Effect of sarcosine on antioxidant parameters and metallothionein content in the PC-3 prostate cancer cell line

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Abstract. Sarcosine is currently one of the most discussed markers of prostate cancer. It is involved in amino acid metabolism and methylation processes that occur during the progression of prostate cancer. In this study, we monitored the effect of the addition of sarcosine (0; 10; 250; 500; 1,000 and 1,500 μM) in a time-dependent manner (0-72 h) on the PC-3 prostate cancer cell line. For the assessment of cell viability, the commonly used MTT test was employed. Furthermore, ion-exchange liquid chromatography was used for the determination of sarcosine content in the PC-3 cells. We also determined metallothionein (MT) levels by chip capillary electrophoresis and Brdicka reaction in the cells treated with sarcosine. Sarcosine levels in the cells increased in a concentration-dependent manner levels increased from only 270 nM with the lowest applied concentration of sarcosine (10 μM) to 106 μM with the highest applied concentration of sarcosine (1,500 μM). There was a marginal change observed in the MT concentration. Finally, the antioxidant activity of the PC-3 cells was determined using five different spectrophotometric methods [2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing ability of plasma (FRAP), free radicals, *N,N*-dimethyl-*p*-phenylenediamine (DMPD) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS)]. A significant negative correlation was observed between DPPH and FRAP (r=-0.68 at P<0.001) and between DMPD and ABST (r=-0.64 at P<0.001). Additionally, as regards the correlation between MT and DPPH, a significant positive trend (r=0.62 at P<0.001) was observed.

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Introduction

Prostate cancer (PCa) is characterised as a non-coordinated proliferation of prostatic cells (1). However, the mechanisms behind tumour progression have not yet been elucidated, although the risk factors of cancer initiation have been defined (2). These include primary genetic predispositions, ethnicity, life style and age (3). Old age has been established as a significant risk factor for PCa (4,5). In addition to these factors, a family history of breast or PCa distinctly enhances PCa risk (6). In terms of ethnicity, a distinct gradient between Afro-Americans and Asians is evident (lower incidence in Asian populations) (7). Apart from these factors, androgens also play an important role in cancer development and progression. Therefore, PCa can be classified into either androgen-independent or androgen-dependent (8,9).

Currently, there is no complex test available for the diagnosis of PCa (10). Usually used tests include digital rectal examination, determination of prostate-specific antigen (PSA) levels (11) and transrectal sonography with a biopsy of prostate tissue. In specific cases, computed tomography (12), magnetic resonance (13) and positron emission tomography may be utilised (14). In this context, potential markers of PCa, which may be considered as a useful tool for earlier diagnosis without clinical examinations, are investigated. Currently, PSA, first described in 1977 (15), is the most perspective marker of PCa. However, it is used for diagnosis, for determining the stage of disease and for monitoring the treatment progression; however, its sensitivity (49-91%) and specificity (68-80%) are not sufficient to confirm diagnosis (16). Novel potential markers,

including alpha-methylacyl-CoA racemase (AMACR) (17), prostate cancer antigen 3 (PCA3) and Annexin A3 (18) have been identified. The most discussed marker of early-stage PCa is the amino acid, sarcosine as described by Sreekumar *et al* (19) (Fig. 1). In spite of the controversy in the scientific community and contradicting views on this marker, the role of sarcosine in methylation processes during cancer progression has been shown (19). A recent study demonstrated the effect of sarcosine on the increasing human epidermal growth factor receptor 2 (HER2/neu) expression levels (20). Therefore, it is important to investigate the function and involvement of sarcosine in PCa initiation and progression. The aim of this study was to investigate the effect of sarcosine on PC-3 PCa cells. PC-3 cells were treated with sarcosine at various concentrations (10; 250; 500; 1,000 and 1,500 μM). In addition, the antioxidant capacity of the PC-3 cells following treatment with sarcosine, as well as the metallothionein (MT) concentration were examined.

Materials and methods

Chemical and biochemical reagents. Ham's F12 medium, fetal bovine serum (FBS) (mycoplasma-free), penicillin/streptomycin and trypsin were purchased from PAA Laboratories GmbH (Pasching, Austria). PBS was purchased from Invitrogen Corp. (Carlsbad, CA, USA). Ethylenediaminetetraacetic acid (EDTA) and all other chemicals of ACS purity were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA), unless stated otherwise.

Cell culture conditions. In this study, the highly metastatic PC-3 prostate cancer cell line derived from bone metastasis was used. Cells were cultivated in Ham's F12 medium supplemented with 7% FBS and antibiotics (penicillin and streptomycin). Cells were cultivated in a MCO-18AIC incubator (Sanyo, Osaka, Japan) at 37˚C under 5% CO_2.

Sarcosine treatment of cell cultures. Immediately after the cells grew to 50-60% confluence, the cultivation medium was replaced by fresh medium to synchronise cell growth. Cells were cultivated for 24 h under these conditions. Subsequently, the culture medium was supplemented with sarcosine (N-methylglycine) diluted to a final concentration 10, 150, 250, 500, 1,000 and 1,500 μM. Treatment was carried out for 0, 6, 12, 24 and 72 h, and samples were collected at these strictly defined time points.

Cell content quantification. Total cell number was analysed using a semi-automated image-based cell analyser (CASY, Roche Innovatis, Basel, Switzerland) according to the manufacturer's instructions. The cultivation medium was removed and the samples were washed twice with 5 ml of ice-cold PBS to maintain only viable cells. Cells were scraped and transferred to clean tubes. Trypan blue solution (Roche Innovatis) was diluted to 0.2% prior to use and added to the samples. The following settings were used in the operating software: cell type, standard cells; dilution, none; process type, standard. All samples were measured in duplicate.

Light microscopy of treated cells. For light microscopy, cells were cultivated directly on glass microscope slides (75x25 mm, thickness 1 mm, Fischer Scientific, Pardubice, Czech Republic) in Petri dishes in the abovementioned cultivation medium as described in 'Cell culture conditions'. Cells were transferred directly onto slides, which were submerged in cultivation medium. Following treatment, the glass microscope slides with a monolayer of cells were removed from the Petri dishes, rinsed with cultivation medium without sarcosine supplementation and PBS buffer and directly used for light microscopy under an inverted microscope (Eclipse TS100; Nikon, Tokyo, Japan). Images were taken using a digital camera (Olympus Camedia C-750, Olympus).

MTT assay. To determine cell viability, MTT assay was carried out. MTT is yellow water-soluble stain (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) that is reduced by living cells to a non-soluble violet formazan precipitate. Cell suspension was pipetted to a microplate (TPP Techno Plastic Products AG, Trasadingen, Switzerland) according to the following scheme: 1st and 12th well with 200 μl medium and 2nd to 11th well with 200 μl cell suspension. The assay was carried out in duplicate. Furthermore, the cells were incubated for 24 h and the media were exchanged. Subsequently, the columns were fed with 200 μl of medium with 50 μl MTT [5 mg/ml in PBS (Invitrogen)] and incubated for 4 h, wrapped in aluminium foil. Subsequently, medium-MTT was exchanged with 200 μl of 99.9% DMSO to dissolve the MTT-formazan crystals. A total of 25 μl of glycine buffer was then added to the wells with DMSO. Plates were read at λ 570 nm (VersaMax Microplate Reader; Molecular Devices, Sunnyvale, CA, USA).

Ion-exchange chromatography. An AAA 400 liquid chromatography apparatus (Ingos, Prague, Czech Republic) was used for the determination of sarcosine concentration. The system consisted of a glassy filling chromatographic column and a steel precolumn, two chromatographic pumps for the transport of elution buffers and derivatization reagent, a cooled carousel for 25 test tubes of 1.5-2.0 ml volume, a dosing valve, a heat reactor, a Vis detector and a cooled chamber for the derivatization reagent. The glassy chromatographic column (i.d. 3.7 mm and 350 mm length) was filled with LG ANB strong catex in sodium cycle (Spolchemie, Ústí nad Labem, Czech Republic) with particles of average size of 12 μm and a netting of 8%. The glassy column was tempered by a thermostat at a temperature ranging from 35 to 95˚C. The precolumn was filled with LG KS0804 ionex (Ingos). Chromatographic columns for the transfer of elution buffers and derivatization reagent function at a flow of 0.01-10 ml/min under a maximum pressure of 40 MPa. The volume of the injected sample was 100 μl with an application accuracy RSD of ~1%. A two-channel Vis detector with a 5 μl flow volume cuvette was operated at wavelengths of 440 and 570 nm. Ninhydrin solution (Ingos) was used as the derivatization reagent. Ninhydrin was dissolved in solution containing 75% (v/v) of the organic solvent, methyl cellosolve (Ingos), and 25% (v/v) of 4 M acetate buffer (pH 5.5). $SnCl_2$ (Lachema, Brno, Czech Republic) was used as a reducing agent. The derivatization reagent was stored under an inert atmosphere (N_2) with cooling at 4˚C. During the analysis, the mobile phase flow was set at 0.3 ml/min under a pressure range of 4.5 to 6.0 MPa. The reactor temperature was set to 120˚C.

Differential pulse voltammetry for Brdicka reaction. Differential pulse voltammetric measurements were performed with a 747 VA Stand instrument connected to 693 VA Processor and 695 Autosampler (Metrohm, Herisau, Switzerland), using a standard cell with three electrodes and a cooled sample holder (4˚C) for the measurement of cells (Julabo F25; Julabo, Seelbach, Germany). A hanging mercury drop electrode (HMDE) with a drop area of 0.4 mm^2 was used as the working electrode. An Ag/AgCl/3M KCl electrode was the reference and a platinum electrode was the auxiliary electrode. For data processing, the VA Database 2.2 (Metrohm) was employed. The analysed samples were deoxygenated prior to the measurements by purging with argon (99.999%) saturated with water for 120 sec. Brdicka supporting electrolyte containing 1 mM $Co(NH_3)_6Cl_3$ and 1 M ammonia buffer [$NH_3(aq) + NH_4Cl$, pH 9.6] was used. The supporting electrolyte was exchanged after each analysis. The parameters of the measurement were as follows: initial potential of -0.7 V; end potential of -1.75 V; modulation time, 0.057 sec; time interval, 0.2 sec; step potential, 2 mV; modulation amplitude, -250 mV; E_{ads} 0 V; volume of injected sample, 25 μl; measurement of cell volume, 2 ml (25 μl of sample and 1,975 μl Brdicka solution).

Capillary electrophoresis-Experion system. Analyses on an automated microfluidic Experion electrophoresis system (Bio-Rad Laboratories, Hercules, CA, USA) were carried out according to the manufacturer's instructions with the supplied chemicals (Experion Pro260 Analysis kit; Bio-Rad Laboratories). A sample (4 μl) was mixed with 2 μl of reducing sample buffer (3.3% mercaptoethanol), and after 3 min of boiling, 84 μl of water were added. After the priming of the chip with the gel and gel-staining solution in the diluted priming station sample, the mixture (6 μl) was loaded into the sample wells. The Pro260 Ladder included in the kit was used as a standard. For operation and standard data analysis, Experion software version 3.10 (Bio-Rad Laboratories) was used.

Spectrophotometric analysis. For the determination of antioxidant activity, a BS-400 automated spectrophotometer (Mindray, Shenzhen, China) was used. It is composed of cuvette space tempered to 37±1˚C, reagent space with a carousel for reagents (tempered to 4±1˚C), sample space with a carousel for the preparation of samples and an optical detector. The transfer of samples and reagents was carried out by a robotic arm equipped with a dosing needle (error of dosage up to 5% of volume). Cuvette contents are mixed by an automatic mixer including a stirrer immediately after the addition of reagents or samples. Contamination is reduced due to its rinsing system, including rinsing of the dosing needle as well as the stirrer by Milli-Q water.

Determination of antioxidant activity by the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) test. The ABST test was carried out as previously described by Sochor *et al* (21,22). Briefly, a 150-μl volume of reagent (7 mM $ABTS^{\bullet}$ and 4.95 mM potassium peroxodisulfate) was incubated with 3 μl of sample. Absorbance was measured at λ 660 nm for 12 min. For the calculation of the antioxidant activity, values determined before the decrease of the absorbance (2nd minute of measurement - A_2) and the last measurement value (12th minute of measurement - A_{12}) were used. The resulting value was calculated in accordance with the following formula: differential absorbance $A = A_{12} - A_2$.

Determination of antioxidant activity by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test. The DPPH test was carried out as described by Sochor *et al* (21,22). Briefly, a 150-μl volume of reagent (0.095 mM $DPPH^{\bullet}$) was incubated with 15 μl of sample. Absorbance was measured at 505 nm for 10 min and the output ratio was achieved by the difference of absorbance at the 10th and 2nd minute of the assay procedure.

Determination of antioxidant activity by the N,N-dimethyl-p-phenylenediamine (DMPD) method. The DMPD test was carried out as previously described by Sochor *et al* (21,22). Briefly, a 160 μl volume of reagent (200 mM DMPD, 0.05 M $FeCl_3$, 0.1 M acetate buffer pH 5.25) was injected into a plastic cuvette with the subsequent addition of 4 μl of sample. Absorbance was measured at 505 nm. The difference between absorbance at the 10th and 2nd minute of the assay procedure was used for the calculation of the antioxidant activity.

Determination of antioxidant activity by the free radical method. The determination of antioxidant activity using the free radical method was carried out as previously described by Pohanka *et al* (23). Briefly, a 150 μl volume of reagent was injected into a plastic cuvette with the subsequent addition of a 6 μl of sample. Absorbance was measured at 450 nm in the 2nd minute of the assay and the 10th minute. The difference of the two absorbances was considered as an output value.

Determination of antioxidant activity by the ferric reducing ability of plasma (FRAP) method. The determination of antioxidant activity using the FRAP method was carried out as previously described by Sochor (21,22). Briefly, a 150 μl volume of reagent was injected into a plastic cuvette with the subsequent addition of 3 μl of sample. Absorbance was measured at 605 nm for 10 min. The difference between the absorbance at the final 10th minute and the 2nd minute of the assay procedure was used for the calculation of the antioxidant activity.

Statistical analysis. Software Statistica 10 (StatSoft, Tulsa, OK, USA) was used for statistical evaluation. T-tests were used to compare levels across groups and correlations were performed to reveal trends between variables. A P-value <0.05 was considered to indicate a statistically significant difference, unless stated otherwise.

Results and Discussion

Cell treatment and viability test. The PC-3 PCa cell line was derived from a metastatic site in the bone and represents a highly aggressive metastatic form of PCa. Compared to the widely used prostate cancer cell lines, DU145 and LNCaP, PC-3 is androgen-independent and does not express PSA (24,25). As mentioned in the Introduction, sarcosine is considered a tumour marker for the diagnosis of PCa. A schematic diagram of the role of sarcosine in the biochemistry of PCa cells adopted from a previous study (26) is shown in (Fig. 1).

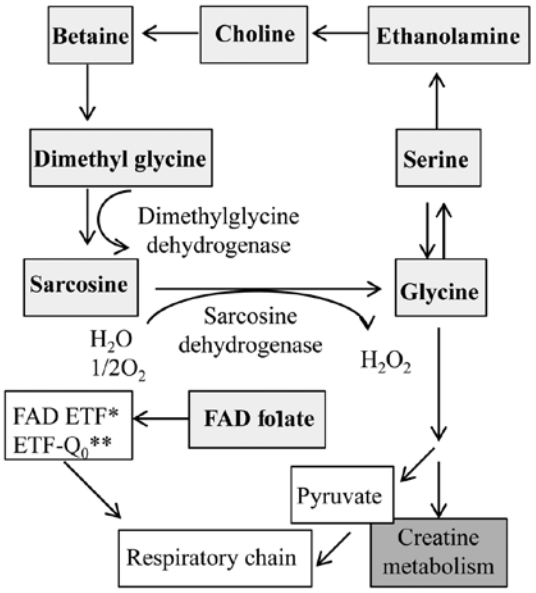

Figure 1. Sarcosine metabolism in the mitochondria. Formed FAD folate is involved in the respiratory chain and glycine is involved in creatine metabolism. *ETF, electron transfer flavoprotein; **ETF-Q_0, ubiquinone oxidoreductase (26).

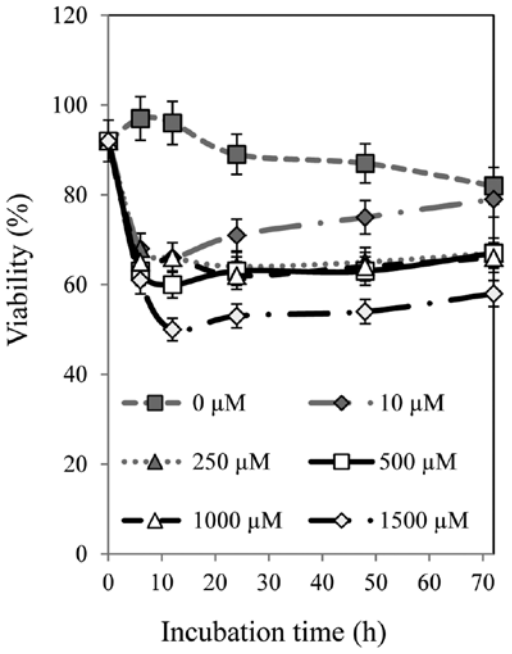

Figure 2. Viability of prostate cancer cells treated with various concentrations of sarcosine (0, 10, 100, 250, 500, 1,000 and 1,500 μM). Influence of selected markers, values are recalculated to determine viability. For measurements, the MTT assay was used as described in 'MTT assay'.

Therefore, the first experiments focused on the determination of cell viability and proliferation following treatment with sarcosine. The commonly used 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay was employed for these tests. For MTT assay, the cells were harvested and re-cultivated in a 96-well plate (5,000 cells/well). After 24 h of growth synchronization, the cells were treated with sarcosine at various concentrations ranging from 10 to 1,500 μM. The cells were further cultivated under these conditions, and samples were taken in the strictly defined time points (6, 12, 24, 48 and 72 h), when viability was evaluated. The results are shown in Fig. 2. Compared to the viability of the control cells (i.e., those not treated with sarcosine), the viability of the sarcosine-treated cells was significantly reduced. The determined IC_{50} value at all the time points was ~325.5 μM. In all the applied concentrations, cell viability was reduced by 30-40% after 10 h of treatment; subsequently, a moderate increase in cell viability (65-80%) compared to the initial viability values was recorded. As shown in Fig. 2, the increasing sarcosine concentration (10-1,500 μM) led to a reduction in cell viability (significance level $P<0.05$) during the first 6-24 h of treatment (34% on average). After 12 h, the decreasing trend in cell proliferation slowly increased compared to the untreated control cells, where the decrease was only moderate and was characteristic of the growth curve of the PC-3 PCa cell line. These results confirm the microscopic observations, where the toxic effect of sarcosine at a high concentration (1,500 μM) was evident. At this concentration, changes in cell morphology (loss of typical shape, formation of round cells and loss of adherence) were determined. At lower concentrations, sarcosine reduced cell viability, although non-significant cellular morphological changes were observed (data not shown).

Sarcosine determination. In the following experiment, both the culture medium and sarcosine-treated cells were analysed for sarcosine content by ion-exchange chromatography (27). In the sarcosine-treated cells, sarcosine content was recalculated to the percentage of viable cells. The sarcosine content in the cells significantly increased until 24 h of treatment at all concentrations (10-1,500 μM). However, after 24 h of treatment, only a moderate sarcosine content increase was recorded. The untreated cells showed a similar tendency compared to the treated cells. These results indicate the possibility of sarcosine biosynthesis by PC-3 cells (19). The obtained results indicated a contrary tendency compared to the sarcosine content in PC-3 cells, i.e., that the concentration of sarcosine in the culture medium increased at all concentrations (10-1,500 μM). This increase is characterised by the directional slopes from each applied concentration (Fig. 3). The most significant increase in sarcosine content was recorded at the highest applied sarcosine concentrations (500-1,500 μM). As regards statistical significance, a significant change in the sarcosine content was observed at the concentrations between 0-1,000 μM vs. 1,500 μM ($P=0.02$). On the other hand, the sarcosine content increased in the culture medium in the case of the untreated control cells.

Determination of MT levels. MT is considered as a possible marker of PCa (28-34). As certain studies have indicated, its levels are elevated in the blood serum of patients suffering from PCa, independent of their state of health (35,36). Chip capillary electrophoresis (Experion) was used for the determination of MT levels. The assumed molecular weight of MT varies from 6 to 15 kDa (37); however, this depends on the type of isoform and the rate of oxidation (38,39). From the Experion records, it is evident that PC-3 cells cultivated for 12 h synthesised MT with molecular weights of 11, 15

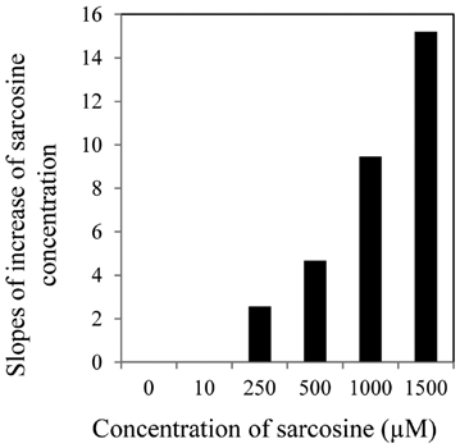

Figure 3. Slopes of increase of sarcosine concentrations (0; 10; 100; 250; 500; 1,000 and 1,500 μM) on the sarcosine concentrations determined in the culture medium.

and 19 kDa (see MT peaks, Fig. 4A). These results are also visible on virtual output (Fig. 4B). The height of these peaks increased depending on the applied sarcosine concentration up to 1,000 μM. On the other hand, a distinct increase in all three peaks was determined for the highest applied concentration of sarcosine (1,500 μM); however, this increase was below the level of statistical significance. This trend is shown in Fig. 4C. Furthermore, PC-3 cells affected by sarcosine were investigated electrochemically using the Brdicka reaction, which is a highly sensitive method for the determination of MT levels (40,41). As shown in Fig. 5A, the MT level was reduced with the highest applied sarcosine concentration (1,500 μM) in a time-dependent manner. This trend confirmed the results obtained by the chip capillary electrophoresis method. As regards the dependence of mentioned variables, we revealed a significant positive correlation between sarcosine and MT (r=0.41 at P=0.03, Fig. 5B). Moreover, no other significant dependencies were identified across variables, including markers of oxidative capacity.

Antioxidant capacity determination. A number of methods have been introduced for the determination of antioxidant activity in the field of chemical and biological analysis (23,42-44). The methods differ according the molecular mechanisms of the particular group of antioxidants (45-47). These mechanisms usually involve the quenching/trapping of the radicals; however, the strictly specific mechanisms of the majority of these antioxidants remain unclear. Therefore, the approaches for the determination of antioxidant capacity are based on various techniques with different chemical principles. In our study, for the determination of the antioxidant capacity of PC-3 cells, we used five different methods, DPPH, TEAC, FRAP, DMPD and free radicals.

The DPPH test is based on the ability of the stable 2,2-diphenyl-1-picrylhydrazyl free radical to react with hydrogen donors and it is still one of the most commonly used methods. In this test, a radical solution is decolourised after reduction with antioxidant (AH) or a radical ($R^{\bullet}$) in accordance with the following scheme: $DPPH^{\bullet} + AH \rightarrow DPPH\text{-}H + A^{\bullet}$, $DPPH^{\bullet} + R^{\bullet} \rightarrow DPPH\text{-}R$ (48). The ABTS radical method is based on the quenching of substances which acts as a hydrogen radical cation created as one electron oxidation of synthetic chromophore $ABTS^{\bullet}$ which is thus reduced and changes its colour, which is monitored as a decrease in absorbance at a preferable wavelength (49). The FRAP method is based on the principle of redox reaction using Fe(III) complexes which are colourless and following reduction, it generates violet-coloured products. DMPD radical cation ($DMPD^{\bullet+}$) is generated through a reaction between DMPD and potassium persulfate and is subsequently reduced in the presence of hydrogen-donating antioxidants, similar to the DPPH test. After the addition of a sample containing antioxidants, $DMPD^{\bullet+}$ radicals are scavenged and as a result of this scavenging, the coloured solution is decolourised (50). The FRAP method is based on the ability of chlorophyllin (the sodium-copper salt of chlorophyl) to accept and donate electrons with a stable change of maximum absorption. This effect is conditioned by an alkaline environment and the addition of a catalyst (21).

All methods were calibrated using the standard compound, Trolox. The obtained results were recalculated to the viable

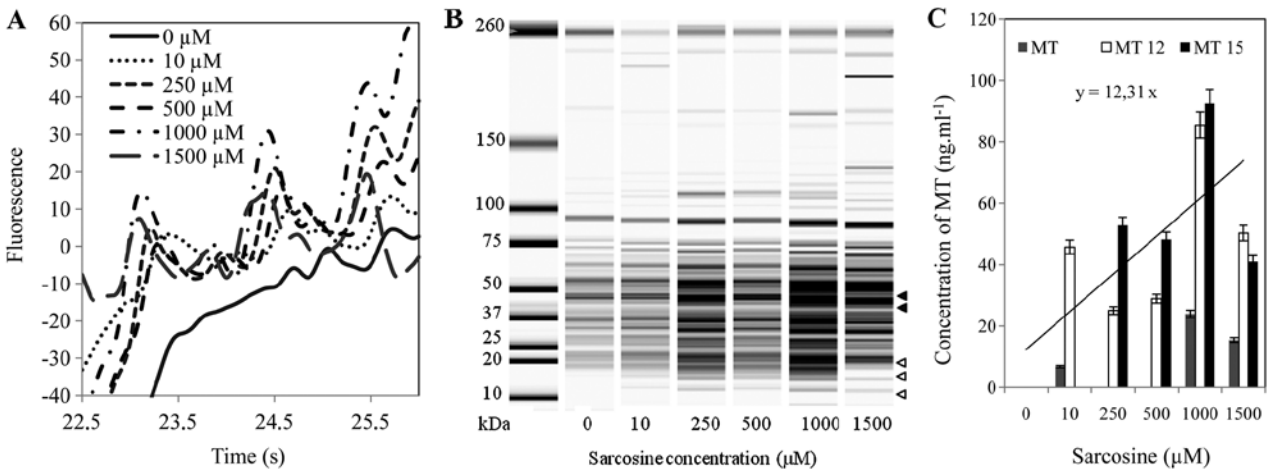

Figure 4. (A) Intensity of metallothionein (MT) peaks with the increasing sarcosine concentration. Capillary electrophoresis was used for all measurements as described in 'Capillary electrophoresis-Experion system'. (B) Virtual gel output. Arrows indicate further analysed peaks corresponding to molecular weight of 9.5 kDa labelled as MT, respectively. (C) Intensity of MT peaks with the increasing sarcosine concentration.

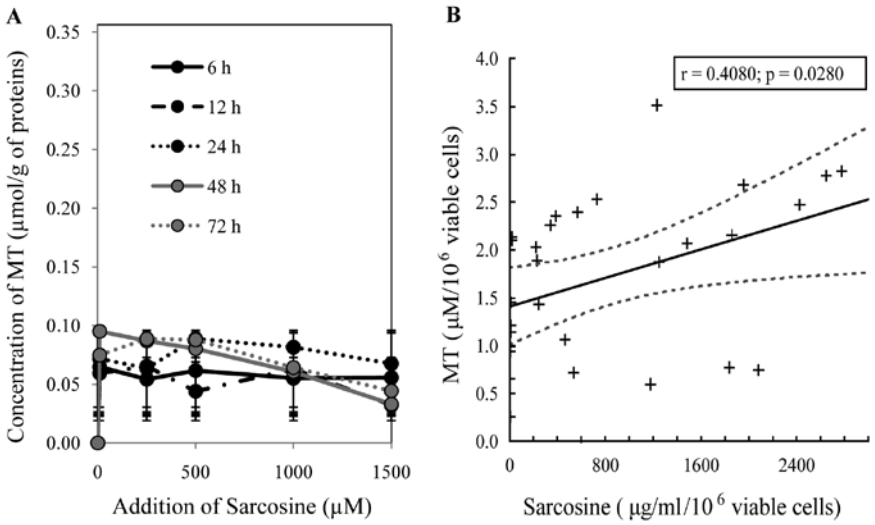

Figure 5. (A) Influence of sarcosine addition on metallothionein (MT) concentration (converted to whole protein concentration). (B) Correlation between sarcosine and MT values obtained by analysis of cells treated with applied concentrations of sarcosine (0, 10, 100, 250, 500, 1,000 and 1,500 μM) (r=0.41 at P=0.03).

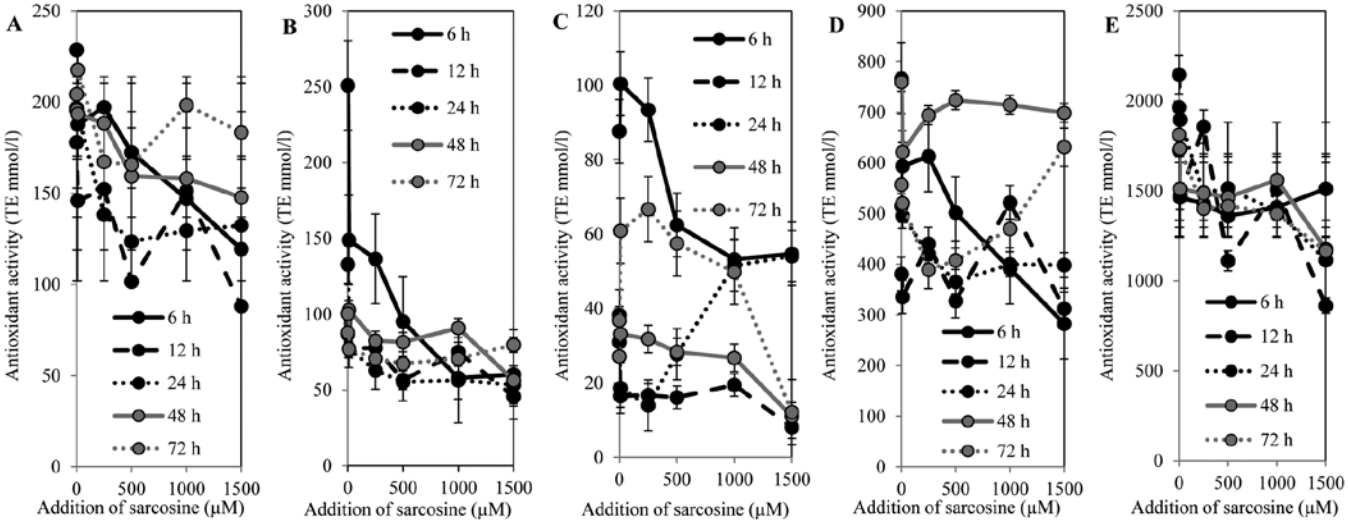

Figure 6. Influence of sarcosine treatment (0, 250, 500, 1,000 and 1,500 μM) on antioxidant capacity [expressed as Trolox equivalent (TE)]. Values were recalculated to determine cell viability. The spectrophotometric methods: (A) free radicals; (B) FRAP; (C) DPPH; (D) ABTS; (E) DMPD was used.

cells (% of viability) that were determined in as described above in 'Spectrophotometric analysis'. The antioxidant capacity determined by the free radical method (Fig. 6A) showed a decreasing tendency in a concentration-dependent manner within the time interval of 0 to 48 h. Subsequently, the antioxidant capacity increased in a time-dependent manner. The highest antioxidant capacity was determined after 72 h of sarcosine treatment in a concentration-dependent manner. However, the antioxidant capacity was reduced by 10% compared to the untreated control cells. The results obtained using the FRAP method correlated with the results obtained by the free radical method (Fig. 6B). A decrease in antioxidant capacity in a concentration-dependent manner was evident. The highest antioxidant capacity was determined in the cells treated with sarcosine for 6 h; subsequently, a decrease was recorded. After 48 h of incubation, an increase in antioxidant capacity in the PC-3 cells treated with high sarcosine concentrations (500-1,000 μM) was observed. On the other hand, the highest sarcosine concentration (1,500 μM) led to a significant reduction in antioxidant capacity. Different results were obtained after 72 h of treatment. The lower sarcosine concentrations (up to 500 μM) led to a reduction in antioxidant capacity, and the higher concentrations led to a significant increase in antioxidant capacity. The DPPH method confirmed the results obtained by the previous two methods (Fig. 6C). The most evident increase in antioxidant capacity was recorded in the untreated PC-3 cells and in the PC-3 cells treated with sarcosine in the concentration of 250 μM at the time points of 6, 12, 48 and 72 h. High sarcosine concentrations (500-1,500 μM) led to a significant reduction in antioxidant capacity. However, the obtained results indicate the role of the duration of the treatment. PC-3 cells incubated for 24 h showed an increasing tendency in antioxidant capacity in a concentration-dependent manner. This fact is particularly

evident in the PC-3 cells treated with 1,000 μM sarcosine. The ABTS method revealed similar results to those obtained by the DPPH method in both the untreated control (0 μM of sarcosine) and treated cells (Fig. 6D). On the other hand, the increasing antioxidant capacity with the increasing sarcosine concentrations in the treated cells for 48 h was evident. The DMPD method showed a decreasing tendency in antioxidant capacity with the increasing sarcosine concentrations within the time points of 12 to 72 h (Fig. 6E). On the other hand, the increase in antioxidant capacity is evident in the cells treated with sarcosine for 6 h. The results indicate the involvement of the compounds with antioxidant activity in the metabolism of the PC-3 cells following sarcosine treatment. The changes in antioxidant capacity demonstrate the rapid response to sarcosine treatment in a time-dependent manner. As regards the correlation of markers of oxidative capacity, we revealed a significant negative trend between DPPH and FRAP (r=-0.68 at P<0.001) and between DMPD and ABST (r=-0.64 at P<0.001). In addition, significant positive trends were observed only between MT and DPPH (r=0.62 at P<0.001).

In conclusion, non-invasive markers for PCa, through which it would be possible to diagnose PCa by urine analysis, are required. The non-protein amino acid, sarcosine, is one of the substances with potential for use in the diagnosis of PCa by urine analysis. However, the exact function of this amino acid in tumour cells is not yet fully understood. In this study, we attempted to cast light on the effects of various sarcosine doses on PC-3 PCa cells and discovered that this compound significantly influences various determined markers.

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