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THE ROLE OF GLUTATHIONE IN PROTECTION AGAINST DNA DAMAGE INDUCED BY RIFAMYCIN SV AND COPPER(II) IONS

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Incubation of calf thymus DNA in the presence of rifamycin SV induces a decrease in the absorbance of DNA at 260 nm. The effect, was found to be proportional to the antibiotic concentration and enhanced by copper(II) ions. In the presence of rifamycin SV and copper(II), a significant increase in thiobarbituric acid-reactive (TBA-reactive) material is also observed. This effect is inhibited to different degrees by the following antioxidants: catalase 77%; thiourea 72%; glutathione (GSH) 62%; ethanol 52%; and DMSO 34%, suggesting that both hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH·) are involved in DNA damage. Rifamycin SV-copper(II) mixtures were also found to induce the production of peroxidation material from deoxyribose and, in this case, glutathione and ethanol were the most effective antioxidant substrates with inhibition rates of 91% and 88% respectively.

Electrophoretic studies show that calf thymus DNA becomes damaged after 20 min. incubation in the presence of both agents together and that the damaged fragments run with migration rates similar to those obtained by the metal chelating agent 1,10-phenanthroline. Normal DNA electrophoretic pattern was found to be preserved by catalase, and GSH at physiological concentrations and by thiourea. No protection is observed in the presence of ethanol or DMSO. The results obtained indicate the involvement of different reactive species in the degradation process of DNA due to rifamycin SV-copper(II) complex and emphasize the role of reduced glutathione as an oxygen free radical scavenger.

KEY WORDS: Rifamycin, copper(II), glutathione, DNA damage, oxygen free radicals.
Abbreviations used: TBA, thiobarbituric acid; TCA, trichloroacetic acid; SOD, superoxide dismutase; DMSO, dimethylsulfoxide; GSH, reduced glutathione.

INTRODUCTION

Quinone containing antibiotics can be activated to free radical semiquinones which either react directly with biological targets molecules such as DNA and RNA or generate the cytotoxic oxygen dependent superoxide and hydroxyl radicals[1].

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Formation of these reactive species has been proposed as the basis for both their antitumour activity and for their cytotoxic secondary effects[1-4].

Rifamycin SV is a lipophilic antibiotic containing a naphthohydroquinone ring and an aliphatic bridge, the latter being responsible for the binding to RNA polymerase and the bacterial DNA-dependent RNA synthesis inhibition[5]. The hydroquinone moiety (QH_2) of rifamycin SV reacts with molecular oxygen to form oxygen intermediates such as superoxide ($O_2 \cdot ^-$), hydrogen peroxide (H_2O_2) and the semiquinone ($QH \cdot$) drug as the oxidation products[6]. Some of the biological effects of rifamycin SV are known to resemble those of the metal chelating agent 1,10-phenanthroline[7] and recent interest in the drug has centered around its reported antiviral[8], anti-inflammatory[9] and immunosupressive properties[10]. It has been proposed that rifamycin SV might have antitumour properties based on the observed inhibition of ascitic Walker 256 carcinosarcoma and of the DNA dependent polymerase of human acute leukemia cells[5].

Also both the rifamycins and tetracyclines have some similarities in producing oxygen radicals, degrading DNA "*in vitro*" as well as modifying inflammatory reactions "*in vivo*"[11]. Recently, it has been shown that rifamycin SV in the presence of copper(II) ions can substantially degrade the DNA molecule and to release fragments containing aldehydic groups[12]. Highly reactive hydroxyl radicals capable of degrading deoxyribose are also formed. The effect was found to be inhibited by catalase and scavengers of $OH \cdot$ radicals such as manitol, formate and thiourea[6].

The damage to DNA was significantly inhibited by catalase but poorly inhibited by scavengers of hydroxyl radicals, emphasizing the importance of H_2O_2 formation and a site-specific formation of hydroxyl radical taking place during DNA damage[12].

Recently, we have shown that incubation of isolated rat hepatocytes in the presence of rifamycin SV and copper(II) results in metabolic as well as in viability alterations of the liver cells. These effects could be partially prevented by catalase while GSH levels revealed a critical threshold in cell susceptibility towards the xenobiotic induced toxicity[13].

The role of low molecular weight thiols such as GSH against free radical induced toxicity represent a research area of growing interest[14]. Radioprotective and free radical scavenger properties of thiol groups have been explained by their ability to interact with hydroxyl ($OH \cdot$) and superoxide ($O_2 \cdot ^-$) radicals and to repair damaged molecules by hydrogen donation to free radical centres formed in DNA[15-18].

It has been suggested that GSH may play an important role repairing DNA and may be one of the substances responsible for the observed increase radioresistance of cells when they are in close contact[19]. There is also ample evidence suggesting that the intracellular sulphydryl group content can influence cell response to antitumour drugs[20] and could be decisive in some chemotherapeutically induced cytotoxicities[21-24].

Consequently, the fact that DNA represents an important target molecule of the reactive species generated during oxidative stress and that GSH plays an important role against free radical-dependent toxicity, prompted the present investigation. The characteristics of the oxidative damage of DNA induced by rifamycin SV and copper(II) and the protection achieved by GSH were investigated.

MATERIALS AND METHODS

Rifamycin SV sodium salt, catalase (bovine liver, thymol free), superoxide dismutase (bovine erythrocyte), DNA (calf thymus, type I), DNA (E. coli, type VIII) and EDTA sodium salt were from the Sigma Chemical Comp. (U.S.A.). Pure grade agarose was from Bio-Rad, England. Thiobarbituric acid, hydrogen peroxide, 1-butanol and reduced glutathione (GSH) were from Merck (Darmstadt, Germany). DNA molecular weight marker was from Boehringer Mannheim (Germany). Trichloroacetic acid was from Panreack, Barcelona (Spain). Metal salts and other chemicals of the highest purity available were obtained from BDH (England). 1,10-phenanthroline was a kind gift from Dr J.M.C. Gutteridge (England).

Substrate solutions were prepared with sterile deionized distilled water (Ibys, Spain) just before use, except for DNA which was dissolved and kept at 4°C for 24 h. GSH, catalase and superoxide dismutase were dissolved in 100 mM sodium phosphate buffer pH 7.4 used for the incubation mixture. Buffer solutions were treated by passing through Chelex 100 resin 100–200, sodium form pre-filled Poly-Prep columns from Bio-Rad (California, USA) The pH of the treated buffers was then adjusted.

Degradation of DNA and Deoxyribose

DNA degradation by rifamycin SV and transition metal ions was studied by the change in optical density at 260 nm, the production of thiobarbituric acid-reactive products, and by the electrophoretic separation of damaged DNA after its incubation under the experimental conditions described below. Degradation of deoxyribose was followed by the release of thiobarbituric acid reactive products measured by spectrophotometry at 532 nm against appropriate blanks as previously described[25].

Incubation mixtures were prepared in new clean plastic disposable tubes and maintained in a shaking water bath at 37°C for 2 hours. Substrates were added in the following order: 400 μl phosphate/saline buffer (100 mM sodium phosphate containing 150 mM NaCl) pH 7.4, 200 μl DNA (1 mg/ml) or 200 μl deoxyribose (10 mM) in distilled water, 200 μl of rifamycin SV (1 mg/ml) and, where indicated, 200 μl of proteins or scavengers. The final volume of the reaction mixture was 1 ml, each tube containing equivalent amounts of buffer and water. Reactions were always started by the addition of rifamycin SV in the presence or absence of copper(II) ions.

Spectrophotometric Investigation of Rifamycin-DNA Interaction and Degradation

DNA absorbance spectra were recorded on a Kontron Uvikon 860 using 1 cm pathiength quartz cuvettes. Reaction mixtures of 1 ml final volume contained in the following order: sodium phosphate buffer pH 7.4, as above, calf thymus DNA (1 mg/ml) and rifamycin SV (1 mg/ml) at the final concentration indicated in Table 1. At the end of the incubation, the reactions were terminated by the addition of EDTA (1 mM) and catalase to a final concentration of 0.06 mg/ml. Tubes were transferred to an ice bath and the DNA was precipitated from solution with NaCl (0.2 M) and ethanol. The pellets were dissolved in sterile distilled water. Aliquots of the resulting mixture were scanned at room temperature from 500 to 180 nm using

TABLE 1
Concentration course effect of rifamycin SV on DNA absorbance at 260 nm

	A 260 nm	%
Control DNA 20 μg	0,309 ± 0,02 (8)	100
+Rifamycin SV 20 [μM]	0,275 ± 0,01 (4)*	89
40	0,251 ± 0,02 (4)**	81
80	0,238 ± 0,03 (7)**	77
120	0,192 ± 0,03 (5)**	62

Concentration-course effect of rifamycin SV on DNA absorbance at 260 nm. Samples of 20 μg/ml of DNA were added to a final volume of 1 ml of phosphate buffer, pH 7.4 alone or in the presence of increasing concentrations of rifamycin SV. Reaction mixtures were scanned against respective blanks from 500 to 180 nm. DNA absorbance units were calculated from the changes of the curve peaks at 260 nm. Results are means ±SD with the number of observations in parenthesis. *$p < 0.05$ **$p < 0.005$.

scan speed of 100 nm/min. Samples scanning was recorded against appropriate blanks containing all the substrates above except for DNA. Absorbance changes were calculated by the decrease in the absorption at 260 nm.

TBA-reactivity Assay

The release of TBA-reactivity as an indication of DNA or deoxyribose degradation, was measured following the procedure described by Quinian and Gutteridge[12]. 1 ml of 1% w/v in 50 mM NaOH of thiobarbituric acid followed by 1 ml of 28% (w/v) trichloroacetic acid (TCA) were added to each tube and mixed. For deoxyribose degradation studies, the addition of TBA was followed by 1 ml of 2.8% (w/v) TCA. For colour development, the mixture was transferred to glass tubes and heated for 15 min. at 100°C and then left to cool. The resulting chromogen was extracted into 1.5 ml of butanol-1-ol by mixing for 2 min. The resulting phases were separated by centrifugation for 15 min. at 2000 r.p.m. The clear organic phase was used for the spectrofluorimetric measurements performed in a Perkin Elmer MPF 44 Spectrofluorimeter at an emission of 553 nm followed by excitation at 532 nm. Fluorescent units were expressed as Relative Fluorescence Intensity (RFI) to a standard of rhodamine B (3 μM) as previously described[26].

Oxygen consumption during rifamycin SV oxidation

Oxygen uptake was measured using a Gilson Oxygraph K-ICC (Middleton, USA). The electrode was calibrated with a 95% saturated air which is equal to 0.95 μmoles of oxygen/ml. The final volume in the reaction vessel was 1.8 ml of phosphate/saline buffer containing rifamycin SV (140 μM) and copper(II) (100 μM) in the presence or absence of either catalase (0,06 mg/ml) or SOD (0,06 mg/ml). Readings were taken from the point of rifamycin SV or metal addition after electrode stabilization.

Electrophoretic separation of damaged DNA

The electrophoretic separation of damaged DNA was performed after incubation of E. coli or calf thymus DNA in the presence of rifamycin SV and copper(II). Incubations were also carried out in the presence of proteins or scavengers at the concentrations indicated in appropriate figures. To induce the degradation of DNA by

1,10-phenanthroline, the experimental conditions described by Gutteridge[27] were adopted. After incubation, 50 μl of each DNA test solution were pre-mixed with 10 μl of a buffered solution containing Ficoll 20%, xylencianol 0.1% and bromophenol blue 0.1%. The resulting mixture was loaded into the wells cut into the agarose gel (0.65% w/v) prepared in 100 mM sodium phosphate buffer, pH 7.8, containing EDTA (5 mM). Gels were run in the above buffer for 8 hr. at 120 mA and afterwards stained in a solution of ethidium bromide (1 μg/ml running buffer). The DNA bands were viewed under uv light and photographed with a Polaroid camera.

RESULTS

Table 1 shows the effect of rifamycin SV on DNA absorbance. A decrease in the absorbance at 260 nm in the presence of increasing concentrations of rifamycin SV alone is observed. The effect is not significant at low concentration (20 μM) while at high concentrations (120 μM) the absorbance decrease is of 38%. In the presence of 100 μM copper(II) the effect on DNA absorbance is significantly enhanced. A decrease of 56% is observed when compared with the value of DNA alone (Figure 1).

Oxygen consumption during rifamycin SV oxidation shows a stimulatory effect by copper(II) and copper/zinc superoxide dismutase and an inhibitory effect by catalase (Table 2). The effect of catalase supports a role for H_2O_2 as a product of rifamycin SV oxidation. However, the presence of other reactive species such as $O_2 \cdot^-$ and OH· cannot be ruled out. A similar effect by both antioxidant proteins

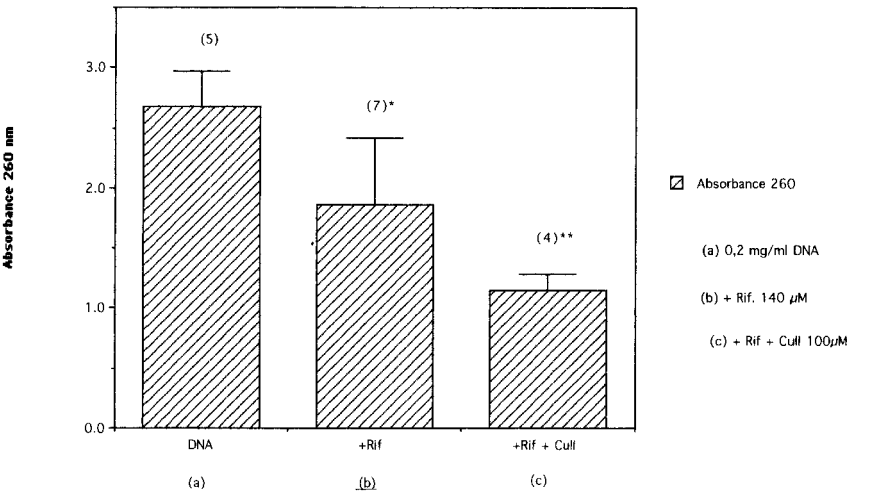

FIGURE 1 Effect of rifamycin SV and copper(II) ions on DNA absorbance at 260 nm. Decrease of DNA absorbance after its incubation in the presence of rifamycin SV and copper(II) ions at 37°C. All samples contained 0.2 ml of calf thymus DNA in phosphate buffer pH 7.4 as previously described in materials and methods section. Diagrams represent the means and SD of absorbance units recorded at 260 nm from (a) DNA alone, (b) DNA plus rifamycin SV 140 μM and (c) DNA plus rifamycin SV plus copper(II) 100 μM. Number of experiments are given in parenthesis *$P < 0.025$ as compared with value in (a). **$P < 0.05$ as compared with value in (b).

was observed when rifamycin SV oxidation was followed by the time-dependent decrease of the absorbance at 525 nm (results not shown).

Rifamycin SV and copper(II) induced damage to DNA and deoxyribose as shown by the release of TBA-reactive material (Tables 3 and 4). Although DNA in the presence of rifamycin SV was able to induce the production of a significant amount of TBA-reactive product, this amount was extremely enhanced when rifamycin SV and copper(II) were added together to the incubation medium. The release of TBA-reactive material was strongly inhibited (77%) by catalase. SOD or heat-denaturated catalase were not able to protect against DNA damage. On the other hand the inhibition induced by GSH, thiourea or ethanol was of 62%, 72% and 52% respectively while desferroxiamine and DMSO were without effect (Table 3). This suggests that in addition to H_2O_2, other reaction mechanisms leading to the formation of OH· radicals may take place during the degradation of DNA by rifamycin SV and copper(II).

When deoxyribose was incubated instead of DNA a significant increase of the TBA-reactive material measured at 532 nm was observed (Table 4). According to

TABLE 2
Effect of catalase and SOD on oxygen consumption during copper(II) catalyzed rifamycin SV oxidation

	μmoles O_2/min.	%	
Control Rifamycin SV	0,032	% increase	% inhibition
+ Copper(II) ions	0,075	+134	–
+ Copper(II) ions + Catalase	0,040	+25	81
+ Copper(II) ions + SOD	0.088	+175	–

The final volume of the incubation mixture was 1.8 ml containing phosphate buffer 100 mM-NaCl 150 mM pH 7.4, rifamycin SV 140 μM, copper sulphate 100 μM, and catalase or SOD 0,06 mg/ml. After rifamycin SV addition, the electrode was allowed to stablise and the reaction started by the presence of copper(II) ions. Percentage inhibition is refered to the effect of rifamycin SV + copper(II). (Mean values of more than four separate experiments are shown).

TABLE 3
Effect of antioxidants on DNA damage by rifamycin SV and copper(II) ions

Sample composition:	TBA-reactivity RFI Units	
DNA (0,2 mg/ml)	95 ± 16 (11)	
+ rifamycin (140 μM) (Blank)	242 ± 54 (12)	
+ rifamycin and Cu(II) (100 μM) (Control)	1544 ±175 (6)	% of Control Inhibition
Control + Catalase (0.06 mg/ml)	323 ± 89 (13)	77
Control + Catalase (heat denaturated)	1289 ±200 (4)	16
Control + SOD (0.06 mg/ml)	1365 ±178 (9)	12
Control + Catalase + SOD (0.06 mg/ml) each	567 ±106 (7)	63
Control + GSH (5 mM)	580 ± 93 (9)	62
Control + Desferroxiamine (1,5 mM)	1049 ±119 (9)	32
Control + Thiourea (50 mM)	439 ±181 (8)	72
Control + Ethanol (50 mM)	739 ±181 (8)	52
Control + DMSO (10 mM)	1018 ±132 (9)	34

Incubation conditions were as described in the materials and methods section. RFI = Relative fluorescence intensity units to a standard solution of rhodamine B, 0.3 mM. Results are means ± SD with the number of observations in parenthesis. % of control inhibition was calculated after substruction of the blank value.

TABLE 4
Degradation of deoxyribose by rifamycin SV and copper(II) ions and the effect of inhibitors

Sample composition	TBA reactivity A 532 nm	% of control inhibition
Deoxyribose (10 mM)	0,48 ± 0,06 (6)	
Deoxyribose + rifamycin (140 μM)(blank)	0,63 ± 0,08 (5)	
Deoxyribose + rifamycin + Copper(II) ions (100 μM) (Control)	1,63 ± 0,14 (4)	
Control + DMSO (10 mM)	1,36 ± 0,26 (5)	28
Control + thiourea (50 mM)	1,13 ± 0,31 (5)	53
Control + GSH (5 mM)	0,72 ● 0,05 (6)	91
Control + ethanol (50 mM)	0,75 ± 0,04 (5)	88

Final concentrations of substrates in reaction mixtures are shown. Results are mean ± SD with the number of experiments in parenthesis. The percentage of inhibition was calculated after substrating the blank.

previous investigations[12], rifamycin SV-copper(II) dependent degradation of deoxyribose can be effectively inhibited by catalase and partially by SOD and albumin, the latter effect being attribuited to non-specific binding properties of the proteins. Here, the antioxidant effects of some of the well known hydroxyl radical scavengers are shown and compared with the effect of GSH. As can be observed in Table 4, GSH and ethanol were the most effective scavengers in the prevention of deoxyribose degradation induced by the metal-antibiotic complex, with inhibitory effects of 91% and 88% respectively. However, under our incubation conditions the protection achieved by 50 mM thiourea was less than previously reported[12], while the lowest inhibitory effect was observed in the presence of DMSO.

Damage to calf thymus DNA by rifamycin SV and copper(II) was also followed by gel electrophoresis assays in which the effect of 1,10-phenanthroline-copper and 2-mercaptoethanol was used for comparison (Figures 2, 3 and 4). In the presence of reducing agents including ascorbate, thiols or 2-mercaptoethanol, the phenanthroline-copper(II) complex is known to produce highly reactive oxygen species such as $O_2 \cdot^-$ and OH· responsible for the degradation of the deoxyribose moiety of DNA. This mechanism has been proposed as a simple model of DNA scission produced "*in vivo*" by several antitumour antibiotics which require metal ion for their action[26]. Figure 2 shows a time-course experiment of the effect of rifamycin SV and copper(II) on DNA visualized by the electrophoretic separation of its damaged fragments. It can be seen that a significant change of the size, as compared with control DNA, is detected after 20 min. of incubation and that the effect increases progressively with time. After 1 hour incubation, DNA damaged fragments of about 2 to 1 Kb size are clearly separated. The resulting fragments resemble in size those obtained by the phenanthroline-copper(II) and 2-mercaptoethanol complex (Figure 4).

The effect of antioxidant enzymes and OH· radical scavengers was also investigated. Catalase but not SOD prevented the degradation of DNA, as observed with the release of TBA-reactive products (Figure 3).

A concentration-course effect study of the prevention by GSH of DNA damage is shown in Figure 4. The inhibition achieved by GSH is observed at concentrations above 0.4 mM and is maximum at 1 mM, although partial inhibition can be detected at a GSH concentration of 0.2 mM. GSH was therefore able to protect DNA from damage in a similar way as other hydroxyl radicals scavengers such as thiourea[28] and DMSO[29,30].

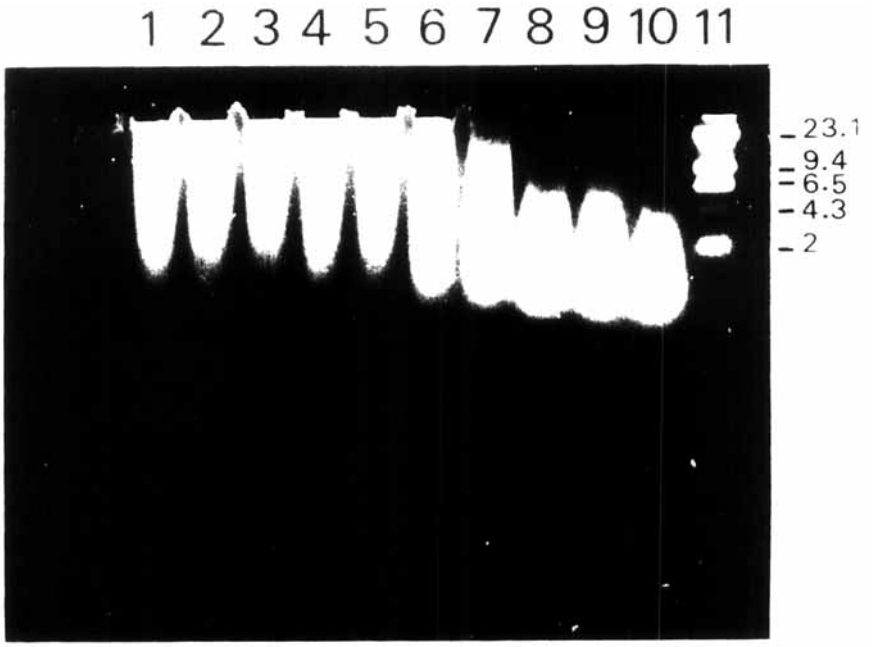

FIGURE 2 Time-course effect of the damaging effect of Rifamycin and copper(II) ions on DNA. The gel shows from left to right: (1) DNA (0,2 mg/ml) alone not incubated. (2) DNA + rifamycin SV + copper(II) ions, not incubated. (3) DNA alone incubated 60 min. (4) to (10) DNA + rifamycin SV + Copper incubated for 30 s., 1, 5, 10, 20, 30 and 60 min. respectively. (11) DNA molecular weight marker lambda HindIII.

DISCUSSION

The degradation of DNA induced by rifamycin SV and copper(II) has been investigated by means of the decrease of absorbance at 260 nm, the release of TBA-reactive material from the entire molecule and from its deoxyribose moiety and by the electrophoretic separation of damaged DNA fragments.

The results obtained indicate that the mechanism of DNA damage induced by rifamycin SV and copper(II) shares some characteristics with other well known free radical producing mixtures which have clearly defined DNA cleavage properties. The similarities between rifamacyn SV and 1,10-phenanthroline in degrading DNA as assessed by different assay systems were previously reported[12,26]. It is known that the inhibitory characteristics of rifamycin SV towards the initiation of RNA synthesis resemble those of the metal chelating agent[7]. Several quinone containing antitumour antibiotics are able to produce DNA damage by interacting and/or free radical release during their oxidation mechanism and it is also known that activated free radical semiquinones can either react directly with biological targets such as DNA or RNA or generate the cytotoxic oxygen dependent superoxide and hydroxyl radicals[1]. The formation of reactive oxygen species to has been proposed as the basis for both the antitumour activity and for the cytoxicity i.e. cardiotoxicity of anthracyclines[31].

A significant decrease of DNA absorbance was observed in the presence of low

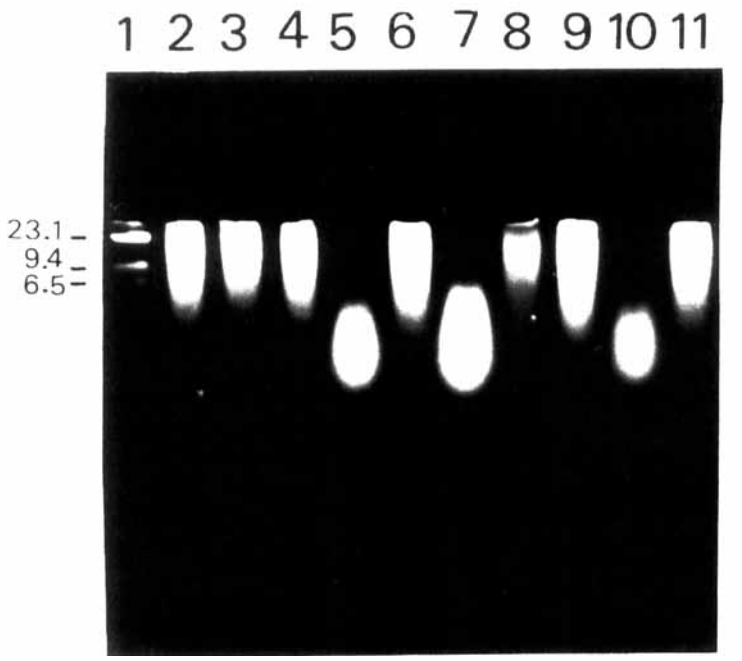

FIGURE 3 Agarose gel electrophoresis separation of damaged DNA induced by rifamycin and copper(II) and the effect of antioxidants. The gel shows from left to right: (1) DNA molecular weight marker lamda HindIII. (2) Not incubated DNA (0,2 mg/ml) alone. (3) As in (2) 1 hour incubation. (4) As in (3) + rifamycin SV. (5) as in (4) + Copper(II). (6) to (11) are as in (5) with the following additions respectively: + catalase (0,06 mg/ml), + SOD (0,06 mg/ml), + GSH (1 mM). + Thiourea (10 mM) + Ethanol (10 mM) and + DMSO (10 mM).

rifamycin SV concentrations (20 μM) the effect being proportional with the increase of the antibiotic concentration. The decrease of DNA absorbance was enhanced by copper(II) ions which in turn stimulated the rate of rifamycin SV oxidation. It has previously been established that under the experimental conditions followed in this investigation, i.e. pH 7.4, copper(II) ions are the most effective of the transition metal ions tested in promoting rifamycin SV oxidation[12]. In all the experiments carried out, including oxygen consumption measurements, catalase but not SOD acts as an effective inhibitor. In addition to the effect of catalase, the hydroxyl radical scavenger thiourea was able to decrease significantly the release of TBA-reactive material from DNA and to prevent the damage to DNA as shown by agarose gel electrophoresis whereas, contrary to previous results[12], the inhibition of deoxyribose degradation was to a lesser extent (50%) even though a higher concentration (50 mM) of the scavenger was used.

GSH exhibited inhibitory characteristics similar to the hydroxyl radical scavengers thiourea and ethanol. At concentrations an order of magnitude lower than utilised for these compounds, GSH decreases the degradation of DNA and of deoxyribose with the inhibition being 62% and 91% respectively. In addition to this, 0.4 mM GSH clearly preserves the normal electrophoretic pattern of DNA. A similar effect was also observed with catalase and thiourea but little or no effect was observed with ethanol or DMSO. It is however unclear whether the inhibition of DNA damage

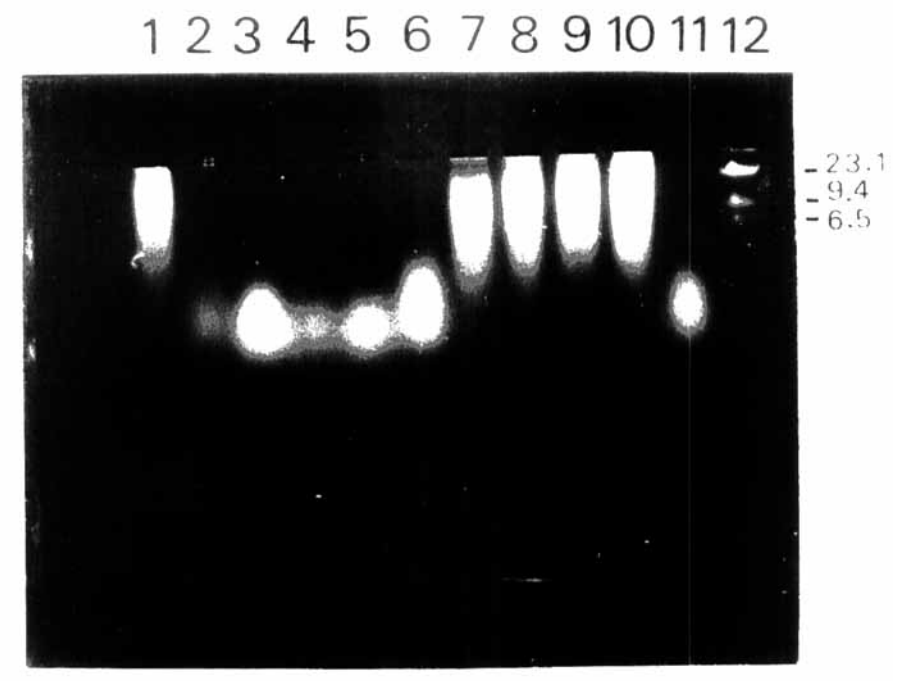

FIGURE 4 Concentration-course effect of GSH on the electrophoretic separation of Rifamycin SV-copper(II) damaged DNA. (1) DNA (0,2 mg/ml) + rifamycin SV. (2) As in (1) + copper(II) (3) to (10) are as in (2) with the following GSH concentrations: 0.025, 0.050, 0.1, 0.2, 0.4, 0.6, 0.8, and 1 mM. (11) DNA + phenanthroline-mercaptoethanol-copper mix (0.1 mM). (12) DNA molecular weight markers as in figure 2.

achieved by GSH is a consequence of its potential free radical scavenging properties. The observed effect requires further experimentation.

Although the role of hydrogen peroxide in the degradation of DNA and deoxyribose by rifamycin SV and copper(II) ions can easily be explained by means of the inhibitory effect of catalase, the involvement of other reactive species is still poorly understood. Hydrogen peroxide by itself was not able to reproduce the effect of rifamycin SV and copper(II) ions (results not shown). This fact, together with the protection of DNA by thiourea and of deoxyribose by ethanol, suggests that OH· radicals formed through a putative copper-catalyzed Fenton type reaction[32] or by semiquinone-dependent hydrogen peroxide reduction[4] might be contributing to the observed damage. The formation of OH· radicals could be also supported by the protection observed in the presence of GSH which in turn can be explained by its reactivity with OH· radicals as previously reported[33].

Glutathione at physiological concentrations was very effective at protecting DNA. GSH may be preventing damage to DNA by removing free copper(II) ions from solution before they can complex with rifamycin SV. Therefore, in addition to its antioxidant capability a chelating effect by GSH cannot be ruled out and could also be important in the protection achieved by the tripeptide since rifamycin SV in the absence of copper(II) does not damage DNA.

The suggestion that GSH plays a protective effect against DNA damage induced

by rifamycin SV and copper(II) is consistent with a large number of previous observations in which its antioxidant and/or free radical scavenger activity has been shown[14]. It has been proposed that GSH levels are important in modulating the cytotoxicity of a number of alkylating agents as well as other free radical release mechanisms such as ionizing radiation and, indeed, an increase of cytotoxicity due to these agents is observed after GSH depletion[23].

The results reported therefore provide further insight into the scavenging properties of GSH and may support the use of the tripeptide as a beneficial agent to improve the therapeutic index of certain cytotoxic drugs in cancer chemotherapy. The effect of rifamycin SV administration on DNA damage in GSH depleted versus normal rats is currently under investigation.

Acknowledgements

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