Protective effect of curcumin on γ-radiation-induced sister chromatid exchanges in human blood lymphocytes

Entissar S. AlSuhaibani

College of Science
King Saud University
P.O. Box 261002
Riyadh 11342, Kingdom of Saudi Arabia
E-mail: ealsuhaibani@hotmail.com

Abstract: The present work evaluates the radioprotective effect of curcumin on γ-radiation-induced genetic toxicity. The DNA damage was analysed by the frequencies of Sister Chromatid Exchanges (SCEs). Human lymphocytes were treated *in vitro* with 5.0 μg/ml of curcumin for 30 min at 37ºC then exposed to 1, 2 and 4 Gy γ-radiation. The lymphocytes were cultured in (RPMI) 1640 tissue culture medium and autologous serum (20%). Phytohemagglutinin and 5-bromodeoxyuridine (10 pM) were added upon the initiation of culture and harvesting was done 72 h after culture initiation. The SCEs were counted and compared with the control lymphocytes, which were untreated with curcumin and exposed to the same γ-radiation doses. At 0 and 4 Gy no significant difference in the frequency of SCEs was found between the control lymphocytes and the lymphocytes pretreated with curcumin. Moreover, at 1 and 2 Gy the incidence of SCEs decreased in the pretreated lymphocytes with curcumin but not in the control lymphocytes. From the results obtained, it can be concluded that curcumin could effectively reduce the clastogenic effects of radiation with a dose of 1–2 Gy in human lymphocytes *in vitro*. However, further studies are needed to clarify the mechanism of action.

Keywords: curcumin; γ-radiation; sister chromatid exchanges; and 5-bromodeoxyuridine; clastogenic effects.

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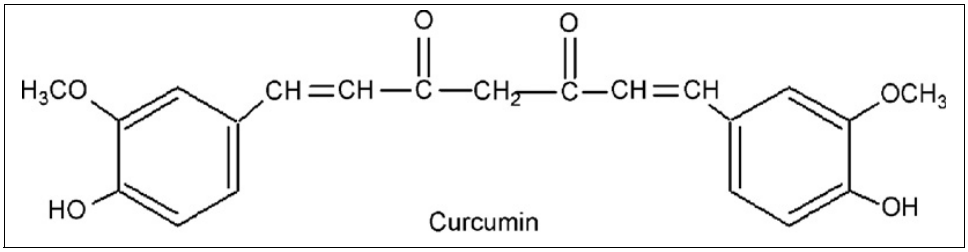
Biographical notes: Entissar S. Alsuhaibani works at King Saud University, Riyadh, Kingdom of Saudi Arabia. She has research interests in the cytogenetic effects of ionising radiation and on the evaluation of genetic damage in subjects living in areas with high background radiation.

1 Introduction

The rhizome of *Curcuma longa* Linn. (turmeric) has been widely used as a yellow colouring agent and spice in many foods, and it has also been used in indigenous medicine for the treatment of inflammatory and other diseases (Nadkarni, 1976). Curcumin (diferuloylmethane, Figure 1) has been identified as the major pigment in turmeric and has been reported to possess both antioxidative and anti-inflammatory activities (Sharma, 1976; Srimal and Dhawan, 1973; Rao *et al*., 1982; Mukhopadhyay *et al*., 1982; Yegnanarayan *et al*., 1976).

Figure 1 Structure of curcumin (bis-1,7-(4-hydroxy-3-methoxyphenyl)-hepta-1,6-diene 3,5-dione)



More recently, reports have shown that curcumin possesses strong antioxidant and anticancer properties (Sharma, 1976; Unnikrishnan and Rao, 1995; Phan *et al*., 2001; Song, 2001; Kalpana and Menon, 2004; Polasa *et al*., 2004; Abraham *et al*., 1993; Inano and Onoda, 2002).

Since the development of the 5-bromodeoxyuridine (BrdU) staining technique for the morphological differentiation of sister chromatids (Perry and Wolff, 1974), many works have been published about SCE induction after *in vivo* and *in vitro* exposure to genotoxic agents (Tucker and Preston, 1996; Tucker *et al*., 1988). The staining of chromosomes after cells have been grown for two replication cycles in the presence of BrdU, which substitutes for thymidine, has made it possible to distinguish the DNA of the two chromatids of each chromosome. When exchanges between chromatids have taken place, these can be seen clearly. The increase in the rate of SCEs reflects the reciprocal interchange of DNA replication products at apparently homologous loci, which involves DNA breakage and reunion (Latt *et al*., 1981).

The present study was conducted using the rate of SCEs in order to examine the effects of curcumin on reducing gamma ray-induced clastogenic effects *in vitro* on human lymphocytes.

2 Materials and methods

Heparinised blood samples were obtained from ten healthy male donors with no drug or radiation treatment one month prior to sampling. Each blood sample was divided into two groups. One group was pretreated for 30 min with curcumin (5.0 μg/ml, Sigma); the other, the control group, was not treated with curcumin. Both groups were exposed to γ-radiation at different doses (1, 2 and 4 Gy) from a ^{60}Co source at room temperature. The radiation dose rate was about 2 mGy/s. The blood cultures were set up with 0.4 ml

of whole blood cultured in 4.5 ml RPMI-1640 (Sigma) medium supplemented with autologous serum (20%), antibiotics (penicillin, 100 iu/m), (streptomycin, 100 μg/ml) and 10 pg/ml of BrdU. Cell cultures were initiated with the addition of 0.1 ml of 5 μg/ml phytohemaglutinin (PHA). The cell cultures were grown in darkness and harvested after 72 h. The SCE analysis was performed according to the standard protocol (Perry and Wolff, 1974). A total of 100 well-spread, second-division metaphases were examined to determine the SCE frequency per sample. The results of the SCEs were expressed as mean ± SD for each sample. The data were analysed using Statistical Package of Social Science (SPSS).

3 Results

The results of the frequencies of SCEs in human lymphocytes treated with curcumin and in the control are presented in Tables 1 and 2 and in Figure 2. The frequencies of SCEs at 0 Gy in the human lymphocytes treated with curcumin and those of controls revealed no observed difference.

Table 1 Effects of different doses of γ-irradiation on the SCE frequencies in human lymphocytes treated with curcumin

	Dose of γ-irradiation (Gy)							
	0		*1*		*2*		*4*	
Case	*SCE*	*X ± SE*	*SCE*	*X ± SE*	*SCE*	*X ± SE*	*SCE*	*X ± SE*
1	1–7	5.71 ± 0.14	3–14	8.34 ± 0.35	4–14	8.95 ± 0.32	6–18	11.82 ± 0.37
2	2–8	5.50 ± 0.29	3–12	7.50 ± 0.29	5–15	9.95 ± 0.32	7–19	12.82 ± 0.37
3	3–9	5.46 ± 0.17	2–11	6.50 ± 0.29	4–13	7.15 ± 0.25	6–20	12.75 ± 0.43
4	4–10	6.42 ± 0.23	4–13	8.50 ± 0.29	3–14	9.37 ± 0.30	7–21	12.75 ± 0.43
5	5–11	4.26 ± 0.22	3–12	7.50 ± 0.29	4–15	10.21 ± 0.35	7–18	13.75 ± 0.43
6	2–9	5.00 ± 0.14	4–11	7.42 ± 0.23	4–16	9.82 ± 0.37	8–21	14.38 ± 0.41
7	4–10	6.60 ± 0.21	3–14	8.34 ± 0.35	5–13	9.96 ± 0.26	6–17	11.34 ± 0.35
8	5–10	4.24 ± 0.21	2–11	8.50 ± 0.29	4–14	10.03 ± 0.34	5–18	11.38 ± 0.41
9	1–7	6.00 ± 0.14	3–10	6.42 ± 0.23	3–15	8.82 ± 0.37	7–19	11.26 ± 0.35
10	1–9	5.32 ± 0.24	4–11	7.42 ± 0.23	4–14	9.14 ± 0.34	5–17	10.82 ± 0.37
Total		5.70 ± 0.07		7.44 ± 0.93^{abc}		9.24 ± 0.11^{def}		12.27 ± 0.13^{ghi}

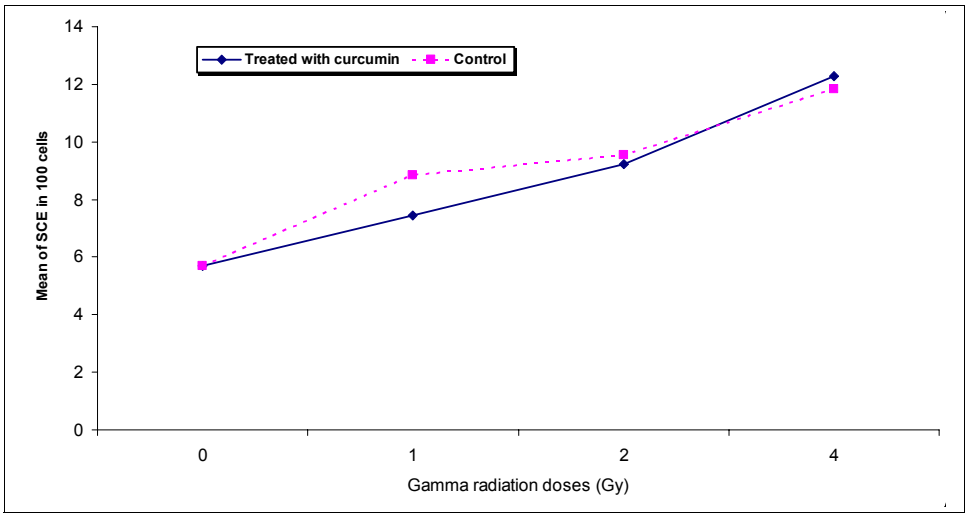
Notes: abc is the mean significant difference between 1 and 0,2,4 at the 0.01 level.
def is the mean significant difference between 2 and 0,1,4 at the 0.01 level.
ghi is the mean significant difference between 4 and 0,1,2 at the 0.01 level.

Table 2 Effects of different doses of γ-irradiation on the SCE frequencies in control human lymphocytes

	Dose of γ-irradiation (Gy)							
	0		*1*		*2*		*4*	
Case	*SCE*	*X ± SE*	*SCE*	*X ± SE*	*SCE*	*X ± SE*	*SCE*	*X ± SE*
1	2–9	5.71 ± 0.14	5–16	10.34 ± 0.35	4–16	8.40 ± 0.39	7–19	11.89 ± 0.37
2	1–10	5.50 ± 0.29	6–14	9.96 ± 0.26	5–17	8.84 ± 0.38	8–20	13.82 ± 0.37
3	3–8	5.46 ± 0.17	4–13	6.68 ± 0.26	4–15	8.37 ± 0.34	7–19	11.11 ± 0.36
4	3–10	6.42 ± 0.23	5–15	10.23 ± 0.35	5–18	9.05 ± 0.41	6–21	13.26 ± 0.47
5	2–8	4.26 ± 0.22	4–16	6.07 ± 0.34	4–16	9.03 ± 0.37	5–19	11.75 ± 0.43
6	3–7	5.00 ± 0.14	5–15	8.86 ± 0.30	4–17	10.38 ± 0.41	7–20	13.38 ± 0.41
7	4–11	6.60 ± 0.21	6–14	9.96 ± 0.26	6–16	10.95 ± 0.32	5–19	9.99 ± 0.44
8	2–9	4.24 ± 0.21	5–16	8.56 ± 0.34	4–17	10.22 ± 0.40	7–18	11.37 ± 0.37
9	4–8	6.00 ± 0.14	4–15	8.56 ± 0.35	4–18	10.75 ± 0.43	6–17	10.26 ± 0.36
10	2–9	5.32 ± 0.24	3–16	9.38 ± 0.41	5–17	9.34 ± 0.38	7–19	11.58 ± 0.38
Total		5.70 ± 0.07		8.86 ± 0.11[abc]		9.53 ± 0.12[def]		11.84 ± 0.13[ghi]

Notes: abc is the mean significant difference between 1 and 0,2,4 at the 0.01 level.
def is the mean significant difference between 2 and 0,1,4 at the 0.01 level.
ghi is the mean significant difference between 4 and 0,1,2 at the 0.01 level.

Figure 2 The distribution of the mean SCEs in the human lymphocytes treated with curcumin and the control (see online version for colours)



The frequency of SCEs in human lymphocytes in both groups is statistically significantly increased ($p < 0.0\ 1$) according to the increase in γ-irradiation doses.

The analysis of the data is presented in Table 3. The independent sample *T*-test results reveal that there is a highly significant decrease in the frequencies of SCEs ($p < 0.01$) at 1 and 2 Gy in the human lymphocytes treated with curcumin compared to the control, and a slight, but statistically insignificant decrease in the frequencies of SCEs at 4 Gy in the human lymphocytes treated with curcumin compared to the control.

Table 3 T-test results between the frequencies of SCEs in the human lymphocytes treated with curcumin and those of controls

Radiation doses	*Group*	*Mean*	*SD*	*T-value*	*Sig.*
1	Treated with curcumin	7.44	2.95	–16.401	0.000**
	Control	9.75	3.34		
2	Treated with curcumin	9.24	3.35	–7.471	0.000**
	Control	10.45	3.86		
4	Treated with curcumin	12.27	3.99	–1.926	0.054
	Control	12.61	4.00		

Note: ** Significant at the .01 level.

4 Discussion

The use of natural dietary antioxidants to prevent chromosomal damage induced by tumour agents is currently eliciting considerable interest (Weijl *et al*., 1997). This study was carried out to investigate the effects of curcumin on induced SCEs in human lymphocytes. This study confirmed the ability of γ-radiation to increase the incidence of SCEs in exposed human lymphocytes, as reported previously in many other investigations that obtained significant SCE increases in different populations occupationally or accidentally exposed to ionising radiation (Gundy *et al*., 1984; Al-Sabti *et al*., 1992; Lazutka and Dedonyte, 1995). Nevertheless, the data are in contrast to the results previously reported where no significant increases in SCE frequency in lymphocytes were detected from people exposed to ionising radiation (Littlefield, 1982; Brandom *et al*., 1990).

However, no data regarding curcumin's genotoxic effects are available. The results of this study *in vitro* indicate that treatment with curcumin is not associated with significant genotoxicity in human lymphocytes. No statistically significant increase in the frequency of SCEs at 0 Gy was found in treated samples as compared with controls. Furthermore, the results showed a radioprotective effect of curcumin at 1 and 2 Gy when applied prior to γ-irradiation (Lusania *et al*., 2000; Tuba and Ilhami, 2008). Similar results were reported by Srinivasan *et al.* (2008), who observed that curcumin caused a significant decrease in DNA damage in primary cultures of isolated rat hepatocytes irradiated with γ-radiation, although it has been recognised that pretreatment with curcumin was less effective against radiation at 4 Gy because its concentration might not be enough to quench all generated free radicals.

Thus, from the results obtained, we observed that pretreatment with curcumin protects the human peripheral blood lymphocytes from γ-radiation-induced SCEs.

However, before the procedure is approved for clinical use, further experimental studies using different cytogenetic and molecular biomarkers at the same time and well-designed clinical studies are needed to clarify the exact mechanisms of its radioprotective action and possible interactions.

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