Differential Genomic Damage in Different Tumor Lines Induced by Prodigiosin

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Abstract. *Prodigiosin is a secondary metabolite produced by Serratia marcercens. As this pigment is suggested to be a cancer drug, genotoxicity studies are necessary. The aim of the present investigation was to evaluate the genotoxic effects of prodigiosin on tumoral and normal cell lines, NCIH-292, MCF-7 and HL-60. A normal line BGMK was used as control. Genomic damage induced by prodigiosin was observed in all tumor lines as well as the control line. The pigment induced the formation of micronuclei in tumor cells. The present data confirm the antitumor potential of prodigiosin. However, these findings also raise concerns regarding its target-specific action, as genotoxic effects on normal cells also occurred.*

Cancer therapies are mainly based on causing DNA damage in tumor cells, as well as the reduction in recognition and repair capacity of these cells. Many chemical compounds have been used in cancer therapy. However, many of these compounds have not yet had their genotoxic mechanisms, induction of cell damage DNA, fully elucidated. The early use of drugs without adequate evaluation can result in a change in the response to therapy, thereby compromising the survival of patients with cancer due to acute or chronic toxicity, as well as the risk of developing resistance to antitumor drugs (1). However, despite their associated severe side-effects, these synthetic drugs are often the only option for chemotherapy, even when they affect normal as well as cancer cells (2). Thus, efforts have been made to find compounds derived from natural products that are equally effective, but less toxic than synthetic products used in the treatment of cancer (3-5). In recent decades, large-scale studies have employed microorganisms, plants, animals and marine organisms in the search for natural antitumor drugs (3, 4).

The natural red pigment prodigiosin is synthesized by the enterobacterium *Serratia marcescens*. This pigment is chemically characterized as an alkaloid that has a linear, tri-pyrrole, flat chemical structure (pyrrole,3-methoxy pyrrole,2-methyl-3-amyl pyrrole) (6). Prodigiosin stands-out among other natural products derived from microorganisms due to its antitumor activity (7, 8). The flat structure of prodigiosin characterizes it as an intercalating agent in the DNA molecule. Intercalating agents can lead to structural changes in DNA and act mainly as inhibitors of topoisomerases I and II. This inhibitory effect can cause DNA damage, which is directly correlated to the level of cytotoxicity. The interaction between prodigiosin and DNA can therefore result in genomic damage, which characterizes this drug as a potential antitumor agent with selectivity (2, 9-12).

The aim of the present study was to determine possible differential genomic damage in tumor cell lines exposed to different concentrations of prodigiosin in comparison to a normal cell line in order to increase knowledge regarding the selective, antitumor effects of this natural product.

Materials and Methods

Cell lines and culture conditions. The following tumor lines were employed in this study: NCIH-292 (mucoepidermoid carcinoma of the lung), MCF-7 (breast adenocarcinoma) and HL-60 (human promyelocytic leukemia). A normal line extracted from monkey liver (BGMK) was used as the control. The Cell Culture and Pharmacological Assay Lab of the Department of Antibiotics of the Federal University of Pernambuco (Brazil) kindly donated all cell lines. The lines were cultivated (2×10^5 cells/ ml) in 25 cm^2 beakers (60 ml) for tissue culture containing Dulbecco's modified Eagle's minimal essential medium (DMEN; Sigma, São Paulo-SP, Brasil) supplemented with 10% fetal bovine serum (Gibco, São Paulo-SP, Brasil), 1% antibiotic solution (penicillin 1,000 UI/ml and streptomycin 250 mg/ml) and 1% L-glutamine (200 mM) and incubated at 37˚C in a humidified atmosphere containing 5% CO_2.

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Table I. *Genome damage indices (DI) and frequencies (DF) in tumor and normal (BGMK) lines after exposure to different concentrations of prodigiosin (1.7, 3.4 and 6.8 μg/ml) for 48 hours p/values refer to comparisons with respective controls of each line (χ^2 test).*

	Cell lines															
	HL-60				NCIH-279				MCF-7				BGMK			
μg/ml	DI	*p*-Value	DF	*p*-Value	DI	*p*-Value	DF	*p*-Value	DI	*p*-Value	DF	*p*-Value	DI	*p*-Value	DF	*p*-Value
0.0	8		7		12		10		9		8		8		6	
1.7	25	<0.001	18	<0.001	14	>0.05	11	>0.05	17	<0.001	13	>0.05	22	<0.001	18	<0.001
3.4	34	<0.001	23	<0.001	28	<0.001	19	<0.001	27	<0.001	20	<0.001	37	<0.001	27	<0.001
6.8	54	<0.001	34	<0.001	55	<0.001	31	<0.001	39	<0.001	26	<0.001	60	<0.001	40	<0.001

DI, Index of genomic damage; DF, Frequency of genomic damage. Accepted level of significance $p<0.05$.

Purification and characterization of prodigiosin. Prodigiosin was previously purified and characterized using UV-Vis spetrophotometry and gas chromatography–mass spectrophotometry, as described in previous studies by Lins *et al*. (13) and Lapenda *et al*. (14).

Genotoxicity assay of prodigiosin in tumor lines. The genotoxic effects of prodigiosin were evaluated using the comet and micronucleus assays, following the methods described by Singh, McCoy, Tice, Schneider (43), Tice *et al*. (15), with modifications, and Fenech *et al*. (16), respectively.

Treatment of cell cultures and exposure time. Approximately 2×10^5 cells/ml of the NCIH-292, Hep-2, MCF-7, HL-60 BGMK lines were cultivated in 24-well microplates (1 ml/well) and incubated at 37˚C in a humidified atmosphere containing 5% CO_2 for 24 hours. Prodigiosin solution was added at a concentration of 1.7, 3.4 or 6.8 μg/ml for each independent treatment for each cell line. The concentrations of prodigiosin employed for the analyses were calculation based on the previous determination of the IC_{50} according to Lapenda *et al*. (14) doses reduced to half (1.7 μg/ml) and doubled (6.8 μg/ml) were also tested. The medium was then aspirated. The cells were washed with 300 μl of a buffer solution (PBS, pH 7.2) and then treated with 300 μL of trypsin/EDTA for fifteen minutes. Next, 600 μl of DEMEN was added to inactivate the typsin, followed by aspiration of the samples, which were transferred to Eppendorf tubes for subsequent processing. Processing began with the samples being washed with 60 μl of buffer solution (PBS, pH 7.2) and centrifuged at 2,000 rpm for 20 min. After centrifugation, the culture medium was discarded and the cells were re-suspended with 60 μl of PBS. The cell solutions were subsequently used in the genotoxic assays.

Micronuclei assay. Two drops of cell suspension were transferred to cleaned and still wet previamante blades, performing a smear being held in a horizontal position until its drying at room temperature. After drying, the slides were fixed with absolute ethanol for 5 min and then washed in water and subjected to the staining using the Giemsa stain (Merck), which was deposited evenly over the blades, for average time of 5 min, and washed again with tap water dried at room temperature. After drying, the slides were taken to the optical microscopy (Microscope bilocular E200 (Prolab, São Paulo-SP, Brazil) for counting a total number of 10^3 cells with micronuclei.

Comet assay. Approximately 15 μl of cell suspension was homogenized in 100 μl of low melting-point agar previously heated in a waterbath to 37˚C. The homogenate was then transferred to glass slides lined with standard agarose, coverslips were placed on the slides which were then incubated for 10 min under refrigeration at 4˚C. The coverslips were then removed and the slides were immersed in a lysis solution for two hours under refrigeration at 4˚C. Next, the slides were submitted to electrophoresis (40 V for 20 min at 300 mA), immersed in a neutralization solution for 15 min and fixed in absolute ethanol for five minutes. After drying, the slides were stored in a refrigerator until staining. Staining was performed using Gel Red (Biotarget GelRed®, Lisboa, Lisboa, Portugal) wing 1 μl of dye homogenized in 1,000 μl of de-ionized sterile water. The slides were analyzed using fluorescence microscopy (Olympus – BX series, Prolab, São Paulo, Brasil). Cell counts were performed. Approximately 100 cells per treated individual were analyzed and scored from 0 to 4 points, depending on the degree of damage in the nucleoid.

Statistical analyses. Comparisons between the Damage Indices (DI), Damage Frequency (DF) and Frequency micronucleated cells (FMC) were performed using the chi-square test (χ^2), individually for treatment/tumor type and the same from the normal BGMK lineage. The significance level for the tests was 0.05. The Proporcioanlidade index (PI) of damage has been established in each treatment compared to their own controls (0.0 mg/ml of prodigiosin), but also in relation to the BGMK line coincident concentrations for all parameters used methodology.

Results

Nearly all cell lines (tumor and normal) exhibited significant differences in genomic damage, as demonstrated by the DIs and DFs determined by the comet assay, in relation to their respective controls (Table I). The exceptions were the NCIH-279 and MCF-7 tumor lines at a prodigiosin concentration of 1.7 μg/ml regarding the DI/DF and DF, respectively. In the comparison of DIs between the treatment and control lines, a statistically significant difference was only found for the MCF-7 line at a prodigiosin concentration of 6.8 μg/ml (Table

Table II. *Genomic damage indices for tumor and normal (BGMK) cell lines after exposure to and different concentrations (DI) of prodigiosin (1.7, 3.4 and 6.8 μg/ml) for 48 h and damage proportionality indices (PI) and p/values refer with the comparisons to control (BGMK) (χ^2 test).*

	Cell line									
	HL-60			NCIH-279			MCF-7			BGMK
μg/ml	DI	PI	*p*-Value	DI	PI	*p*-Value	DI	PI	*p*-Value	DI
0.0	8	1.0	>0.05	12	1.5	>0.05	9	1.1	>0.05	8
1.7	25	1.1	>0.05	14	1.5	0.1>*p*>0.05	17	1.3	0.1>*p*>0.05	22
3.4	34	1.1	>0.05	28	1.3	0.1>*p*>0.05	27	1.4	0.1>*p*>0.05	37
6.8	54	1.1	>0.05	55	1.1	>0.05	39	1.5	*p*<0.01*	60

DI, Index of genomic damage; DF, frequency of genomic damage; PI, index of proportionality damage. Accepted level of significance $p<0.05$.

Table III. *Genomic damage frequencies for tumor and normal (BGMK) cell lines after exposure to different concentrations (DI) of prodigiosin (1.7, 3.4 and 6.8 μg/ml) for 48 hours, damage proportionality indices (PI) and p/values refer withy the comparisons to control (BGMK) (X^2 test).*

μg/ml	Cell line									
	HL-60			NCIH-279			MCF-7			BGMK
	FD	PI	*p*	DF	PI	*p*	DF	PI	*p*	DF
0.0	7	1.2	>0.05	10	1.6	0.1>*p*>0.05	8	1.3	>0.05	6
1.7	18	1.0	>0.05	11	1.6	>0.05	13	1.4	0.1>*p*>0.05	18
3.4	23	1.2	>0.05	19	1.4	0.1>*p*>0.05	20	1.3	>0.05	27
6.8	34	1.2	>0.05	31	1.3	>0.05	26	1.5	0.1>*p*>0.05	40

DF (Index of genomic damage, DF (Frequency of genomic damage), PI (Index of proportionality damage), Accepted level of significance $p<0.05$.

Table IV. *Micronucleated cells (MC) / 1000 tumor and normal (BGMK) cells after exposure to different concentrations of prodigiosin (1.7, 3.4 and 6.8 μg/ml) for 48 h.*

	Cell line							
	BGMK		MCF-7		NCIH-279		HL-60	
μg/ml	MC	FMN	MC	FMN	MC	FMN	MC	FMN
0.0	4	0.0040	6	0.0060	4	0.0040	6	0.0060
1.7	12	0.0120	8	0.0120	17	0.0170	16	0.0160
3.4	21	0.0210	18	0.0210	29	0.0290	30	0.0300
6.8	38	0.0380	27	0.0380	40	0.0400	48	0.0480

MC, Micronuclei cells; FMN, frequency of cells micronuclei.

II). Regarding the DFs, no tumor line differed significantly from the control lines at any of the concentrations of prodigiosin evaluated (Table III).

Tables II and III display the PIs for each tumor line in relation to the control for both the DIs and DFs. All tumor lines exhibited a smaller proportion of damage in comparison to BGMK cells for both parameters at all concentrations of prodigiosin. The only exception was DI and DF regarding the HL60 line at a concentration of 1.7 μg/ml. Table IV shows the number of micronucleated cells and respective frequencies per cell line and treatment. Statistically significant differences in micronucleated cells were found for MCF-7 (6.8 μg/ml of prodigiosin), NCIH-279 (3.4 μg/ml) and HL60 (3.4 and 6.8 μg/ml) (Table V) cells. In the comparison of the PIs between the control concentration of prodigiosin (0.0 μg/ml) and the different treatments, as well as among treatments, regarding the three methodological parameters employed (Table VI), the highest indices were found for micronucleated cells.

Table V. *p-Values for comparisons of micronucleated cell frequency in different tumor cell lines in comparison with control (BGMK) (χ^2 test).*

Cell line	BGMK			
	Concentration (μg/ml)			
	0.0	1.7	3.4	6.8
MCF-7	$p>0.05$	$p>0.05$	$p>0.05$	$p<0.05$*
NCIH-279	$p>0.05$	$0.1>p>0.05$	$p<0.05$*	$p>0.05$
HL-60	$p>0.05$	$p>0.05$	$p<0.05$*	$p<0.001$*

*Significant values; **highly significant values; significance level: 0.05.

Discussion

Its selective cytotoxicity against cancer cells, p53-independent pro-apoptotic effect and its anti-metastatic activity place prodigiosin among those compounds with considerable potential as a cancer drug (2). However, clarifications regarding its molecular/cellular targets, as well as its effects at the cell and organism levels are needed for prodigiosin to be used as a therapeutic product with ensured efficacy and selectivity. Thus, the present results contribute to knowledge regarding this potential antitumor agent.

The analyses regarding the quantification of genomic damage induced by prodigiosin in different tumor cell lines demonstrated significant differences regarding the exposure of these cell lines to different concentrations of the drug. All lines except NCIH-329 with regard to the DI and DF and MCF-7 with regard to the DF at a prodigiosin concentration of 1.7 μg/ml, exhibited concentration-dependent genomic damage, with the largest effects occurring on treatment with 6.8 μg/ml of prodigiosin for each cell line. These effects were similar by the two methods employed. However, differential responses were found in some treatments specific to each line in comparison to the control line (BGMK) in both the comet assay and micronuclei test. DNA damage and its repair mechanism depend on the origin of the tumor (17). Thus, each line will be considered independently below.

HL60. Regarding the DIs for the degree of damage in the nucleoids analyzed during the comet assay, the normal cell line (BGMK) demonstrated approximately 10% greater genomic damage compared to the HL60 tumor cell line, as indicated by the damage PI (1.1), despite the lack of statistically significant differences at prodigiosin concentrations of 3.4 and 6.8 μg/ml. With regard to the DF, HL60 cells exhibited a lower frequency of damage (PI=1.2) at concentrations of 3.4 and 6.8 μg/ml in comparison to BGMK.

The lower DFs found for HL60 in comparison to the control cell line suggest a relationship with the resistance to genotoxic treatment triggered in these tumor cells. The oncogenic BCR/ABL tyrosine kinase plays two main complementary roles in cancer (17). First of all, BCR/ABL allows for cell proliferation in the absence of growth factors, protection against apoptosis in the absence of external survival factors, tissue invasion and metastasis (18). The second characteristic of BCR/ABL in malignant blood-related diseases concern its capacity to make cells resistant to genotoxic therapy (17, 19-23). This characteristic is due to the fact that BCR/ABL-positive cells can repair DNA damage more quickly, can promptly activate checkpoints of the cell cycle, thereby allowing greater DNA repair time and can activate cell protection mechanisms against pro-apoptotic signaling pathways that are normally activated by injury to DNA. Using the comet assay, Skorski (17), Majsterek and colleagues (24) and Slupianek and colleagues (23) found that genomic damage was repaired more efficiently in BCR/ABL-positive cells in comparison to control cells. Thus, the lower frequency of genomic damage evidenced by this assay in the present study at prodigiosin concentrations of 3.4 and 6.8 μg/ml on the HL60 blood tumor line in comparison to BGMK suggest this BCR/ABL action.

Although the DF was lower in the HL60 line in comparison to the control, the DI was indicative of damage of a greater magnitude (greater damage per cell), which could contribute to the effective action of the drug. This efficacy was also demonstrated by the greater frequency of micronucleated cells in the HL60 line. In the evaluation of macro-damage, as evidenced by the frequency of micronucleated cells, the HL60 line exhibited significant differences ($p<0.001$) in comparison to BGMK for prodigiosin concentrations of 3.4 and 6.8 μg/ml, with respective PIs of 1.4 and 1.7 regarding this parameter. However, this raises the question of how one can explain the greater incidence of micronucleated cells and higher DIs in the HL60 line in contradiction to the supposed induction of resistance to genotoxic therapy. According to Skorski (17), normal cells have strongly regulated DNA repair mechanisms associated with apoptotic pathways. Cells with excessive or irreparable damage that represents a threat to genomic stability are eliminated through these pathways. However, BCR/ABL-positive cells, such aleukemia cells, can accumulate more DNA damage following genotoxic therapy, thereby increasing the chance of harmful genetic errors, despite being more efficient at surviving genotoxic damage by modulating sensitivity to damage through the facilitation of DNA repair and the inhibition of the induction of apoptosis (23). As the reliability and efficiency of DNA repair mechanisms are not complete even in normal cells, the likelihood of BCR/ABL-positive cells accumulating DNA errors is greater due to the greater occurrence of damage and the impairment of DNA repair mechanisms (17). The greater genomic damage in these is cells is likely due to the increase in the formation of reactive oxygen species stemming from the mitochondria and NADPH

Table VI. *Proportionality indices (PI) between control prodigiosin concentration (0.0 μg/ml) and different treatments as well as among treatments regarding damage indices (DI), damage frequencies (DF) and number of micronucleated cells (MC).*

	Methodological parameters														
	DI					FD					MC				
		PIt					PIt							PIt	
		PIc					PIc					PIc			
μg/ml	ID	8	22	37	60	FD	6	18	27	40	MC	4	12	21	38
0.0	8	-	-	-	-	6	-	-	-	-	4	-	-	-	-
1.7	22	2.7	-	1.7	2.7	18	3.0	-	0.4	2.2	12	3.0	-	1.7	3.2
3.4	37	4.6	1.7	-	1.6	27	4.5	0.4	-	1.5	21	5.2	1.7	-	1.8
6.8	60	7.5	2.7	1.6	-	40	6.7	2.2	2.2-		38	9.5	3.2	1.8	-

PIt, Proportionality index among treatments; PIc, proportionality index in relation to control (0.0 μg/mL); MN, micronucleated cells.

oxidase induced by BCR/ABL (25, 26). Thus, prodigiosin administered at concentrations of 3.4 and 6.8 μg/ml in the present study to determine its effectiveness as an antitumor agent in the presence of promyelocytic leukemia (HL60) cells may cause greater genome damage in tumor cells, as demonstrated by the DIs reported above.

NCIH-279. Genomic damage in the NCIH-279 line was lower in comparison to the control at all concentrations of prodigiosin evaluated in terms of the DF, despite the non-significant differences found regarding the DI and DF at all concentrations. In micronucleus analysis, NCIH-279 demonstrated a greater frequency of micronucleated cells in comparison to BGMK at the prodigiosin concentration of 3.4 μg/ml. The non-significant difference at the highest concentration (6.8 μg/ml) was likely due to the considerable increase in micronucleated cells in the normal line in the presence of the drug. Based on these findings, the action of prodigiosin in lung tumor cells demonstrates greater damage per cell in this type of tumor rather than an increase in the number of damaged cells. Thus, higher concentrations of this drug are necessary, which can compromise normal cells, as demonstrated by the frequency of micronucleated cells in the normal line at a concentration of 6.8μg/ml.

The capacity to repair DNA damage in normal cells diminishes the risk of malignant transformation. However, malignant cells can repair DNA damage induced by genotoxic therapy, making these cells less sensitive to this type of cancer drug (1). Approximately 25 genes for repair of genotoxic damage have been described, along with their transcription factors (27). Changes in groups of genes can lead to an increase in genomic instability in the tumor or, contrarily, can lead to resistance to genotoxic therapy (17, 28-30). There is a considerable amount of evidence that perturbations in DNA-repair pathways are common in lung cancer, changing the resistance of the affected cells to a large number of chemotherapeutic drugs (28). The *BRCA1* gene is among the genes likely acting on resistance to genomic damage by chemotherapeutic drugs in lung cancer due to its functions in DNA repair, including repair through the excision of nucleotides and breaks in double-stranded DNA (29). Thus, the different DFs found in the present study for the NCIH-279 line, with lower values in the tumor cells, suggest the action of genes involved in resistance to genotoxic damage. The explanation suggested for the greater frequency of micronucleated cells in the leukemia cell line may also be applied to the NCIH-279 line.

MCF-7. Interestingly, the MCF-7 line had lower DI and DF in comparison to the control at all concentrations, with statistically significant differences at the concentration of 6.8 μg/ml regarding both the DI and frequency of micronuclei, with respective PIs ranging from 1.1 to 1.5 and 1.2 to 1.5, respectively. The lower DI and DF values indicate the main cause in the failure of chemotherapy for this type of cancer: multidrug resistance (31, 32).

Mechanisms of resistance to therapeutic agents are generally not the direct consequences of cellular malignant transformation, but may be the result of the selection of tumor cell clones that develop protective mechanisms, thereby diminishing the incidence of DNA damage through an increase in the metabolism or efflux of the drug (33-35). P-Glycoprotein, which is a product of the multidrug-resistance 1 gene, is among these possibilities. This glycoprotein serves as an ATP-dependent pump associated with the lower efficiency of chemotherapy in breast cancer (31). Thus, although prodigiosin is a potent inducer of DNA damage due to its flat molecular structure which enables intercalation in the DNA

molecule, the efflux mechanisms for the drug in normal mammary cells may contribute to its lower efficacy, as demonstrated by the DIs and DFs reported in the present study.

However, prodigiosin was capable of inducing significant damage at 6.8 μg/ml in terms of macro-injuries as demonstrated by the greater incidence of micronucleated cells in this tumor cell line in comparison to the control (BGMK). Breast tumor cells (MCF-7) may exhibit dysregulation in biological pathways, such as the cell cycle, DNA replication, DNA repair and activation of the p53 pathway (32), which involve changes in specific genes (36-40). This dysregulation may lead to genomic instability induced by genotoxic agents (17). Therefore, although MCF-7 cells have known resistance to chemotherapeutic treatment, the administration of prodigiosin at a concentration of 6.8 μg/ml can cause greater genomic damage in this cell line that cannot be repaired by the cell, as demonstrated herein by the greater frequency of micronuclei in the MCF-7 cells in comparison to the control cells (BGMK). Thus, the purpose of antitumor therapy through the use of prodigiosin can be achieved at this concentration to cause greater genomic damage in tumor cells and activate genome damage-induced apoptosis.

BGMK. The present findings demonstrate the effects on the normal cell line (BGMK) and raise questions regarding the actual selectivity of prodigiosin. For all parameters analyzed (DI, DF and micronuclei), the BGMK cells exhibited considerable genotoxic effects at all concentrations of prodigiosin in comparison to the control concentration, as demonstrated by the PIs, which ranged from 2.75 to 7.5 for the DI, 3.0 to 6.66 for the DF and 3.0 to 9.5 for micronucleated cell frequency. Among the treatments, the PIs also demonstrated higher dose-dependent values. In some treatments, the normal cells exhibited greater genomic damage in comparison to the tumor cell lines, as discussed above. These findings suggest that prodigiosin has a worrisome effect on normal cells, placing the organism as a whole at risk.

Antitumor therapies with genotoxic agents have been associated with the risk of toxicity. Genotoxic therapies are generally directed to cells that are active in the cell cycle, such as the majority of cancer cells. However, genotoxic agents can also affect, albeit to a lesser degree, all cells capable of dividing (1). The main concern regarding genotoxic therapy is the patient's quality of life, which can be affected during and following treatment with genotoxic agents, as some patients can become considerably ill, resulting in the suspension of treatment, and others may die as a result of the severe inhibition of the growth and function of normal cells (1). The greater DIs and frequencies, as well as the frequencies of micronucleated cells found in the BGMK line point to the harmful effects of prodigiosin on normal cells; its selectivity to tumor cells was not demonstrated by the present findings.

Another likely mechanism of action of prodigiosin that may have contributed to the effects found in all tumor cell lines evaluated in the present study concerns the re-activation of p53. Hong and colleagues found that prodigiosin can reactivate transcriptional activity dependent on the p53 family in colon tumor cells (41,42). According to the authors, the mechanism of action of prodigiosin occurs through restoration of p53 signaling in tumor cells with p53 hotspot mutations, with little or no detectable toxicity/genotoxicity in normal human fibroblasts. Prodigiosin induced the expression of p73 and interrupted its interaction with the mutant p53 protein, thereby saving the deficiency of the p53 pathway and promoting antitumor effects. The interruption of the mutant p53/p73 interaction was specific to prodigiosin. However, the controversial selective action of prodigiosin for the treatment of cancer found in the present study demonstrates the need for further studies to clarify the action pathways of this drug and ensure its administration in genotoxic antitumor therapy.

Conclusion

Prodigiosin induced genomic damage in the different tumor lines analyzed, thereby demonstrating the potential antitumor action of this natural compound. However, the findings also raise concerns regarding its target-specific action, as genotoxic effects on normal cells also occurred. Thus, further studies are required to gain a better understanding of the action of prodigiosin in possible target cells and potentiate the use of this drug in antitumor therapy.

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