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Enhancement by caffeine of sister-chromatid exchange frequency induced by antineoplastic agents in human lymphocytes

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Summary. The SCE frequency induced by Thiotepa and the effect of this antineoplastic drug in combination with caffeine have been studied in cultures of human peripheral blood. Caffeine was found to enhance SCE and breakage frequencies induced by Thiotepa in human lymphocytes.

Caffeine has been reported to be without effect on DNA repair mechanisms in human cells[2-4] but we have already reported that this substance enhances the effect of several SCE-inducing chemicals in cultured human lymphocytes[5]. Caffeine which is known to increase the frequency of chromosome aberrations induced by UV and many chemical substances in rodent and plant cells, apparently reduces the frequency of SCEs produced by UV, MMC, 4-NQO and Triaziquone in rodent cells[6,7] but in human lymphocytes following treatment with MMC, 8-Methoxypsoralen plus UV-light[8], 4-NQO or Trenimon the effect of post-treatment with caffeine is to enhance SCE formation[5]. Caffeine does not potentiate chromosome damage induced in human lung embryonic (LU 106) cells by Thiotepa and MMC[9] although caffeine post-treatment does potentiate the chromosome aberration frequency induced by MMC in a normal human fibroblast strain and in 2 *Xeroderma pigmentosum* strains (XP4LO and XP7TA) but at different threshold values[10]. I now report the effect of caffeine on SCE rate and breakage frequency in human lymphocytes following treatment with Thiotepa, a trifunctional cross-linking agent. Results are also presented concerning the time factor in caffeine potentiation of the MMC-induced SCE frequency in these cells.

Material and methods. SCEs were demonstrated in human lymphocytes from normal subjects by growing PHA stimulated cultures of whole blood in the presence of 4 µg/ml BUDR for 72 h. Thiotepa was given with BUDR at 18 h after initiation of the culture with PHA. Caffeine at 100 µg/ml (5×10^{-4} M) was added at 18 h of culture life. The effect of adding caffeine at different times after treatment with MMC was investigated by introducing the caffeine (5×10^{-4} M) immediately after treatment, 18 h post-treatment and 48 h post-treatment. In these experiments MMC was added at the time of initiation of the culture with PHA and the cultures harvested, as usual, at 72 h of culture life.

Results and discussion. The results are shown in table 1 and indicate that when caffeine is added to the cultures exposed to Thiotepa at doses sufficiently great to induce SCEs the effect of the caffeine is synergistic, the SCE level achieved being consistently much greater than that expected by the simple addition of the effect by the Thiotepa and caffeine. A synergistic effect can also be seen for chromosome aberrations (table 2). Caffeine produces a relative reduction in the number of cells that reach 2nd and later divisions. Potentiation of MMC-induced SCE level was found when caffeine was present for any of the 3 periods tested but the extent of synergism varied being highest when caffeine was introduced at 18 h (54 h post-treatment) after initiation of the culture (table 3).

The difference between these results on human lymphocytes and the findings on rodent material[6,7] might be accounted for by the different cell types used. In cultured rodent cells in which there is little excision repair caffeine interferes with post-replication repair[11] which may be responsible for the drop found in SCE frequency. Kato[6] concluded that SCEs were the result of errors in a repair process that includes a caffeine sensitive step. Vogel and Bauknecht[7] refrained from drawing conclusions about their rodent data because they were distorted by the treatment (agent plus caffeine) influencing the cell cycle and reducing the mitotic index, which they consider may distort any influence of caffeine on the incidence of SCEs mediated by post-replication repair. In the present experiments using human lymphocytes, although the rate at which the cells are cycling appears to be reduced by Thiotepa and caffeine (table 2), the treatment results in an enhancement, instead of suppression, of SCE frequency. The finding that caffeine

Table 1. Comparison of the effect of Thiotepa on SCE rates in the presence and absence of caffeine

Thiotepa concentration (ng/ml)	Without caffeine		100 µg/ml caffeine	
	Mean SCE/cell	No. of cells	Mean SCE/cell	No. of cells
Control	7.3	53	8.7	75
3	7.7	60	10.3	60
30	14.8	53	18.1	60
60	22.6	39	35.7	60
150	35.7	60	57.7	60
300	59.2	55	69.2	22

Table 2. Comparison of the effect of Thiotepa (150 ng/ml) on breakage rates in the presence and absence of caffeine (100 µg/ml)

Treatment	No. of cells analyzed	Cells (aberrant cells) in 1st division	in 2nd division	in subsequent division	Aberrations Gaps	Breaks	Other aberrations (fragments, dicentric rings)	Total	Aberrant cells No.	%
Control	123	18 (2)	70 (5)	35 (2)	-	-	9	9	9	7.3
Caffeine	133	5 (2)	87 (9)	41 (5)	2	1	13	16	16	12.0
Thiotepa	150	26 (6)	94 (17)	30 (5)	3	2	23	28	28	18.7
Thiotepa + caffeine	150	53 (20)	87 (25)	10 (4)	8	3	46	57	49	32.7

Table 3. SCEs observed in 72 h cultures after treatment with 8 ng/ml MMC and followed by various durations of caffeine (100 µg/ml) treatment

Treatment	Mean SCE/cell	No. of cells
MMC	22.4	112
MMC + caffeine (at the initiation of culture)	29.2	52
MMC + caffeine (18 h after initiation of culture)	39.0	29
MMC + caffeine (48 h after initiation of culture)	26.7	60
Control	7.9	60
Caffeine (18 h after initiation of culture)	9.1	60

produces a relative reduction in the number of cells that reach 2nd and later divisions by 72 h is not surprising, since caffeine has been shown to inhibit or delay mitosis in a wide range of cell types acting either alone or in combination with other agents, although in a few cases an increase in mitotic index has been reported[12]. Caffeine exhibits 2 distinct actions in connection with cell viability. In some systems it is itself lethal usually at high concentrations (e.g. 10^{-2} M caffeine kills all cells in human lymphocyte culture[12]), while in others it is synergistic.

The highest synergism with post-caffeine treatments upon MMC-induced SCE rates, occurred when caffeine was introduced at 18 h. This observation is in a good agreement with results reported for chromosome aberrations by Hartley-Asp[13] who found that the frequency of these induced in rat-kangaroo cells by MMC was enhanced much more when caffeine was given 6 h after the inducer treatment than when caffeine post-treatment followed immediately after the treatment with the inducer. The observation that the lowest potentiation occurs when caffeine is introduced 48 h after initiation of the culture (24 h duration) is in agreement with results reported by Kihlman et al.[14], which show that the potentiation of chemically induced aberrations by caffeine is strongly manifested only when it is present during the 1st growth phase after treatment of plant cells with these agents. Sturelid and Kihlman[15] using root tips of *Vicia faba* found that whereas the Thiotepa induced chromosome damage was potentiated much more when the caffeine post-treatment was given after a 5-h interval than when it followed immediately after the inducer, the induced damage by monofunctional alkylating agents was not enhanced more by a delayed than by an immediate post-treatment with caffeine. Sturelid and Kihlman[15] suggest that the time dependence of caffeine potentiation is characteristic of agents that produce crosslinks in DNA. The present results with MMC, a crosslinking agent, may be compared with these reports of chromosome aberrations and support Sturelid and Kihlman's[15] suggestion. In the present experiments with Thiotepa, caffeine was given immediately after the inducer treatment. Thus the effect of caffeine upon Thiotepa-induced SCE levels in these experiments may represent an underestimate.

Although there is little evidence to suggest that caffeine which appears to have high affinity for partially denatured DNA[16], does have an effect on excision repair in human cells[17], a small effect in the efficiency of this repair system may be sufficient to bring about an increase of the incompletely repaired lesions by the time the cells reach S phase. We have already suggested that in human lymphocytes undertaking DNA repair after being damaged by alkylating agents, caffeine may interfere with excision repair and this might lead to the increase in the number of unrepaired lesions at the S phase which may subsequently give rise to SCEs and chromosome aberrations[5].

There is evidence to suggest that the main action of caffeine in the enhancement of antitumour effects of X-rays and alkylating agents in the hamster[18–20] and of 1,3-bis(2 chloroethyl)-1-nitrosourea against murine L 1210 leukaemia[21,22] is inhibition of DNA repair. Post-treatment with caffeine diminishes 4-NQO induced lung tumour production in the mouse and this antitumour action is also attributed to the ability of caffeine to inhibit DNA repair in mouse cells[23]. Although the relationship between SCEs, chromosome aberrations and DNA repair mechanisms is still speculative, SCEs are being increasingly used as highly sensitive indicators of chromosome damage and/or subsequent repair[5,6,24,25]. Cancer therapy in many instances is designed to produce severe damage to the neoplastic cell. However if an agent penetrates a cell and produces damage, its biological effect may be minimized by the cell's capacity for repair[19]. If caffeine does interfere with DNA repair in man, as seems likely, it should be possible to increase the effectiveness of the antineoplastic agent by inhibiting the excision repair system. Further studies in this direction should be prove rewarding.

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The effect of protein-energy malnutrition on appositional bone growth in the rat[1]

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Summary. During protein-energy malnutrition appositional bone growth in the seventh caudal vertebra of the rat slows and finally ceases. During rehabilitation appositional growth begins agains and attains a rate in excess of that of the controls. This may account for alterations in skeletal proportions resulting from malnutrition.

Protein-energy malnutrition in the weanling rat is characterized by growth failure, with suppression of longitudinal bone growth[3,4]. Studies from this laboratory have demonstrated that longitudinal bone growth slows significantly by the 1st week of severe malnutrition and by the 2nd week has ceased[5]. Others[6] have reported that malnutrition is accompanied by alterations in bone width, relative to length. This may be due to a differential effect of malnutrition on appositional and longitudinal bone growth. The experiments reported here were undertaken to examine the effect of protein-energy deficiency on appositional bone growth in the rat, as assessed by the deposition of fluorescent dyes in the caudal vertebrae[7].

Methods. Male Sprague-Dawley rats (Biobreeding Laboratories, Ottawa, Canada) weighing approximately 150 g each were housed in plastic cages and fed either the 18% lactalbumin (control) or the 0.5% lactalbumin (protein-deficient) diet described by Edozien[8]. After consuming the protein-deficient diet for 12 weeks, some animals were rehabilitated for an additional 4 weeks on the control diet. Controls remained on the control diet throughout the experiment. Food and water were available ad libitum. Food consumption was not recorded.

Control and experimental animals were given tetracycline and DCAF (2,4-bis(N,N′-di′(carboxymethyl)aminomethyl)fluorescein) according to the schedule shown in the table. Both were administered at 50 mg/kg b.wt, by i.p. injection. Animals were sacrificed 6 days after receiving the last injection of dye (groups I and IV at 2 weeks, 6 days; groups II and V at 12 weeks, 6 days; and groups III and VI at 16 weeks, 6 days). The 7th caudal vertebra of each animal was removed, cleaned of adhering tissue, and fixed in buffered formol-saline. The bones were embedded in methylmethacrylate and undecalcified sections were cut transversely with a Gillings-Hance rotary diamond disc. Sections were mounted on glass slides, ground to a thickness of approximately 20 μm, and photographed with fluorescence optics. Tetracycline and DCAF deposition can be distinguished, as the former has a yellow fluorescence, while the latter has a green fluorescence. Photographic prints (50× enlargements) were prepared, and the distance between fluorescent lines adjacent to the periosteal surface was measured at 20 locations on each section, as described by Hammond and Storey[7]. The mean width of each growth band was calculated and appositional bone growth (μm/week) was estimated as the distance between adjacent fluorescent lines, divided by the number of weeks between the administration of the dyes.

Results. Protein-energy malnutrition resulted in an early cessation of weight gain, followed by gradual weight loss; weight gain was rapid during rehabilitation (figure 1). Appositional bone growth of control animals was rapid during the 1st week, but was considerably less when averaged over the first 4 weeks. A further decline is seen between the 1th and 12th weeks and during the periods of 12–14 and 14–16 weeks (figure 2). However, the protein-energy malnourished animals showed a subnormal rate of appositional bone growth as early as the 1st week, and this is further reduced during the first 4 weeks, as well as from the 4th to the 12th weeks. During the first 2 weeks of rehabilitation no appositional bone growth is measurable, but during the next 2 weeks there is a resurgence of appositional growth, much in excess of that of the controls of the same age.

Discussion. It is well recognized that in many animal species, including man, protein and/or energy deficiency is accompanied by a suppression of skeletal growth. Some workers[3,9] have emphasized that even under conditions of severe malnutrition skeletal growth slows, but does not cease. There is some indication that longitudinal bone growth is related to body weight, while bone width is more closely related to age[6], suggesting that longitudinal bone growth may be more severely affected by malnutrition than would be bone width. Thus appositional bone growth may be expected to continue during periods of growth delay, resulting in alterations in the proportions of the long bones.

Schedule of administration of tetracycline (t)* and DCAF (D)* to control, protein-energy deficient, and rehabilitated rats

Group	No.	Time (weeks consuming diet)					
		0	1	4	12	14	16
		Controls					
I	2	T	D				
II	5	T		D	T		
III	5				T	D	T
		Restricted			Rehabilitated		
IV	4	T	D				
V	4	T		D	T		
VI	6				T	D	T

* 50 mg/kg b.wt, i.p.