Accepted: January 6, 2018
Published: January 9, 2018

Abstract

Background: The neutrophils (PMN) are our main blood cells to combat fungi, bacteria, and fibrin. For normal function, an activated PMN generates a certain concentration of reactive oxygen species (ROS). If the generated blood ROS concentration is too low, then fungi, bacteria or fibrin might threaten the life of the patient, and it could be of great medical interest to stimulate PMN by physiologic drugs. Granulocyte-Colony Stimulating Factor (G-CSF) is a cell hormone that increases the cell number of PMN and that stimulates the individual PMN. The blood ROS generation assay (BRGA) is an innovative physiologic test to monitor the ROS generation of PMN in blood. Here the ROS generating action of G-CSF on normal PMN is quantified. *Material and Methods*: 40 µl 0 - 10.3 ng/ml (final conc.) G-CSF (in 5% human albumin) in black Brand® 781608 high quality polystyrene F-microwells was incubated in triplicate with 125 µl Hanks' balanced salt solution (HBSS; modified without phenol red) and 10 µl normal citrated blood. Immediately (BRGA) or after 60 min (BRGA-60-) 10 µl 5 mM luminol sodium salt in 0.9% NaCl and 10 µl 0 or 36 µg/ml zymosan A in 0.9% NaCl was added. The photons were counted within 0 - 318 min (37˚C) in a photons-multiplying microtiter plate luminometer. At about 0.5 t-max_n (0.5 fold the time to normal maximum) the approx. SC200 of G-CSF was determined. *Results and Discussion*: The approx. SC200 of G-CSF on normal blood ROS generation was 0.2 µg/l (=20 IU/ml). In clinical situations where an increased blood ROS generation is pharmacologically required, few micrograms of G-CSF could be a sufficient dosage for an adult patient. The BRGA helps to find out the correct stimulating G-CSF dosage for each individual. An enhanced PMN function could favor a better clinical outcome in situations of wanted increase of the innate immunology or in cellular fibrinolysis. G-CSF plasma concentrations of 0.1 - 1 µg/l might favor singlet oxygen generation without immunosuppression or cell fragment-

induced thrombin generation.

Keywords

Singlet Oxygen ($^1\Delta O_2$), Reactive Oxygen Species (ROS), Excited Carbonyl (R-C = O*), Photon (hν), Phagocytes, Neutrophils (PMN), BRGA, G-CSF

1. Introduction

The main cells of innate immunology are the phagocytes (neutrophils = PMN, monocytes = MØ, dendritic cells = DC). Drugs that enhance the PMN function are of great clinical relevance in many diseases where PMN are needed against the disease [1] [2]. The present study aimed to analyze the drug granulocyte-colony stimulating factor (G-CSF) in the blood ROS generation assay (BRGA) [3] [4], an innovative test for whole blood ROS generation working with luminol-enhanced photons emission primarily by diluted whole blood PMN [5] [6] [7], stimulated by typical pathophysiological septic concentrations of the fungal compound zymosan A (ZyA; 1 - 2 µg/ml).

2. Material and Methods

40 µl 0 - 10.3 ng/ml (0 - 974 IU/ml) G-CSF (final conc.) (2nd International WHO Standard, human rDNA derived, protein expressed in *E. coli*; NIBSC, Potters Bar, UK; article nr. 09/136; 1000 ng G-CSF (containing less than 10 ng LPS [8]), 10 mg arginine, 10 mg phenylalanine, 5 mg trehalose, 2 mg human albumin, 0.01% Tween 20® dissolved in 500 µl H_2O followed by 500 µl 5% human albumin (CSL Behring, Marburg, Germany) in black high quality flat bottomed polystyrene microwells (Brand, Wertheim, Germany; article nr. 781608), diluted with 5% human albumin, were incubated in triplicate with 125 µl Hanks' balanced salt solution (HBSS; modified without phenol red; SAFC Biosciences-Sigma, Deisenhofen, Germany; article nr. 55037C-1000 ML) and 10 µl freshest normal blood anticoagulated with 11 mM sodium citrate (within 30 min after withdrawal). Immediately (BRGA) or after 60 min (BRGA-60-) 10 µl 5 mM luminol sodium salt (Sigma, Deisenhofen, Germany) in 0.9% NaCl and 10 µl 0 or 36 µg/ml zymosan A (Sigma) in 0.9% NaCl were added. The photons were counted within 0 - 318 min (37˚C) in a photons-multiplying microtiter plate luminometer (LUmo; anthos, Krefeld, Germany) with an integration time of 0.5 s per well. The intra-assay coefficients of variation were less than 10%. At about 0.5 $t\text{-}max_n$ (0.5 fold the time to normal maximum) the approx. SC200 of G-CSF was determined.

HBSS consisted of 185.4 mg/l $CaCl_2 \cdot 2H_2O$, 200 mg/l $MgSO_4 \cdot 7H_2O$, 400 mg/l KCl, 60 mg/l KH_2PO_4, 350 mg/l $NaHCO_3$, 8000 mg/l NaCl, 90 mg/l Na_2HPO_4, 1000 mg/l glucose, pH 7.0 - 7.4. Expressed in molarity, the concentrations of the HBSS components are: 1.3 mM Ca^{2+}, 0.8 mM Mg^{2+}, 5.8 mM K^+, 143 mM Na^+, 144 mM Cl^-, 1.6 mM SO_4^{2-}, 0.4 mM $H_2PO_4^-$, 0.6 mM HPO_4^{2-}, 4.2 mM HCO_3^-,

5.6 mM glucose.

3. Results

In albumin samples, the BRGA maximum of 2389 RLU/s was reached after 124 min. In NaCl samples, the maximum of 1694 RLU/s was reached after 137 min. At 318 min, the blood ROS generation was 51% or 37% of the maximum, respectively (Figure 1).

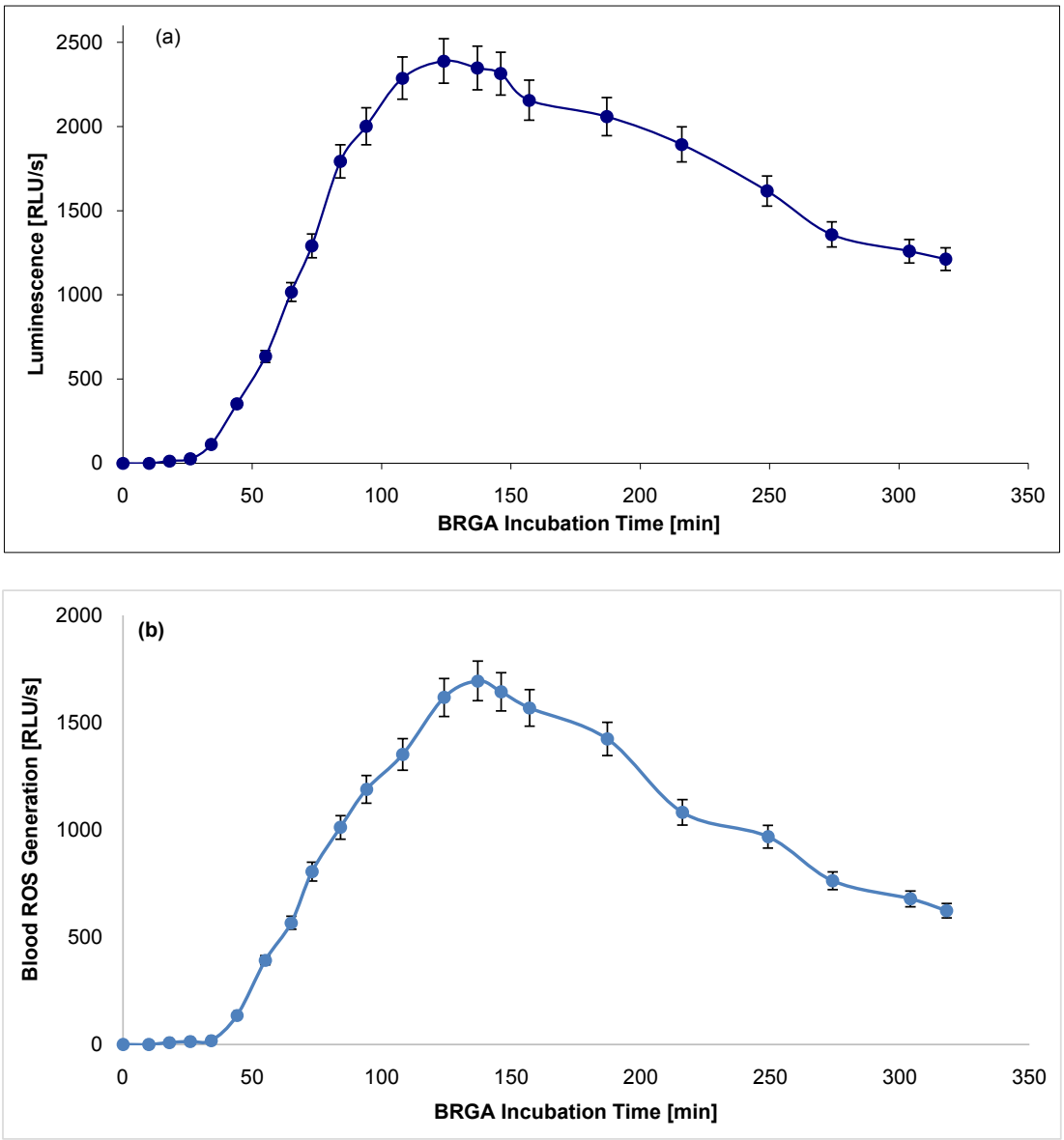

Figure 1. Blood ROS generation in presence of albumin or 0.9% NaCl in BRGA. 40 µl 5% human albumin (Figure 1(a)) or 0.9% NaCl for control (Figure 1(b)) in black Brand® 781608 high quality polystyrene F-microwells were incubated in triplicate with 125 µl Hanks' balanced salt solution (HBSS; modified without phenol red) and 10 µl normal citrated blood. 10 µl 5 mM luminol sodium salt in 0.9% NaCl and 10 µl 36 µg/ml zymosan A in 0.9% NaCl were added. The photons were counted within 0 - 318 min (37˚C) in a photons-multiplying microtiter plate luminometer (LUmo). In albumin samples the maximum of 2389 RLU/s was reached after 124 min, in NaCl samples the maximum of 1694 RLU/s was reached after 137 min. At 318 min the blood ROS generation was 51% or 37% of the maximum, respectively. The experiment was repeated twice, the standard deviations were <10%.

In albumin samples, the BRGA-60-maximum of 6502 RLU/s was reached after 84 min. In NaCl samples, the maximum of 6254 RLU/s was reached after 84 min, too. At 264 min, the blood ROS generation was 43% or 22% of the maximum, respectively (Figure 2). This means that a protein-poor environment facilitates the down-regulation of the ROS generation.

In the BRGA, the approx. SC200 was 0.2 ng/ml G-CSF (=20 IU/ml) (Figure 3). In the BRGA-60-, there appeared an approx. IC50 of 2 ng/ml G-CSF. Higher conc. of G-CSF again stimulated the ROS generation (Figure 4). This means that

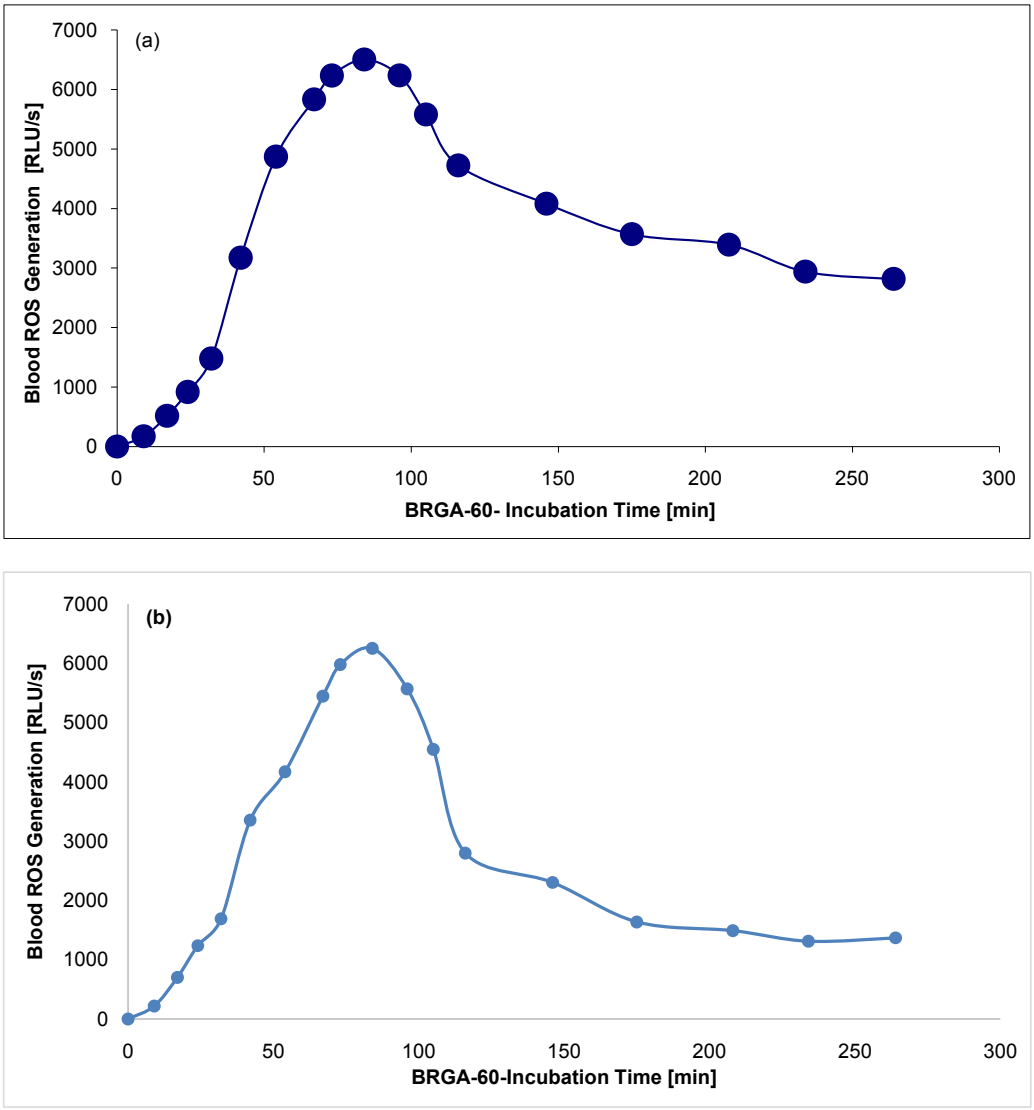

Figure 2. Blood ROS generation in presence of albumin or 0.9% NaCl in BRGA-60-. 40 µl 5% human albumin (Figure 2(a)) or 0.9% NaCl for control (Figure 2(b)) in black Brand® 781608 high quality polystyrene F-microwells were incubated in triplicate with 125 µl Hanks' balanced salt solution (HBSS; modified without phenol red) and 10 µl normal citrated blood. After 60 min 10 µl 5 mM luminol sodium salt in 0.9% NaCl and 10 µl 36 µg/ml zymosan A in 0.9% NaCl were added. The photons were counted within 0 - 264 min (37˚C) in a photons-multiplying microtiter plate luminometer (LUmo). In albumin samples the maximum of 6502 RLU/s was reached after 84 min, in NaCl samples the maximum of 6254 RLU/s was reached after 84 min, too. At 264 min the blood ROS generation was 43% or 22% of the maximum, respectively. The experiment was repeated twice, the standard deviations were < 10%.

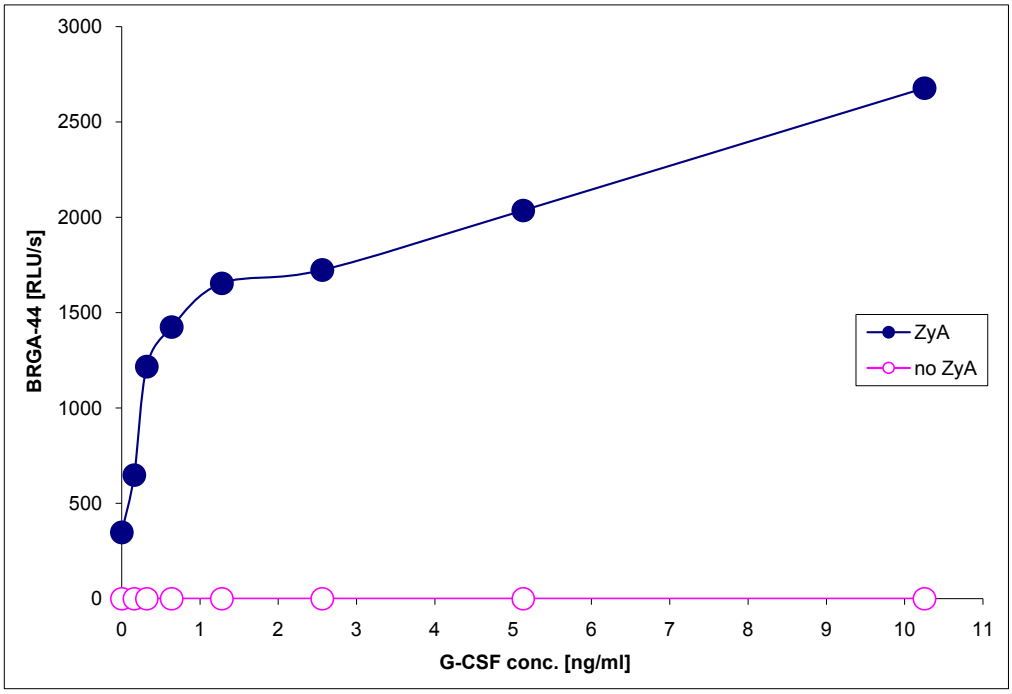

Figure 3. Approx. SC200 of G-CSF in BRGA. 40 µl 0 - 10.3 ng/ml (final conc.) G-CSF (in 5% human albumin) in black Brand® 781608 high quality polystyrene F-microwells were incubated in triplicate with 125 µl Hanks' balanced salt solution (HBSS; modified without phenol red) and 10 µl normal citrated blood. 10 µl 5 mM luminol sodium salt in 0.9% NaCl and 10 µl 0 or 36 µg/ml zymosan A in 0.9% NaCl were added. The photons were counted at 44 min (37˚C); approx. SC200 = 0, 2 ng/ml = 20 IU/ml.

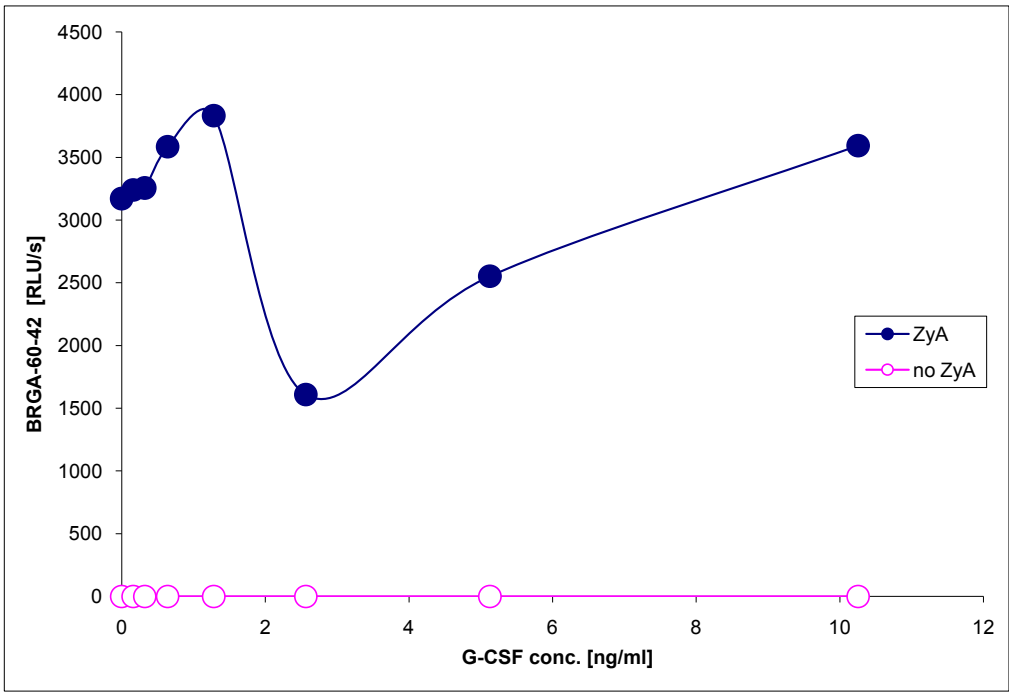

Figure 4. Approx. IC50 of G-CSF in BRGA-60-. 40 µl 0 - 10.3 ng/ml (final conc.) G-CSF (in 5% human albumin) in black Brand® 781608 high quality polystyrene F-microwells were incubated for 60 min (37˚C) in triplicate with 125 µl Hanks' balanced salt solution (HBSS; modified without phenol red) and 10 µl normal citrated blood. 10 µl 5 mM luminol sodium salt in 0.9% NaCl and 10 µl 0 or 36 µg/ml zymosan A in 0.9% NaCl were added. The photons were counted at 42 min (37˚C). There appeared an approx. IC50 of 2 ng/ml G-CSF. Higher conc. of G-CSF again stimulated the ROS generation.

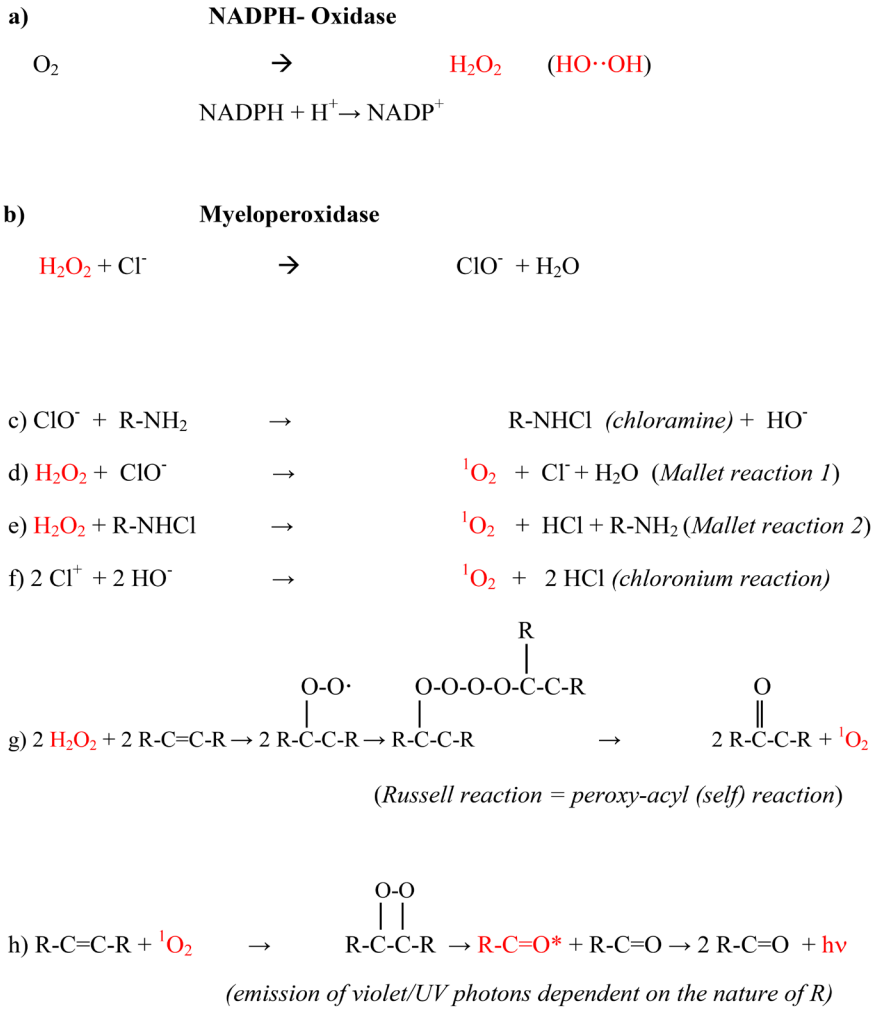

Figure 5. Biogenesis of ROS/photons by activated neutrophils.

within the first incubation time of one hour (37˚C) in the BRGA-60-, G-CSF seems to be inactivated to some extent.

4. Discussion

By contrast, in the BRGA, very low concentrations of G-CSF stimulate blood ROS generation. This could be of pharmacologic interest: in clinical situations where an increased blood ROS generation is pharmacologically required, few micrograms of G-CSF could be a sufficient dosage for an adult patient. The BRGA helps to find out the correct stimulating G-CSF dosage for each individual. An enhanced PMN function could favor a better clinical outcome in situations of wanted increase of the innate immunology or in cellular fibrinolysis [9]-[17].

The normal plasma concentration of G-CSF is about 25 ± 20 pg/ml, and in acute infections, the G-CSF concentration can increase up to about 100 fold [18] [19] [20]; upon subcutaneous injection of 300 µg filgrastim, the G-CSF plasma concentration has increased about 1000 fold (blood half-life about 4 h), activating on neutrophils the CD11b/CD18 expression and the respiratory burst, on

monocytes/dendritic cells the generation of immune suppressive interleukin-10, on endothelial cells the release of von Willebrand factor and F8, on hepatocytes the release of fibrinogen [21]. There could be an enhanced generation of thrombin/systemically circulating micro-thrombi [14] [22]. Thus, respective blood hemostasis, a G-CSF dosage of about 300 µg seems to be "too much of a good thing". The present work indicates that a G-CSF plasma concentration around 1 ng/ml (injection of about 3 µg G-CSF, *i.e.* 100 fold less than currently used) might favour the physiologic singlet oxygen generation (**Figure 5**) against pathogens without pathologic thrombin generation or immune suppressive side effects [23]-[47]. The BRGA is a powerful tool to compare new analogues of G-CSF (e.g. the *E. coli* product filgrastim or the CHO product lenograstim). Dose-finding studies are highly indicated to establish the range of beneficial G-CSF concentrations for each individual patient.

References

[1] Stief, T.W. (2008) Neutrophil Granulocytes in Hemostasis. *Hemostasis Laboratory*, **1**, 269-289.

[2] Seguchi, H. and Kobayashi, T. (2002) Study of NADPH Oxidase-Activated Sites in Human Neutrophils. *Journal of Electron Microscopy*, **51**, 87-91. https://doi.org/10.1093/jmicro/51.2.87

[3] Stief, T. (2013) The Routine Blood ROS Generation Assay (BRGA) Triggered by Typical Septic Concentrations of Zymosan A. *Hemostasis Laboratory*, **6**, 89-98.

[4] Stief, T. (2013) Pathophysiologic Routine Blood Tests for the Generation of Reactive Oxygen Species. *Hemostasis Laboratory*, **6**, 141-153.

[5] Stief, T.W. (2013) Reactive Oxygen Species Generation in Diluted Whole Blood Anticoagulated by Citrate, EDTA, or Heparin. *Hemostasis Laboratory*, **6**, 155-174.

[6] Nathan, C.F. (1987) Neutrophil Activation on Biological Surfaces. Massive Secretion of Hydrogen Peroxide in Response to Products of Macrophages and Lymphocytes. *Journal of Clinical Investigation*, **80**, 1550-1600. https://doi.org/10.1172/JCI113241

[7] Goodridge, H.S., Wolf, A.J. and Underhill, D.M. (2009) Beta-Glucan Recognition by the Innate Immune System. *Immunological Reviews*, **230**, 38-50. https://doi.org/10.1111/j.1600-065X.2009.00793.x

[8] Stief, T. (2013) Quantification of Active LPS in Unknown Matrices by the Oxidative Limulus Test. *Hemostasis Laboratory*, **6**, 343-350.

[9] Stief, T.W. and Fareed, J. (2000) The Antithrombotic Factor Singlet Oxygen/Light ($^1O_2$/hv). *Clinical and Applied Thrombosis / Hemostasis*, **6**, 22-30.

[10] Stief, T.W., Fu, K., Doss, M.O. and Fareed, J. (1999) The Anti-Thrombotic Factor Singlet Oxygen ($^1O_2$) Induces Selective Thrombolysis *in vivo* by Massive Phagocyte Infiltration into the Thrombus. *XVII Congress of the International Society on Thrombosis and Haemostasis*, 14-21 August 1999, Washington DC.

[11] Stief, T.W. (2000) The Blood Fibrinolysis/Deep-Sea Analogy: A Hypothesis on the Cell Signals Singlet Oxygen/Photons as Natural Antithrombotics. *Thrombosis Research*, **99**, 1-20. https://doi.org/10.1016/S0049-3848(00)00213-9

[12] Stief, T.W. (2003) The Physiology and Pharmacology of Singlet Oxygen. *Medical Hypotheses,* **60**, 567-572. https://doi.org/10.1016/S0306-9877(03)00026-4

[13] Stief, T.W. (2004) Regulation of Hemostasis by Singlet-Oxygen ($^1\Delta O_2$). *Current Vascular Pharmacology*, **2**, 357-362. https://doi.org/10.2174/1570161043385420

[14] Stief, T. (2013) Micro-Thrombi Stimulate Blood ROS Generation. *Hemostasis Laboratory*, **6**, 315-325.

[15] Ringelstein, E.B., Thijs, V., Norrving, B., Chamorro, A., Aichner, F., Grond, M., Saver, J., Laage, R., Schneider, A., Rathgeb, F., Vogt, G., Charissé, G., Fiebach, J.B., Schwab, S., Schäbitz, W.R., Kollmar, R., Fisher, M., Brozman, M., Skoloudik, D., Gruber, F., Serena Leal, J., Veltkamp, R., Köhrmann, M. and Berrouschot, J. (2013) AXIS 2 Investigators. Granulocyte Colony-Stimulating Factor in Patients with Acute Ischemic Stroke: Results of the AX200 for Ischemic Stroke Trial. *Stroke*, **44**, 2681-2687. https://doi.org/10.1161/STROKEAHA.113.001531

[16] Lu, F., Nakamura, T., Toyoshima, T., Liu, Y., Shinomiya, A., Hirooka, K., Okabe, N., Miyamoto, O., Tamiya, T., Keep, R.F. and Itano, T. (2014) Neuroprotection of Granulocyte Colony-Stimulating Factor during the Acute Phase of Transient Forebrain Ischemia in Gerbils. *Brain Research*, **1548**, 49-55. https://doi.org/10.1016/j.brainres.2013.12.010

[17] Welte, K. (2014) G-CSF: Filgrastim, Lenograstim and Biosimilars. *Expert Opinion on Biological Therapy*, **14**, 983-993. https://doi.org/10.1517/14712598.2014.905537

[18] Kawakami, M., Tsutsumi, H., Kumakawa, T., Abe, H., Hirai, M., Kurosawa, S., Mori, M. and Fukushima, M. (1990) Levels of Serum Granulocyte-Stimulating Factor in Patients with Infections. *Blood*, **76**, 1962-1964.

[19] De Haas, M., Kerst, J.M., van der Schoot, C.E., Calafat, J., Hack, C.E., Nuijens, J.H., Roos, D., van Oers, R.H. and von dem Borne, A.E. (1994) Granulocyte Colony-Stimulating Factor Administration to Healthy Volunteers: Analysis of the Immediate Activating Effects on Circulating Neutrophils. *Blood*, **84**, 3885-3894.

[20] Pauksen, K., Elfman, L., Ulfgren, A.K. and Venge, P. (1994) Serum Levels of Granulocyte-Colony Stimulating Factor (G-CSF) in Bacterial and Viral Infections, and in Atypical Pneumonia. *British Journal of Haematology*, **88**, 256-260. https://doi.org/10.1111/j.1365-2141.1994.tb05015.x

[21] Anderlini, P. and Champlin, R.E. (2008) Biologic and Molecular Effects of Granulocyte Colony—Stimulating Factor in Healthy Individuals: Recent Findings and Current Challenges. *Blood*, **111**, 1767-1772. https://doi.org/10.1182/blood-2007-07-097543

[22] Kang, H.J., Kim, H.S., Zhang, S.Y., Park, K.W., Cho, H.J., Koo, B.K., Kim, Y.J., Soo Lee, D., Sohn, D.W., Han, K.S., Oh, B.H., Lee, M.M. and Park, Y.B. (2004) Effects of Intracoronary Infusion of Peripheral Blood Stem-Cells Mobilised with Granulocyte-Colony Stimulating Factor on Left Ventricular Systolic Function and Restenosis after Coronary Stenting in Myocardial Infarction: The MAGIC Cell Randomised Clinical Trial. *The Lancet*, **363**, 751-756. https://doi.org/10.1016/S0140-6736(04)15689-4

[23] Subramaniam, R., Barnes, P.F., Fletcher, K., Boggaram, V., Hillberry, Z., Neuenschwander, P. and Shams, H. (2014) Protecting against Post-Influenza Bacterial Pneumonia by Increasing Phagocyte Recruitment and ROS Production. *The Journal of Infectious Diseases*, **209**, 1827-1836. https://doi.org/10.1093/infdis/jit830

[24] Sun, K. and Metzger, D.W. (2014) Influenza Infection Suppresses NADPH Oxidase-Dependent Phagocytic Bacterial Clearance and Enhances Susceptibility to Secondary Methicillin-Resistant *Staphylococcus aureus* Infection. *The Journal of Immunology*, **192**, 3301-3307. https://doi.org/10.4049/jimmunol.1303049

[25] Sun, K., Torres, L. and Metzger, D.W. (2010) A Detrimental Effect of Interleukin-10

on Protective Pulmonary Humoral Immunity during Primary Influenza a Virus Infection. *Journal of Virology*, **84**, 5007-5014. https://doi.org/10.1128/JVI.02408-09

[26] Kanlop, N., Thommasorn, S., Palee, S., Weerateerangkul, P., Suwansirikul, S., Chattipakorn, S. and Chattipakorn, N. (2011) Granulocyte Colony-Stimulating Factor Stabilizes Cardiac Electrophysiology and Decreases Infarct Size during Cardiac Ischaemic/Reperfusion in Swine. *Acta Physiologica*, **202**, 11-20. https://doi.org/10.1111/j.1748-1716.2011.02259.x

[27] Stief, T. (2013) Neutrophils' Photons and Opsins (Preface). In: *Photonic Hemostasis*, *Physiology of Light Signals in the Neutrophil*, Nova Science Publishers, New York, vii-xvii.

[28] Stief, T. (2013) Blood Neutrophils See UV Light: 340 nm Primes ROS Generation Nearly Half as Strong as 405 nm. *Hemostasis Laboratory*, **6**, 389-403.

[29] Fuhler, G.M., Blom, N.R., Coffer, P.J., Drayer, A.L. and Vellenga, E. (2007) The Reduced GM-CSF Priming of ROS Production in Granulocytes from Patients with Myelodysplasia Is Associated with an Impaired Lipid Raft Formation. *Journal of Leukocyte Biology*, **81**, 449-457. https://doi.org/10.1189/jlb.0506311

[30] Olivetta, E., Pietraforte, D., Schiavoni, I., Minetti, M., Frederico, M. and Sanchez, M. (2005) HIV-1 Nef Regulates the Release of Superoxide Anions from Human Macrophages. *Biochemical Journal*, **390**, 591-602. https://doi.org/10.1042/BJ20042139

[31] Hoggatt, J. and Pelus, L.M. (2014) New G-CSF Agonists for Neutropenia Therapy. *Expert Opinion on Investigational Drugs*, **23**, 21-35. https://doi.org/10.1517/13543784.2013.838558

[32] Bath, P.M., Sprigg, N. and England, T. (2013) Colony Stimulating Factors (Including Erythropoietin, Granulocyte Colony Stimulating Factor and Analogues) for Stroke. *The Cochrane Database of Systematic Reviews*, **6**, CD005207.

[33] Moazzami, K., Roohi, A. and Moazzami, B. (2013) Granulocyte Colony Stimulating Factor Therapy for Acute Myocardial Infarction. *The Cochrane Database of Systematic Reviews*, **5**, CD008844. https://doi.org/10.1002/14651858.CD008844.pub2

[34] Stief, T. (2006) G-CSF Enhances Cellular Fibrinolysis. *Clinical and Applied Thrombosis/Hemostasis*, **12**, 122. https://doi.org/10.1177/107602960601200123

[35] Gong, Y. and Hoover-Plow, J. (2012) The Plasminogen System in Regulating Stem Cell Mobilization. *Journal of Biomedicine and Biotechnology*, **2012**, Article ID: 437920. https://doi.org/10.1155/2012/437920

[36] Kuritzkes, D.R. (2000) Neutropenia, Neutrophil Dysfunction, and Bacterial Infection in Patients with Human Immunodeficiency Virus Disease: The Role of Granulocyte Colony—Stimulating Factor. *Clinical Infectious Diseases*, **30**, 256-260. https://doi.org/10.1086/313642

[37] Hartung, T. (1999) Granulocyte Colony—Stimulating Factor: Its Potential Role in Infectious Disease. *AIDS*, **13**, S3-S9.

[38] Hübel, K., Dale, D.C. and Liles, W.C. (2002) Therapeutic Use of Cytokines to Modulate Phagocyte Function for the Treatment of Infectious Diseases: Current Status of Granulocyte Colony-Stimulating Factor, Granulocyte-Macrophage Colony-Stimulating Factor, Macrophage Colony-Stimulating Factor, and Interferon-Gamma. *The Journal of Infectious Diseases*, **185**, 1490-1501. https://doi.org/10.1086/340221

[39] Chirullo, B., Sgarbanti, R., Limongi, D., Shytaj, I.L., Alvarez, D., Das, B., Boe, A., DaFonseca, S., Chomont, N., Liotta, L., Petricoin, E.I., Norelli, S., Pelosi, E., Garaci,

E., Savarino, A. and Palamara, A.T. (2013) A Candidate Anti-HIV Reservoir Compound, Auranofin, Exerts a Selective "Anti-Memory" Effect by Exploiting the Baseline Oxidative Status of Lymphocytes. *Cell Death & Disease*, **4**, e944. https://doi.org/10.1038/cddis.2013.473

[40] Stief, T.W., Slenczka, W., Renz, H. and Klenk, H.D. (2001) Singlet Oxygen ($^1O_2$) Generating Chloramines at Concentrations That Are Tolerable for Normal Hemostasis Function Inactivate the Lipid Enveloped Vesicular Stomatitis Virus in Human Blood. 3*rd Symposium on the Biology of Endothelial Cells*, *Pathophysiology of the Endothelium*: *Vascular and Infectious Diseases*, Giessen, 24-26 May 2001, Abstr. D10.

[41] Stief, T.W. (2008) Hemostasis Tolerable Singlet Oxygen—A Perspective in AIDS Therapy. *Hemostasis Laboratory*, **1**, 21-40.

[42] Stief, T.W. (2003) Singlet Oxygen—Oxidizable Lipids in the HIV Membrane, New Targets for AIDS Therapy? *Medical Hypotheses*, **60**, 575-577. https://doi.org/10.1016/S0306-9877(03)00046-X

[43] Stief, T.W. (2010) Singlet Oxygen and Thrombin Generation: 0.5-1 mM Chloramine as Anti-Viral Therapy. *Hemostasis Laboratory*, **3**, 311-324.

[44] Li, C., Lu, L., Zhang, J., Huang, S., Xing, Y., Zhao, M., Zhou, D., Li, D. and Meng, A. (2015) Granulocyte-Stimulating Factor Exacerbates Hematopoietic Stem Cell Injury after Irradiation. *Cell & Bioscience*, **5**, 65. https://doi.org/10.1186/s13578-015-0057-3

[45] Carrão, A.C., Chilian, W.M., Yun, J., Kolz, C., Rocic, P., Lehmann, K., van den Wijngaard, J.P., van Horssen, P., Spaan, J.A., Ohanyan, V., Pung, Y.F. and Buschmann, I. (2009) Stimulation of Coronary Collateral Growth by Granulocyte Stimulating Factor: Role of Reactive Oxygen Species. *Arteriosclerosis*, *Thrombosis*, *and Vascular Biology*, **29**, 1817-1822. https://doi.org/10.1161/ATVBAHA.109.186445

[46] Stief, T. and Cimpean, C.M. (2013) Singlet Oxygen—Oxidized Human Albumin Stimulates Blood ROS Generation. *Hemostasis Laboratory*, **6**, 423-435.

[47] Weiss, S.J., Lampert, M.B. and Test, S.T. (1983) Long-Lived Oxidants Generated by Human Neutrophils: Characterization and Bioactivity. *Science*, **222**, 625-628.