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P60 QUINACRINE AS AN INDUCER OF DICENTRICS, RINGS AND MARKER CHROMOSOMES IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES

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Quinacrine dihydrochloride (QDH), with an extensive history of medicinal usage, especially as an antimalarial drug, has recently been used as a non-invasive transvaginal chemosterilant in the human female and more than 80,000 women have been sterilized in several developing countries. Quinacrine, like many other aminoacridine compounds, forms reversible complexes with double stranded DNA by intercalation of the three planar rings and has been reported to be mutagenic in bacteria. However, except for one preliminary report, there are no data on the clastogenic or mutagenic effect(s) of quinacrine in the mammalian systems. In view of lack of information on its genotoxicity, human exposure to quinacrine for the purpose of female sterilization, has come under severe criticism from several national and international agencies including WHO. Therefore, it was considered of profound interest to investigate the chromosomal damaging ability of quinacrine, if any, directly in human cells. Experiments have been carried out in peripheral blood lymphocytes from normal healthy female volunteers to assess effect of QDH on chromosome aberrations, sister chromatid exchanges (SCEs) in metaphases and micronuclei (MN) in cytokinesis blocked micronucleus assay. G_0 and G_1 lymphocytes were exposed to quinacrine at 0.6, 3 and 6 µg/ml in 48 and 72 h cultures and G_2 lymphocytes to 0.6, 1.5, 3 µ/ml in 72 h cultures. Effect of quinacrine on mitotic spindle inhibition was studied in the absence of colcemid treatment, by exposure of 72 h lymphocytes cultures to 0.45 µg/ml of the drug at 69h. At 6 and 3 µg/ml concentration, QDH was found to be cytotoxic in G_0 lymphocytes. Surprisingly, Quinacrine not only induced dicentrics and rings but it was found to exhibit a very interesting cytogenetic profile. (i) It enhanced effectively cell proliferation as evidenced by the increased mitotic index (10-30%) in all treated groups. (ii) It behaved as a mitotic spindle inhibitor like colcemid and induced 10-14% C-metaphases. (iii) In G_0 lymphocytes at 0.6 µg/ml, a dose level that can be encountered during therapy, QDH induced dicentrics, marker chromosomes, inversions, and peculiar ring configurations, chromosome pulverization, centromere separation, haploid, polyploid and endoreduplicated cells and banded chromosomes. It also increased the MN frequencies at 0.6 µg/ml, though no increase in SCEs was evident at this dose level. Chemical mutagens, except few radiomimetic chemicals like bleomycin, do not produce dicentrics and only aberrations like chromatid breaks, gaps and exchanges are encountered. To our knowledge, this is perhaps the first report to show that QDH also induces such major chromosomal changes as dicentrics and rings and shares unique properties, ranging from cell proliferation to spindle inhibition. The molecular mechanisms leading to cytogenetic effects quinacrine, as reported here, remain to be elucidated. However, in view of this important positive cytogenetic evidence in human cells, a detailed genetic epidemiology study, including monitoring of the health profile, reproductive outcome and neoplastic changes, if any, in the exposed women in developing countries is warranted.

P62 ENHANCEMENT OF MITOMYCIN C INDUCED MICRONUCLEI AND SISTER CHROMATID EXCHANGES BY ASCORBIC ACID (VITAMIN C) IN HUMAN LYMPHOCYTES *IN VITRO*

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Ascorbic acid (Vitamin C) is well known as an important physiologic antioxidant and free radical scavenger and is believed to protect other antioxidants such as Vitamin E, Vitamin A and Glutathione. Present as ascorbate in most biological settings, ascorbic acid (AA) is also one of the few molecules whose concentration inside the cell can be altered by over ten fold by dietary regimen. AA has been reported to exhibit both genotoxic as well as antigenotoxic effects in various test systems. During the present study modulation of mitomycin C induced clastogenesis by L-ascorbic acid has been evaluated in human peripheral blood lymphocytes. Various cytogenetic markers eg. chromosome aberrations (CA), sister chromatid exchanges (SCEs) in metaphases and micronuclei (MN) in cytokinesis blocked binucleate cells (CBBN) have been used during the study. The various treatment regimen used included (i) AA 200 µg/ml, 24h (ii) MMC 0.1 and 0.2 µg/ml 25h 26h (iii) AA 24h MMC 25h (iv) AA 24h MMC 26h. Chromosome aberrations were scored in hundred first division metaphases in BrdU incorporated 72h cultures. The incidence of SCE was determined in fifty second division metaphases. PRI was calculated from the cell cycle kinetics data of the number of metaphases in the M1, M2, M3 and M4 counted in two hundred metaphases. One thousand binucleated cells per dose point were scored to evaluate the MN frequencies. NDI for each treated group were determined by scoring the number of mono, bi, tri, tetra and higher number of nucleated cells in one thousand lymphocytes. Reduction in the % BN, NDI and increase in MN frequency was noticed in the MMC treated groups. Increase in MN, SCE frequencies, NDI and PRI was noticed in all AA treated groups. Data show that in the 24h AA treatment regimen, a potentiation upwards of 30-64% of MMC induced MN frequencies and 28-61% of MMC induced micronucleated binucleate cells was observed. A 5-14 fold increase in the number of cells with multiple micronuclei was also observed. SCE analysis in the 24h AA treated regimen revealed and enhancement of 13-32% in the 25h and 13-27% in the 26h MMC treated groups respectively. The clastogenic effect of MMC in human lymphocytes *in vitro* seems to be potentiated by ascorbic acid. The potentiation observed has to be viewed in the light of metal ion catalyzed autooxidation of AA in oxygenated media and the existence of an antioxidant system *in vivo* which inactivates oxyradicals before their interaction with DNA.

P213

Quinacrine induces dicentrics, rings and marker chromosomes in human peripheral blood lymphocytes in vitro

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Quinacrine dihydrochloride (QDH), with an extensive history of medicinal usage as an antimalarial drug, has been used in recent years as a non-invasive transvaginal chemosterilant in the human female and over 80,000 women have been exposed in several developing countries. In view of lack of adequate data on its mutagenicity evaluation in mammalian system(s), human exposure to QDH for female sterilization has come under severe criticism from national and international agencies including WHO. Therefore, search for any effect(s) of QDH on human chromosomes, particularly in females, was considered highly desirable. Peripheral blood samples obtained from healthy female volunteers were grown as whole blood cultures under standard conditions. G_0 and G_1 lymphocytes were exposed to QDH at 0.6, 3 and 6 μg/ml in 48 and 72 h cultures and G_2 lymphocytes to 0.6, 1.5 and 3 μg/ml in 72 h cultures. QDH at 6 and 3 μg/ml was found to be cytotoxic in G_0 lymphocytes. Surprisingly, in G_0 lymphocytes at 0.6 μg/ml, a dose level that can be encountered during therapy, QDH induced dicentrics, marker chromosomes, inversions, peculiar ring configurations, chromosome pulverization, banding, centromere separation, euploidy and endoreduplication. Like colcemid, QDH was found to inhibit mitotic spindle and induced 10–14% C-metaphases (in the absence of colcemid) at 0.45 μg/ml in 72 h cultures treated at 69 h. The drug also acted as an effective cell proliferating agent, as it increased the mitotic index (10–30%) among all treated groups. QDH also increased MN frequency at 0.6 μg/ml, though SCEs remained unaffected at this concentration. Unlike radiation, but for few exceptions such as bleomycin, chemical mutagens do not produce dicentrics and the aberrations induced are mostly of the chromatid type. In view of the profound cytogenetic evidence reported here, a detailed genetic epidemiology study including monitoring of the health profile, reproductive outcome and neoplastic changes etc. among the women exposed to quinacrine for sterilization is warranted.

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Quinacrine dihydrochloride, the non-surgical female sterilant induces dicentrics, rings, and marker chromosomes in human peripheral blood lymphocytes treated in vitro: a preliminary report

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Abstract

During the last decade, quinacrine dihydrochloride (QDH) has been promoted for clinical trials as a much needed non-surgical female sterilant, largely in the Third World. Recently, however, these human trials have come under severe criticism due to lack of adequate evidence of biological safety of QDH, particularly of its genotoxicity in mammalian systems. In the present study, the cytogenetic analysis of QDH-treated human lymphocytes, grown as whole blood cultures in vitro, surprisingly showed a wide range of chromosomal aberrations. At a concentration of 3.0 and 6.0 μg/ml in culture, QDH was cytotoxic, as shown by the very few analyzable metaphases that could be observed. G_0 lymphocytes, treated with 0.6 μg/ml QDH, exhibited chromosome aberrations including dicentrics, ring configurations, translocations, inversions, and marker chromosomes. Near haploid, polyploid, and endoreduplicated cells were also observed. All the rings appeared to be formed as a result of telomere fusion/association. Twenty percent of the dicentrics observed also indicated telomere fusion/association in the D and G groups of chromosomes. Overall, a frequent involvement of chromosomes 1, 2, and 3 in both unstable and stable chromosome rearrangements was also observed. Exposure of 72-h cultures to 0.45 μg/ml QDH at 69 h resulted in an accumulation of C-metaphases, suggesting that probably QDH behaves as a mitotic spindle inhibitor. The G_2 lymphocytes from two donors exposed to 0.6, 1.5 or 3.0 μg/ml of QDH showed no increase in chromatid aberrations in two donors. However, QDH at 0.6 μg/ml increased the frequency of micronucleated binucleate cells. No increase in sister chromatid exchanges was observed at this concentration. Though preliminary, these observations demonstrate the chromosome damaging ability of QDH in human lymphocytes treated in vitro. Surprisingly, like ionizing radiation, QDH acted by an S-phase-independent mechanism, unlike most of the chemical mutagens. These results warrant detailed investigations on the cytogenetic effects of QDH in vitro, as well as among women exposed to this agent during clinical trials for non-surgical sterilization. The interesting cytogenetic profile of QDH deserves to be pursued and the underlying mechanisms, in particular, the DNA topoisomerase II inhibitory effect, if any, needs to be elucidated.

Keywords: Quinacrine dihydrochloride; Human peripheral blood lymphocytes; Sterilization

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1. Introduction

Quinacrine, initially introduced as an antimalarial drug before World War II [1], is endowed with various therapeutic properties, and exhibits diverse biological effects in the mammalian systems [2,3]. The local sclerosing action of quinacrine used for treatment of recurrent pleural and pericardial effusions [2] was later found to occlude the fallopian tubes by Zipper et al. [4] during the 1970s. The installation of quinacrine slurry, and finally its pelleted form, has been propagated as a much-needed, simple, and non-invasive devise for transcervical sterilization of women [4]. During the last 10 years, about 80–100,000 women of reproductive age have been exposed to quinacrine dihydrochloride (QDH) pellets as single or repeated application in highly populated Third World countries including Vietnam, Brazil, China, and the Indian subcontinent [5].

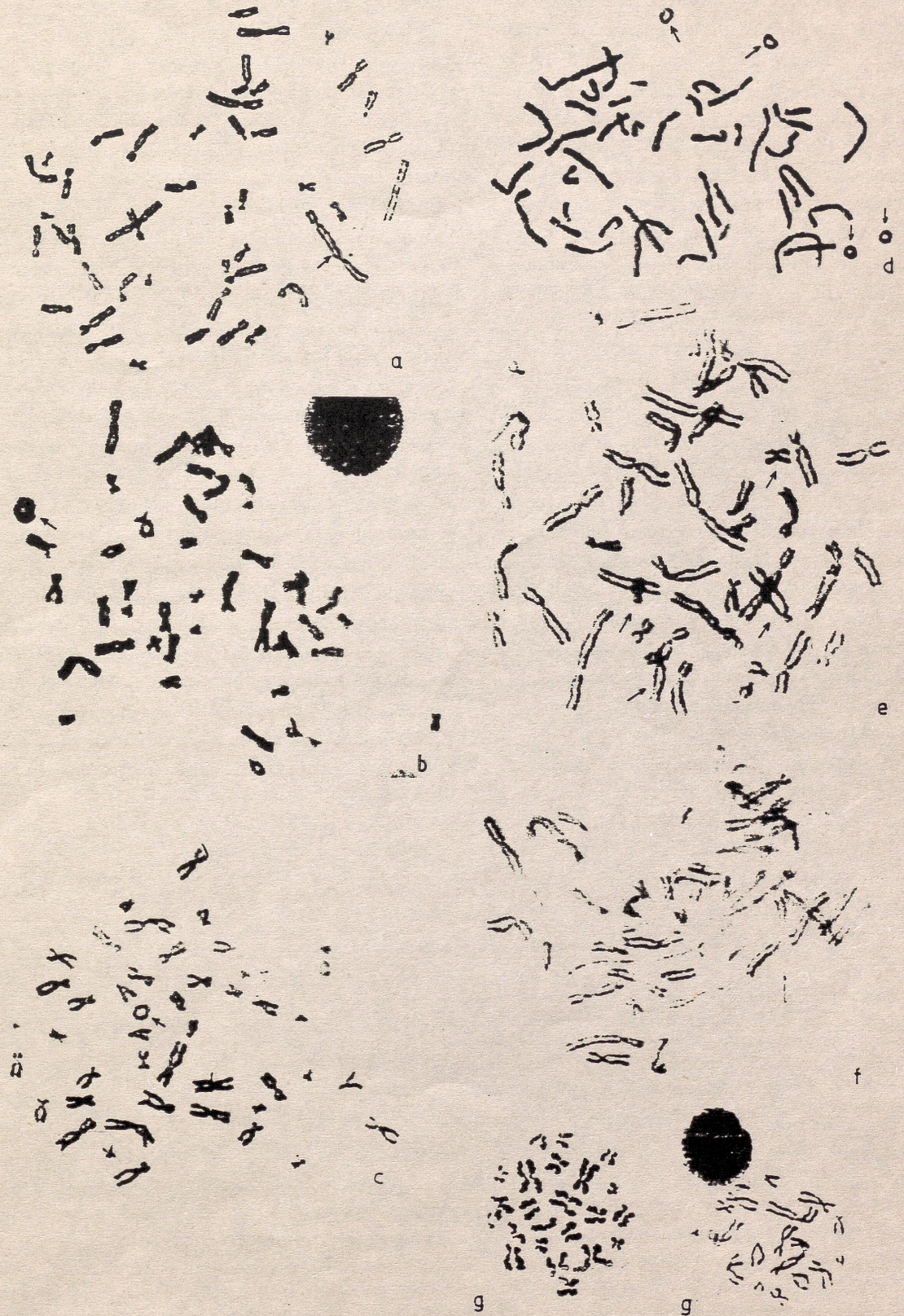
In India, clinical trials involving over 10,000 women have been undertaken in various parts of the country by enthusiastic clinicians in their attempts to find a much-desired, simple, practical, and economic mode of population control. However, during an organized effort to launch a National programme on QDH pellet trials in India, it was realized that a lot of work still needs to be done with respect to the genetic toxicological evaluation of this drug, keeping in mind its entirely new application and known ability to intercalate with the human DNA. Several national and international forums have expressed criticism of clinical trials due to the lack of adequate evidence on the safety of QDH as an irreversible sterilizing agent in young women [6]. In view of the above, and taking into consideration the lack of adequate studies on genotoxicity of QDH, it was decided to assess its possible cytogenetic effects, directly on the human genome, using the easily accessible peripheral blood lymphocytes exposed in vitro as whole blood cultures. The main objective of this preliminary study is to report a wide range of chromosomal aberrations/cytogenetic changes induced by QDH in lymphocytes of female subjects.

2. Materials and methods

Heparinised peripheral blood samples were obtained from three healthy adult female volunteers (22, 43, and 45 years of age), who agreed willingly to donate the samples. Whole blood cultures were initiated by a standard procedure used in our laboratory, as reported elsewhere [7]. No antibiotics were added to the cultures at any stage. Hams' F10 medium, 200 mM L-glutamine, 10% fetal bovine serum, 0.2 ml reconstituted PHA, and 0.3 ml whole blood per 5 ml of culture volume were used. The cultures were terminated after 48 or 72 h at 37°C as the case may be, following a final 3-h treatment with colcemid at a concentration of 0.02 μg/ml and harvested following the conventional procedure involving hypotonic 0.075 M KCl treatment, fixation with methanol–acetic acid (3:1) and air drying on chilled wet slides.

QDH (Sigma) ($C_{23}H_{30}ClN_3O \cdot 2HCl$, M_W 472.9) dissolved in distilled water was used throughout the study. G_0 lymphocytes were exposed to QDH at a final concentration of 0.6, 3, and 6 μg/ml in 48-and 72-h cultures. The G_2 lymphocytes were treated at 69 h with 0.6, 1.5, and 3 μg/ml in 72-h cultures. Donors 1 and 2 (48 h; 0.6, 3, and 6 μg/ml), donors 2 and 3 (72 h; 0.6, 3, and 6 μg/ml as well as 0.6, 1.5, and 3 μg/ml G_2) cultures were run in parallel. The drug was present in the medium until harvest. Initially, 500 cells were scored for chromosome aberrations per donor. However, once the cytogenetic profile of quinacrine was found to be interesting, a total of 1500-well spread metaphases were examined per donor. Effect on mitotic spindle inhibition was studied in the absence of colcemid treatment by exposing the 72-h cultures to 0.45 μg/ml QDH at 69 h.

Fig. 1. Cytogenetic profile seen after the 0.6 μg/ml QDH treatment of human lymphocytes in vitro in whole blood cultures. (a) Dicentric No. 1/C group chromosome; (b) No. 2 chromosome ring; (c) D group chromosome ring; (d) acentric rings; (e) chromosome rearrangements, translocation D/C group chromosomes, C group chromosome deletion, dicentric C/D group chromosome, fragment; (f) despiralisation; (g) near haploid cells.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For the micronuclei (MN) study, the method reported elsewhere was followed [8]. Cytochalasin B was added at 24 h after the initiation of cultures to arrest cytokinesis at a final concentrations of 6 μg/ml. A mild hypotonic treatment with cold 0.8% KCl for 5 min preceded the fixation [9]. To demonstrate sister chromatid exchanges (SCEs), BrdU was added at a final concentration of 10 μg/ml at the initiation of cultures. Differential staining for SCEs was carried out as previously described [9]. From each subject, a total of 1000 binucleated cells were scored to determine the frequency of micronucleated cells and 50-s metaphases were analyzed for SCEs.

3. Results

Metaphase analysis of QDH-treated cultures revealed a wide range of chromosomal changes in the human lymphocytes. The drug was cytotoxic at 3 and 6 μg/ml concentration, as very few analyzable metaphases were observed in these samples. Treatment of G_0 lymphocytes with 0.6 μg/ml QDH, a dose level that could be encountered during therapy, surprisingly, produced various chromosome-type aberrations including dicentrics, translocations, inversions, and marker chromosomes. Unlike ionizing radiation, chemical mutagens generally do not produce dicentrics, but for a few exceptions such as bleomycin, they mostly induced chromatid-type aberrations. Another conspicuous finding was the presence of peculiar ring configurations (RC) particularly in the 72-h cultures. Normally, rings are rarely observed. However, in the present study, it appeared as if these were being formed by the sticky ends of chromosomes resulting from QDH treatment. Certain metaphases showed centromeric separation, chromatin despiralisation, and in some cases, chromosome pulverisation. Other major alterations include polyploid, endoreduplicated, and even near haploid cells. The cytogenetic profile of QDH in human lymphocytes is shown in Fig. 1. QDH also enhanced cell proliferation at 0.6 μg/ml as evidenced by an increase in mitotic index (10–30%) among all the treated samples (cells scored = 1000). G_2 lymphocytes from two donors exposed to 0.6, 1.5, and 3 μg/ml did not show any increase in chromatid aberrations.

In the absence of colcemid, the 72-h lymphocyte cultures treated with QDH at a concentration of 0.45 μg/ml at 69 h exhibited about 10–14% C-metaphases (data based on scoring of 1000 cells), thus, showing a mitotic spindle inhibitory effect (like colcemid). And so, the cytogenetic profile of QDH was found to be concentration and exposure time-dependent. QDH could be characterised as an S-phase-independent agent, since it produced mostly chromosome-type aberrations. The main objective of the

Table 1
Cytogenetic effects of QDH in human peripheral blood lymphocytes in vitro

Donor (culture duration)	QDH (μg/ml)	Type and frequency of aberrations per 100 metaphase cells							
		DIC	RIN	REA	AF	Ctb	Haploid cells	Polyploid cells	ER
1 (48 h)	0.0	0	0	0	0	0.40 (6)	0	0	0
	0.6	0.47 (7)	0	0.47 (7)	0.67 (10)	0.67 (10)	0.06 (1)	0.13 (2)	0.20 (3)
2 (48 h)	0.0	0	0	0	0	0.53 (8)	0	0	0
	0.6	0.40 (6)	0.06 (1)	0.20 (3)	0.26 (4)	0.73 (11)	0.13 (2)	0	0.20 (3)
2 (72 h)	0.0	0	0	0	0	0.53 (8)	0	0	0
	0.6	0.13 (2)	0.26 (4)	0.20 (3)	0.06 (1)	0.73 (11)	0.20 (3)	0.06 (1)	0.06 (1)
3 (72 h)	0.0	0	0	0	0	0.46 (7)	0	0	0
	0.6	0.33 (5)	0.13 (2)	0.13 (2)	0.13 (2)	0.60 (9)	0.20 (3)	0.06 (1)	0.13 (2)

DIC: dicentrics; RIN: rings; REA: rearrangements (translocations, inversions, and marker chromosomes); AF: acentric fragments rings and double minutes; Ctb: chromatid breaks; ER: endoreduplication.
The cultures were harvested at 48 or 72 h. QDH was added to achieve a final concentration of 0.6 μg/ml of culture, which remained in the culture until harvest. A total of 1500 cells (metaphases) were scored from each donor. The figures within parentheses denote the number of events observed.

Table 2
A detailed analysis of unstable and stable chromosome rearrangements

Donor (culture duration)	Total no. of dicentrics			Rings[a]	Chromosome involved	Chromosome rearrangements	
		With fragment[b]	Without fragment[b]			No.	Nature of events
1 (48 h)	7	C/C	2/3	0	0	7	Translocation — B/1 chromosome
		C/C	G/G (TA)				Translocation — D/C group chromosome (Marker)
		1/C	B/C				Inversion — No. 3 chromosome
			D/D (TA)				Inversion — C group chromosome
							Deletion — C group chromosome
							Deletion — D group chromosome
							Deletion — D group chromosome
2 (48 h)	6	2/C	1/C	1	1 — No. 2 chromosome	3	Translocation — 1/2 chromosome
		2/C	D/G (TA)				Inversion — No. 3 chromosome
		C/D	C/C				Small metacentric marker
2 (72 h)	2	0	1/C	4	1 — D group chromosome	3	Translocation D/C group chromosome (Marker)
			C/C		1 — D group chromosome		Inversion — No. 1 chromosome
					1 — D group chromosome		Inversion — C group chromosome
					1 — No. 3 chromosome		
3 (72 h)	5	F/F	C/C	2	1 — No. 3 chromosome	2	Translocation — B group chromosome (Marker)
		C/C	G/G (TA)		1 — No. 1 chromosome		Inversion — C group chromosome
			2/C				

[a]All the rings are without fragments, and appeared to be formed as a result of telomere fusion/association (TA).
[b]Chromosomes involved.

present communication is to report a cytogenetic profile of QDH. It is too early to present any quantitative data since dose–response relationship with adequate sample size remains to be established. A shortcoming of this communication relates to the lack of chromosome aberrations, particularly dicentrics in the control (untreated) cultures (Table 1). The baseline frequency of dicentrics reported from the normal populations shows a wide range of variation [10] ranging from 0.2 to 2.1 per 1000 cells [11,12]. Based on the compilation of over 60 investigations (comprising 2000 subjects and 211,661 cells), Lloyd et al. [13] estimated the average spontaneous incidence of dicentrics to be 0.55 per 1000 cells. During our earlier studies, the incidence of dicentrics has been comparable, i.e., 2 dicentrics per 5000 cells or 0.40 per 1000 cells [8]. Recently, Lloyd et al. [14] have reported a spontaneous incidence of dicentrics to be about 1 per 1000 cells, and that of centric rings as low as 1 in 60,000 cells.

In QDH-treated (0.6 μg/ml) lymphocytes, the incidence of dicentrics at 48- and 72-h cultures was 4.3 and 2.3 per 1000 cells, respectively. About 12 of the dicentrics (60%) were without fragments (Table 2). In about four of such dicentrics (20%), a clear telomere fusion/association of the D and G groups of chromosomes was observed. Likewise, RCs in QDH-treated samples were in large numbers (7/6000 cells), particularly in 72-h cultures (6/3000 cells), taking into account the rare occurrence of rings reported in the literature for control populations. All the rings likewise appeared to be formed as a result of telomere fusion/association. Among the seven rings observed, D group chromosomes seem to be involved in three cases, chromosome No. 3 in two instances, chromosomes Nos. 1 and 2 in one instance each. Thus, the frequent involvement of chromosomes 1, 2 and 3 in both unstable and stable chromosome rearrangements was apparent (Table 2). QDH at the same concentration (0.6 μg/ml) also increased the frequency of MN in the cytochalasin B blocked binucleated lymphocytes. The frequency of MN observed in control culture was 0.005 and 0.032 per cell in 0.6 μg/ml quinacrine-treated culture from the single sample analyzed. The frequency of binucleate cells in control and treated was 53.5%, 59.5%, and NDI 1.81, 2.27, respectively. However, no increase in SCEs was observed at this concentration. A small but significant increase in SCEs has been reported in human lymphocytes treated with 0.5 μg/ml quinacrine in vitro [15].

4. Discussion

Despite an organized effort for clinical trials resulting in the exposure of about 100,000 women across the world, an astonishingly limited attention has been paid to the safety and genotoxicity evaluation of QDH, particularly in mammalian systems. A critical analysis of the data on the toxicity and mutagenicity of QDH reviewed recently [3,16,17] revealed the incompleteness of these studies. Quinacrine, like other aminoacridines, is a frameshift mutagen, known to induce mutations in *Salmonella* and *Escherichia coli* [16]. In silk worm, it has been reported to produce mosaic-type mutations among females in a specific locus test [16] but did not induce sex-linked recessive lethals in Drosophila [16]. The effect of QDH in CHO and other mammalian cells varies from being clastogenic [18] to antimutagenic [16] and even radiosensitising [16]. In mice bone marrow erythroblasts, however, quinacrine induced lysis of the nucleus resulting into MN-like structures, which led to a controversy resolved by fluorescence; but increased chromosome aberrations was not evident [16]. A single dominant lethal mutation study based on few animals also gave equivocal results in mice [16]. Likewise, in an in vivo study of quinacrine exposed monkeys, 20 banded metaphases analyzed per specimen was an extremely small sample to arrive at any reliable conclusions in respect of chromosomal damage in the bone marrow of the animals [19]. Quinacrine has not been evaluated for carcinogenicity at all.

To our knowledge, in the only human report in humans published so far, an abnormal marker chromosome was found in peripheral blood lymphocytes of patients taking 0.3 g of quinacrine for 14 days for the treatment of protozoan infection [20]. The present investigation is, perhaps, the first one to show that quinacrine induces stable and unstable chromosome aberrations in human lymphocytes in vitro and features unique properties, ranging from modulation of cell proliferation to spindle inhibition. The population exposed to QDH during clinical trials for steril-

ization is the most appropriate material to assess the ability of this drug to induce any genetic damage in humans in vivo. In addition to cytogenetic studies, detailed genetic investigations including the monitoring of the health profile, reproductive outcome, and for neoplastic changes is warranted to discern any health risk to the exposed population.

Additionally, the cytogenetic profile of QDH seems to be extremely interesting, in respect of preponderance of the formation of ring-like configurations and dicentrics. QDH seems to promote telomere fusion/association as several ring-like configurations were formed without any evidence of fragments (Fig. 1). In the present study, the RCs and some of the dicentrics observed in QDH-treated cultures seemed to have arisen by end-to-end fusions and in their formation, no loss of chromosomal material could be recognized. However, though a rare phenomenon, the telomere fusion/association of human chromosomes resulting in dicentric formation often becomes indistinguishable from the classical dicentrics. Such a phenomenon has been described in patients of ataxia telangiectasia, in SV 40 transformed epidermal fibroblasts and kidney cells, human embryonic fibroblasts undergoing senescence and in various tumour cells and even from an apparently normal individual [21,22]. Aphidicolin, an inhibitor of DNA polymerase, also enhances telomeric association in treated cultures [22,23]. Telomere association is being considered as a potential source of new stable cytogenetic rearrangements that may have a role in oncogenic transposition and tumor etiology or might confer a selective growth advantage on tumor cells. In a very recent study, telomerase activity and length of telomeres of peripheral blood mononuclear cells, obtained from 124 healthy individuals aged 4–95 years revealed that 65% of those aged ≥ 40 years, had relatively stable but very low telomerase activity [24]. Inhibitors of DNA topoisomerase II, which may give rise to DNA DSBs, also induce chromosomal aberrations in all phases of the cell cycle, acting by an 'S'-independent mechanism, similar to ionizing radiation [25]. In the light of emerging evidence from diverse sources converging to show the involvement of telomerase activity, telomeric associations, topoisomerase II, and chromosome stickiness in the induction of chromosomal rearrangements [26–30], it would be interesting to elucidate the mechanisms underlying the cytogenetic effects of QDH, particularly the formation of ring-like configurations. Taking into consideration its cytogenetic profile, it would also be interesting to examine the effect of quinacrine on topoisomerase II and DNA repair enzymes. A recent prospective clinical study of 200 women monitored in Indonesia, 6, 12, 24, and 48 months after quinacrine and ibuprofen treatment has concluded that the intrauterine insertion of quinacrine pellets is a safe, acceptable, and effective method of non-surgical female sterilization [31]. This is an extension of earlier such papers published, which advocated and promoted the use of quinacrine as a female chemosterilant without looking into long-term safety aspects. In the light of our observations, long-term safety aspects of quinacrine should be probed. (This work was presented during the 13th Intl. Chromosome Conference held at Ancona, Numana, Italy on September 8–12, 1998 and appeared as an abstract in Cytogenetics and Cell Genetics [32].)

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