

Modulation of oxidative stress/antioxidative defence in human serum treated by four different tyrosine kinase inhibitors

Marija Mihajlovic^a, Branka Ivkovic^b, Biljana Jancic-Stojanovic^c, Aleksandra Zeljkovic^a, Vesna Spasojevic-Kalimanovska^a, Jelena Kotur-Stevuljevic^a and Dragana Vujanovic^d

Recent findings implied the significance of reactive oxygen species (ROS) as a part of tyrosine kinase inhibitors (TKIs) pharmacological activity. Evidences also suggested that toxic effects of TKIs were related to ROS production. The results regarding benefits of vitamin E usage alongside with prescribed TKIs therapy are ambiguous. We aimed to examine oxidative stress and antioxidative defense in human serum treated with four different TKIs and their possible interactions with hydrosoluble vitamin E analog (Trolox). An in-vitro experiment with serum pool as a substitute model was performed. Different parameters of oxidative stress and antioxidative defense were measured in serum pool with and without addition of TKIs (axitinib, crizotinib, nilotinib, and imatinib), before and after addition of Trolox. Z score statistic was used for calculation of Prooxidative and Antioxidative scores. The highest oxidative potential was recorded for samples incubated with imatinib and nilotinib, while the lowest damaging scores were observed for crizotinib and axitinib (nilotinib vs. imatinib, $P < 0.05$; axitinib vs. imatinib, $P < 0.01$; crizotinib vs. imatinib, $P < 0.001$). The best capability for antioxidative protection was seen in samples with nilotinib,

then with imatinib, while the lowest level was obtained in samples with crizotinib and axitinib (imatinib and axitinib vs. nilotinib, $P < 0.05$ for both; crizotinib vs. nilotinib, $P < 0.01$; axitinib vs. imatinib, $P < 0.05$, crizotinib vs. imatinib, $P < 0.01$). Our results demonstrated the opposite effects of Trolox in combination with imatinib and nilotinib. Usage of antioxidant in combination with TKIs should be carefully evaluated in each specific case. *Anti-Cancer Drugs XXX: 000–000* Copyright © 2020 Wolters Kluwer Health, Inc. All rights reserved.

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Departments of ^aMedical Biochemistry, ^bPharmaceutical Chemistry, ^cDrug Analysis and ^dToxicology, Academic Danilo Soldatović, Faculty of Pharmacy, University of Belgrade, Serbia

Correspondence to Dragana Vujanovic, PhD, Department of Toxicology, Academic Danilo Soldatovic, Faculty of Pharmacy, University of Belgrade, POB 146, 11000 Belgrade, Serbia
Tel: +381 11 3951 249; fax: +381 11 3972 840;
e-mail: dragana.vujanovic@pharmacy.bg.ac.rs

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Introduction

It has been demonstrated that carcinogenesis and metastasis development are directly dependent on functioning of delicate system of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs) [1,2]. Studies have shown that changes of extracellular signaling, induced by different autocrine and paracrine mediators, DNA mutations, or genetic reciprocal translocation can lead to altered functioning of PTKs and thus affect constitutive signal transduction that underlies etiopathogenesis of different cancers [3]. Additionally, PTKs were stressed out as a major group of oncogenes responsible for the development of various malignant disorders [2]. Tyrosine kinase inhibitors (TKIs) act by competing ATP association to tyrosine kinase catalytic site, thus disabling transfer of γ -terminal phosphate group to targeted proteins and consequently inhibiting phosphorylation of many signaling molecules [4]. As a result, TKIs can stop vital processes in tumor cells through termination of signaling pathways [4]. Studies have shown that TKIs can interfere

with cell cycle regulation, cellular division, proliferation, and differentiation [2]. Because PTKs have an important role in vasculogenesis and angiogenesis, the use of TKIs leads to the reduction of tumor mass, thereby reducing the possibility of malignant cells dissemination and development of metastases [4].

TKIs represent a highly effective group of medicines with beneficial effects in treatment of several aggressive types of leukemia. Based on the US Food and Drug Administration approval report from 2013, TKIs have multiple targets and can be used in treatment of various cancers [5]. For example, axitinib, one of the TKIs representatives, is an inhibitor of vascular endothelial growth factor's receptor [5]. Due to its size, axitinib is capable to fit in the kinase domain and thereby to cause inhibition of signal transduction pathways, such as vascular endothelial growth factor/vascular endothelial growth factor's receptor (VEGF/VEGFR) 2 and 3, with consequent reduction of several important enzymes phosphorylation

[5,6]. Studies on renal cancer showed that hereditary background can significantly enhance the formation of hypoxia-inducible factor (HIF)-1 α / HIF- β complexes, which aggravate the disease by inducing transcription of different growth factors, predominantly VEGF and platelet-derived growth factor (PDGF) [6]. Therefore, axitinib is known as a therapy for advanced renal cell carcinoma, which is mostly used when the first line of systemic therapy fails [5].

While axitinib targets process of angiogenesis, the echinoderm microtubule-associated protein-like 4 (EML4)-anaplastic lymphoma kinase (ALK) fusion oncogene, known as one of the causes of altered kinase activity in lung cancer, represents a target place for crizotinib [7]. Crizotinib is mostly used in treating ALK-positive non-small-cell lung carcinoma patients [7]. It was found that the (EML4)-ALK chimeric protein has constitutively expressed tyrosine-kinase activity, which is induced by dimerization or oligomerization of ALK [7]. This activity, as well as mesenchymal epithelial transition growth factor (c-MET) inhibition, makes crizotinib an efficient proapoptotic agent and therapy of choice for this type of cancers [7]. A broader spectrum of kinases activity is targeted by nilotinib [BCR-Abl; PDGFR; stem cell factor receptor – KIT; colony stimulating factor receptor-1 (CSF-1R); discoidin domain receptor (DDR1)], as well as by imatinib (BCR-Abl; DDR; KIT; PDGFR; CSF-1R) [8]. The abnormal fusion protein kinase BCR-Abl is over-expressed in chronic myeloid leukemia (CML) due to chromosomal aberration known as the Philadelphia chromosome [8]. This is the main target for both aforementioned medicines. Nilotinib is considered as beneficial in treatment of those CML patients who are either resistant or intolerant to imatinib [8]. Additionally, although imatinib is registered for treatment of patients with gastrointestinal stromal tumors, nilotinib showed antiproliferative activity in cells with KIT mutations, implying its usefulness in this condition as well [8]. The mechanisms of action of these two drugs are closely related, but minor changes in Abl binding suggest the advantage of nilotinib over imatinib [8].

Based on the aforementioned findings, TKIs were at first considered as highly promising therapy approach, but then significant resistance to these drugs was recorded [9]. Previous research studies conducted with an aim to overcome this therapeutic limitation, pointed toward a dual role of oxidative stress in cancer and its association with PTKs. Namely, higher production of reactive oxygen species (ROS) was shown in solid tumors, due to tissue hypoxia, higher metabolic status, and intensified cell cycle [10]. However, it has also been demonstrated that several cytostatic drugs increase the formation of ROS, thus leading to irreversible oxidative damage of malignant cells and consecutively to their death [11]. A research by Sun *et al.* [12] has shown the interrelation between c-Abl tyrosine kinase and apoptosis induced by oxidative stress, while Li

and Pang [13] recorded that PTPs, due to cysteine residue in their structure, are susceptible to oxidative stress modifications and subsequent decrease of activity. Besides, Koptyra *et al.* [14] proposed a mechanism by which BCR/ Abl oncogenic tyrosine kinase self-mutagenesis, induced by ROS, could provoke resistance to TKIs. Additionally, it has been shown that activation of nuclear factor- κ B signaling pathway relies on tyrosine phosphorylation, which is a process that can be strongly influenced by redox imbalance [15]. In summary, a great interest arose regarding the role of oxidative stress in tumorigenesis and cancer treatment, because novel findings point to intriguing possibility that increased production of ROS could be a part of cytotoxic activity of the applied cytostatic therapy, rather than an adverse effect of these drugs.

A hypothesized dual role of ROS in cancer development and progression puts into question potential benefits of supplemental use of antioxidants during cancer treatment. Previously, it has been assumed that the use of antioxidants in parallel with cytostatics can be beneficial for cancer patients, in terms of preventing drug-induced oxidative damage of healthy tissues [16]. However, in spite of significant efforts, there is no definitive conclusion regarding this issue [16]. Recently, it has been suggested that the administration of antioxidants during cancer therapy should be carefully weighed with respect to the cancer type, general condition of the patient, type of the prescribed anticancer therapy, and type and dosage of antioxidant [17].

In this study, we aimed to explore the influence of four TKIs: nilotinib, crizotinib, axitinib, and imatinib on different parameters of oxidative stress in human serum pool and to evaluate differences in the level of induced oxidative stress. In addition, we sought to explore antioxidative capacity of a hydrosoluble vitamin E analog Trolox and changes in ROS levels in serum concomitantly incubated with specific TKIs and vitamin E analog.

Materials and methods

Materials

Serum pool was formed by collecting human serum samples obtained from healthy subjects who underwent regular medical check-up examinations and laboratory testing (Department of Clinical Chemistry, Faculty of Pharmacy, Belgrade, Serbia). After initial collection, samples were homogenized by stirring at the magnetic stirrer for 2 h, and then aliquoted and frozen at -80°C . The entire study protocol was designed according to the Helsinki Declaration and approved by the local ethical committee (The Ethics committee of Faculty of Pharmacy, approval number 2262/2).

Methods

Advanced oxidation protein products (AOPPs) were assessed by spectrophotometric analysis of formed compounds after the influence of potassium iodine and glacial

AQ3

acetic acid (pH adjusted medium of 7.4) on oxidatively changed proteins. According to Witko-Sarsat *et al.* [18] absorbance was measured at 340 nm. Chloramine T was used for calibration. Total oxidative status (TOS) levels were obtained by measuring ferrous ion oxidation in the reaction of ferrous ammonium sulfate and o-dianisidine dihydrochloride in glycerol solution with different oxidants from serum samples. Measurement of ferrous ion oxidation (from the ferrous ion–o-dianisidine complex) was accomplished by forming colored complex between ferric ion and xylenol orange under acidic conditions of the reaction. Calibration was done by usage of hydrogen peroxide solutions in range of concentrations of 10–200 mol/L [19]. Paraoxonase 1 (PON1) activity was measured kinetically by using paraoxon as substrate, according to Richter and Furlong's instructions [20]. PON1 activity and paraoxon conversion to p-nitrophenol were quantified at 405 nm. Ellman's method was applied for total SH-groups (SHGs) evaluation [21]. According to the conditions in our laboratory, the method was modified by Kotur-Stevuljevic *et al.* [22]. The absorbance of formed p-nitrophenol in the reaction of thiol compounds with dinitrodithiobenzoic acid was measured at 412 nm and reduced glutathione (GSH) (conc. range 0.1–1.0 mM) was used as a standard. Total antioxidative status (TAS) measurement was done by Erel's recommendations. Hydrogen peroxide under acidic conditions induces oxidation of 2, 2'-azino-bis 3-ethylbenzothiazoline-6-sulphonic acid (ABTS) chromogene [23], thus forming a colored solution of ABTS⁺ radical cation. Antioxidants in the serum pool cause reagent discoloration, which can be assessed as a decrease in absorbance at 660 nm, thereby providing valuable information of serum's total antioxidative capacity. Prooxidative–antioxidative balance was measured according to Hamidi Alamdari's method. 3, 3', 5, 5'-tetramethylbenzidine and its cation were used for simultaneously determination of prooxidants and antioxidants. Calibration was done with hydrogen peroxide as a prooxidant, while uric acid was used for antioxidative standardization [24].

Statistic

According to the Shapiro–Wilk test for assessment of normality of data, our values followed skewed data distribution. Our results are presented as medians with interquartile range; therefore, the nonparametric assessment was done by employing Kruskal–Wallis statistical analysis. For further evaluation of observed differences between groups, the Mann–Whitney test was applied. A comparison for paired samples was done by using the Wilcoxon statistical analysis. SPSS statistical package (v.22.0; IBM, Chicago, Illinois, USA) was employed for data analysis.

Summary scores

In order to estimate cumulative impact of different ROSs on serum biomolecules, we have used Z score statistics,

which enables us to concomitantly evaluate parameters measured in different concentration ranges and with different units. Moreover, by using Z score statistics, we can simultaneously weight the influence of different prooxidants and antioxidants. In general, Z score is assessed as difference between the value of the estimated parameter in the analyzed sample and the mean value of the same parameter in general population, divided by the population SD. The Oxy score is calculated as the difference of the prooxidative score (mean Z score of the measured prooxidant parameters: AOPP, TOS, and PAB) and the antioxidative score (mean Z value of the measured antioxidant parameters: TAS, SHG, and PON1). A larger Oxy score indicates weaker antioxidative protection and prevalence of prooxidative processes.

Results

We have used four cytostatic drugs: imatinib, crizotinib, axitinib, and nilotinib as 80 mg/L dimethyl sulfoxide DMSO solutions, which were added to the serum pool. We compared prooxidative potential of these four substances in biological material by measuring several prooxidants and antioxidants (Table 1). In order to counteract increased oxidative stress in pool samples, we added 50 µmol/L of 2 mM Trolox and then measured the same parameters of oxidative stress and antioxidative defense. The obtained results are presented in Fig. 1. Regarding prooxidative influence of TKIs on proteins, measured as AOPP, imatinib and nilotinib showed the strongest effects, because we found significantly higher concentrations of AOPP after serum incubation with these two drugs when compared with others (Fig. 1a). Besides, the addition of Trolox did not cause any changes at the AOPP level in samples incubated with any of four TKIs (Fig. 1a). The similar pattern was seen in the case of TOS, with crizotinib and axitinib showing the lowest increase in this parameter (Fig. 1b). Moreover, Trolox addition at least in part suppresses damaging influence of imatinib and nilotinib, expressed through changes in the TOS level (Fig. 1b). Prooxidative–antioxidative balance measured as PAB showed the similar imprint as for TOS and AOPP, but with significantly higher imatinib prooxidant activity (Fig. 1c). However, the PAB level was significantly lowered after Trolox addition (Fig. 1c). On the other side, our results demonstrated that the interaction of nilotinib with vitamin E analog significantly increased prooxidative activity of this cytostatic drug (Fig. 1c). Regarding antioxidant levels, we found that incubation of serum with axitinib and nilotinib was associated with less prominent changes in total SHG content, when compared with imatinib and crizotinib (Fig. 1d). Incubation with Trolox induced a significant increase in SHG only in a sample with imatinib, while we observed a decrease in the SHG level in combination of vitamin E analog with other three drugs (Fig. 1d). TAS was significantly higher in the presence of nilotinib, while the incubation with

T1

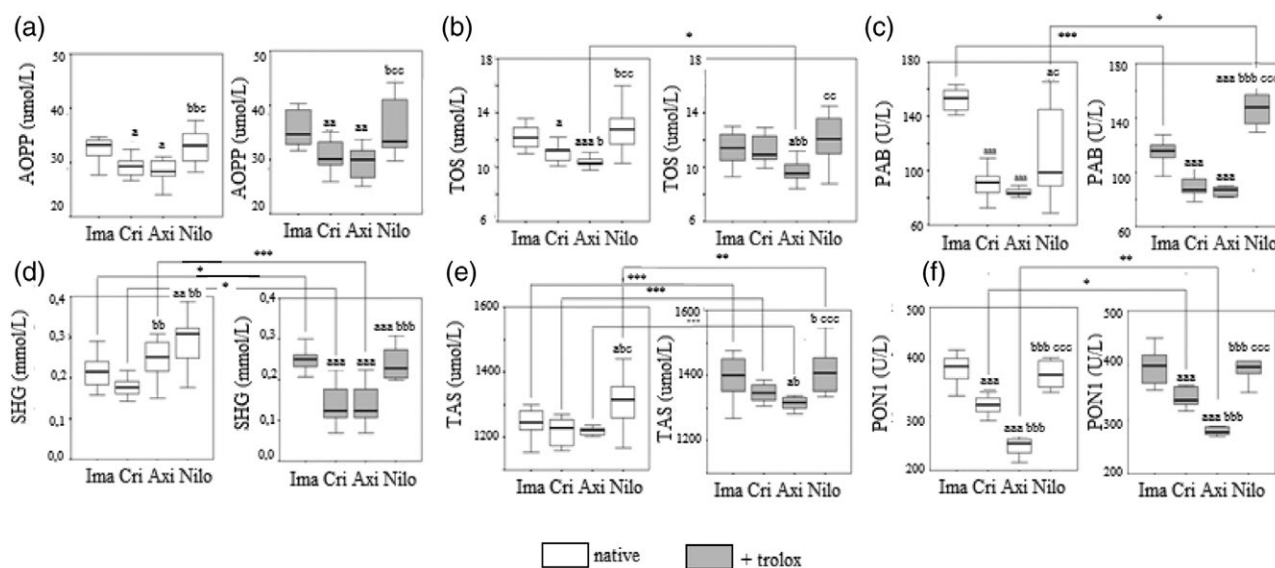
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Table 1 Redox status parameters in serum pool after the 2-h incubation at 37°C with cytostatic drugs

Parameter	Imatinib	Crizotinib	Axitinib	Nilotinib	P value
AOPP (μmol/L)	33.2 (30.9–33.7)	28.6 (27.2–30.9) ^a	28.7 (26.35–32.8) ^a	31.6 (28.7–33.4) ^{b,c}	<0.05
TOS (μmol/L)	12.8 (11.8–12.9)	11.3 (10.9–11.8) ^a	10.5 (9.8–10.6) ^{a,b}	12.5 (11.1–13.5) ^{b,c}	<0.05
PAB (U/L)	145 (143–153)	90 (78–102) ^{aa}	83 (82–86) ^{aa}	95 (79–108) ^{a,b,c}	<0.01
PON (U/L)	402.5(362–420.5)	317.5 (296–340) ^{aaa}	250 (231–257) ^{aaa,bbb}	360 (350–385) ^{bbb,ccc}	<0.001
SHG (mmol/L)	0.178 (0.165–0.196)	0.203 (0.166–0.228)	0.254 (0.200–0.272) ^b	0.278 (0.225–0.326) ^{a,bb,c}	<0.01
TAS (μmol/L)	1289.5 (1254–1299.5)	1220 (1186–1242)	1205 (1190–1215)	1234.5 (1188–1320) ^{b,c}	<0.05

P-Kruskal–Wallis ANOVA with post-hoc Mann–Whitney U test: a, significant difference vs. imatinib; b, vs. crizotinib; c, vs. axitinib (one letter – $P < 0.05$; two letters – $P < 0.01$; three letters – $P < 0.001$).

AOPP, advanced oxidation protein product; PAB, ; PON1, paraoxonase 1; SHG, SH group; TAS, total antioxidative status; TKI, tyrosine kinase inhibitor; TOS, total oxidative status.

Fig. 1

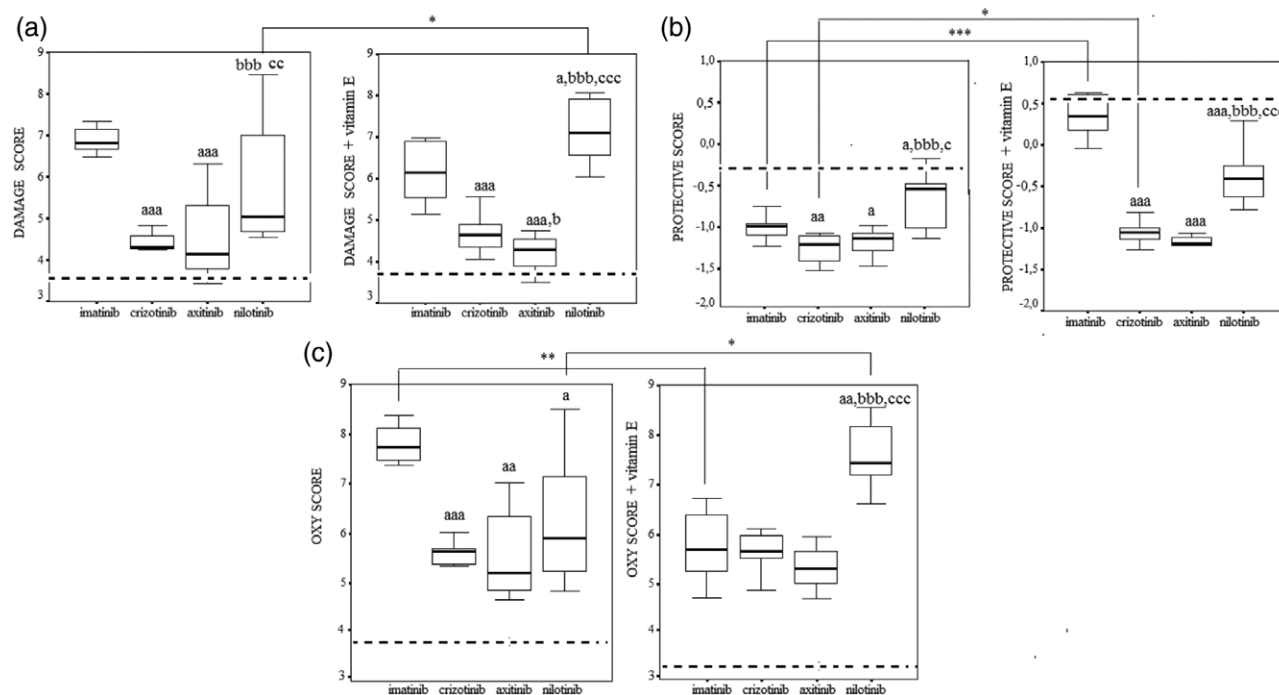
Prooxidative effects of TKI on the redox status parameters, that is, prooxidants and their product's and antioxidative protection elements: (a) AOPP, (b) TOS, (c) PAB, (d) SHG, (e) TAS, (f) PON1. Letters a, b, and c designate significant difference compared to imatinib, crizotinib and axitinib, respectively. Number of corresponding letters labels the achieved level of statistical significance, that is, one letter $P < 0.05$, two $P < 0.01$, three $P < 0.001$. Asterisks mean significant difference between the sample containing TKI compared with the same sample after the Trolox addition. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. AOPP, advanced oxidation protein product; PAB, ; PON1, paraoxonase 1; SHG, SH group; TAS, total antioxidative status; TKI, tyrosine kinase inhibitor; TOS, total oxidative status.

Trolox was associated with an increase in this marker in the samples with all four drugs (Fig. 1e). We have noticed the most intriguing results for PON1 enzymatic activity. Namely, we observed higher PON1 activities in samples incubated with imatinib and nilotinib, when compared with crizotinib and especially to axitinib (Fig. 1f). This significant decrease in PON1 activity in the crizotinib and axitinib-incubated samples was significantly ameliorated following the addition of Trolox (Fig. 1f). Finally, in order to estimate which of the analyzed cytostatic drugs can induce the strongest prooxidative effects, we calculated three summary scores using the above-mentioned redox status parameters. Damaging score is an average of prooxidative damaging capability calculated by z-score statistics. The highest damaging potential was recorded in the sample incubated with imatinib, while the lowest damaging scores were observed for crizotinib and

axitinib (Fig. 2a). Regarding antioxidative protection, the best average capability was seen in the sample incubated with nilotinib, then with imatinib, while the lowest level was obtained in samples incubated with crizotinib and axitinib (Fig. 2b). Oxy score evaluation showed that the incubation with imatinib reached significantly highest Oxy score compared with other TKIs (Fig. 2). Moreover, the most prominent difference in Oxy score was recorded in comparison of imatinib and crizotinib (Fig. 2b). After Trolox addition, damaging score was shifted toward higher values in the nilotinib sample. On the other side, addition of this antioxidative vitamin significantly increased protective score of imatinib and crizotinib, but had no influence on either axitinib or nilotinib sample. Also, Trolox caused significant decrease in the Oxy score of the imatinib sample, while increase in the nilotinib sample.

F2

Fig. 2



Summary scores: damage (a), protective (b), and Oxy (c) for TKIs: imatinib, crizotinib, axitinib, and nilotinib – comparison of native serum pool and pool with addition of vitamin E. Dashed line represents the baseline level of the examined markers in native serum, without and with Trolox. Letters a, b, and c designate significant difference compared to imatinib, crizotinib, and axitinib, respectively. Number of corresponding letters labels the achieved level of statistical significance, that is, one letter $P < 0.05$, two $P < 0.01$, three $P < 0.001$. Asterisks mean significant difference between the sample containing TKI compared to the same sample after the Trolox addition. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. TKI, tyrosine kinase inhibitor.

Discussion

Given a variety of functions and importance of physiological processes mediated by PTKs family of proteins, as well as enrollment of nonreceptor PTKs in cell division processes, cytokine receptor activation, and cytoskeleton rearrangements [25]. TKIs exhibit numerous mechanisms of anticancerous activity. In general, TKIs are inhibitors of different growth factors, including VEGF, PDGF, epidermal growth factor, fibroblast growth factor, and others. In addition, they act as multitarget drugs and may lead to deterioration of function of IL2 T cell inducible kinase, or they can diminish the capability of malignant cells to evade the immune response [26,27]. Several TKIs can induce damages of DNA via ROS, making cancer cells more prone to the activity of natural killer cells [28].

Recently, it has been suggested that crizotinib could have an additional mechanism of acting, which comprises induction of $O_2^{\bullet-}$ production [29]. Such mechanism enables crizotinib to act by enhancing oxidative stress attack on the targeted cells. ROS-mediated apoptosis of melanoma cells, induced by imatinib, was also recorded [30]. Moreover, it has been documented that the aldehyde lactate dehydrogenase-mediated protection system against ROS can diminish positive effects of several TKIs and lead to the development of TKIs resistance

[29]. In contrast, Petrola *et al.* [31] have demonstrated that tyrosine kinase Abl-fused protein, as the main pathological initiator of CML, leads to higher ROS production and thereby to the disease aggravation and induction of genetic instability and resistance to first generation of TKIs. These disagreements imply necessity to explore the association between TKIs use and ROS production and its clinical significance.

Our results showed that the four examined TKIs exhibit prooxidative actions and the highest prooxidative potential are related to imatinib and nilotinib treatment, because elevated levels of AOPP could not be suppressed even after incubation with powerful antioxidative agent, such as Trolox (Fig. 1a). Determination of TOS led to the same conclusion, although partial influence of Trolox on this marker was registered (Fig. 1b). To further explore this subject, we measured PAB levels and the obtained results pointed again to prooxidative effects of imatinib and nilotinib (Fig. 1c). Findings of Ciarcia *et al.* [32] are in concordance with our study. These authors showed that the highest oxidative potential is carried by the second-generation TKIs, especially nilotinib, because it has stronger effect on ROS formation than imatinib. Additionally, authors proposed a mechanism of this action, which comprises the activation of

Ca-mediated apoptosis by ROS species [32]. Surprisingly, determination of PAB after incubation of samples with Trolox indicated that vitamin E analog, in parallel with nilotinib treatment failed to induce lowering of ROS. Moreover, prooxidative activity was even strengthened in concomitant presence of nilotinib and Trolox (Fig. 1c). Such findings once more confirm the complexity of these interactions, emphasizing the need to correctly define possible useful or harmful effects of antioxidant supplementation to a regular cytostatic therapy.

Determination of a chemical mechanism by which oxidative reactivity of different TKIs is achieved is challenging process which has to include the analyses of involved chemical structures and their possible interactions. It is known that imatinib's adverse effects originated from its iminoquinone structure. Many efforts were made in order to reduce negative effects which are developed as result of imatinib's molecular interactions with different organic compounds. Detrimental effects of imatinib's iminoquinone core on DNA could be a consequence of imatinib's capability to produce free radicals and to induce disbalance between origination of ROS and antioxidative protection system. Importance of redox status in imatinib's metabolism is emphasized by the fact that GSHs protective pathway enables elimination of iminoquinone derivatives. Our results showed that after Trolox addition, imatinib's prooxidative effects significantly declined. Based on these findings, we can assume that a competition of quinoid structures of vitamin E analog and imatinib can cause elimination of prooxidants after Trolox addition, due to its higher affinity for unpaired electrons of free radicals.

In contrast, Teppo *et al.* [29] suggested that not every TKI is related with higher levels of ROS production. Namely, it has been shown that few TKIs can be associated with higher levels of GSH, implying increased antioxidative defense [29]. Our findings are in disagreement with that hypothesis, given the observed reduction of SHG caused primarily by imatinib and crizotinib (Table 1, Fig. 1d). Additionally, such depletion of SHG can lead to induction of ROS-dependent apoptosis of malignant cells, implying enrollment of TKIs in decreasing tumor antioxidative defense. However, it should be noticed that ample of evidences indicate detrimental role of TKI-induced oxidative stress, not only on malignant cells, but also on healthy cells and tissues. Although the primary goal of cancer therapy is selective targeting, the toxic effects of TKIs on healthy cells are observed. Paech *et al.* [33] showed undesirable effects of different TKIs in different hepatocyte cell lines, implying that cytotoxicity of these medicines is associated with ROS formation. Additionally, the authors [33] demonstrated potent adverse effects of TKIs, because the impairment of vital cells function such as mitochondrial oxygen consumption and glycolysis, were noted. Several studies showed

the involvement of ROS in cardiotoxicity as well [34]. Although these effects are mainly associated with the dosage of administered drugs, the role of ROS in TKIs-mediated effects was once more stressed out. Thus, all available evidences suggest that subtle differences in the level of induced oxidative stress and variable response to applied antioxidants might be important for characterization of TKIs and direction of these drugs toward specific pathological condition and individual patients.

Our results also confirmed the antioxidative potential of Trolox because an elevation of TAS was observed after addition of vitamin E analog in samples containing all four drugs (Fig. 1e). According to these results, we can hypothesize that parallel use of vitamin E during TKIs treatment ensures antioxidative protection for healthy cells. On contrary, a study by Pédebosq *et al.* [35] demonstrated that vitamin E supplementation during TKIs therapy can be considered as a fault, because it is preferable target will be TKIs, and therefore, it could decrease cytostatic and cytotoxic effects of TKIs. Taken altogether, the decision about vitamin E usage and adequate dosage and timing during cytostatic therapy should be carefully contemplated, with an aim to ensure prooxidative activity of cytostatic medication, but to avoid that damage of healthy tissues due to increased levels of ROS.

The most interesting results of our study are related to PON1 activity. PON1 is an enzyme carried by high-density lipoprotein (HDL) particles and it is considered as the most potent antioxidant within HDL, whose activity is directed toward prevention of lipoprotein oxidation [36]. Studies investigating PON1 genetic polymorphisms were already conducted in patients with chronic myeloid and chronic lymphocytic leukemia finding R risk allele (PON1Q192R polymorphism) and the MM (PON1L55M polymorphism) genotype more frequent and consequently related to higher tendency for development of chronic lymphocytic leukemia [37]. Interestingly, our analysis showed the highest levels of PON1 activity in samples incubated with the most potent prooxidants (imatinib and nilotinib; Fig. 1f). We can hypothesize that these drugs may influence HDL particles functioning and PON1 antioxidative capacity, while another possibility is that the observed increase in PON1 activity occurs as a compensatory response to elevated ROS. These assumptions need further confirmations.

Finally, we were interested in comparison of overall effects of four examined drugs. In order to summarize all prooxidative and antioxidative effects, we opted for calculation of adequate scores [38,39]. Our results (Fig. 2) suggest that imatinib might be the most promising agent when considering oxidative damaging as potential cytotoxic mechanism. Interestingly, nilotinib, being an alternative to imatinib, had comparable damage score (Fig. 2a), but its protective score was significantly higher than the one attributed to imatinib, which could

be important when deciding which drug should be used (Fig. 2b). Moreover, our results demonstrated opposite effects of Trolox in combination with imatinib and nilotinib, once more emphasizing that the use of antioxidant in combination with cytostatic medicines should be carefully evaluated in each specific case.

Conclusion

Our study emphasized the significance of oxidative reactivity of different TKIs, in relation to complexity of their structures. Although enhancement in ROS production can be interpreted as a part of their pharmacological activity, it could also deteriorate the delicate prooxidative/antioxidative balance in healthy cells. Future research studies should be directed toward the examination of these complex interactions with particular reference to the role of vitamin E and its possible beneficial or harmful impact on activity of different TKIs. Our results suggest that additional investigations should be conducted in the field of TKIs drug structure – pharmacological action – reactivity in biological systems. Current findings should be further explored not only in serum pool as a substitute model but also in actual clinical settings. Also, the obtained results indicate the need for the estimation of malignant patients' redox status before, during and after the therapy in order to accurately assess the patients' response to TKIs therapy and to adjust the therapeutic approach.

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Conflicts of interest

There are no conflicts of interest.

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