Sister chromatid exchanges induced by methylxanthines contained in coffee, tea and cocoa

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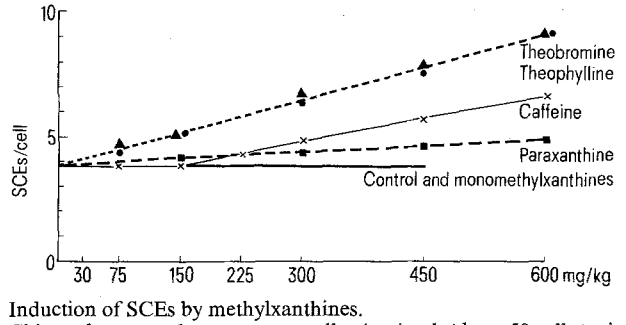
Summary. Methylxanthines consumed daily by most humans were investigated for induction of sister chromatid exchanges (SCE). Effects observed in this highly sensitive in vivo system decreased in the order theophylline/theobromine > caffeine > paraxanthine > monomethylxanthines.

Caffeine (1,3,7-trimethylxanthine), theophylline (1,3-dimethylxanthine) and theobromine (3,7-dimethylxanthine) cause various mutagenic activities in in vitro systems. These effects can be most evidently induced by caffeine, and less distinctly by theophylline and theobromine[1-5]. In several short-term mutagenicity tests on mammals these 3 methylxanthines did not show mutagenic effects[6,7]. Doses of caffeine administered to mice for some days or chronically did not exhibit mutagenic effects in the dominant lethal test[8,9], and no induction of specific locus mutations was found in about 64,000 offspring of caffeine-treated males or females[10]. Caffeine has synergistic effects with mutagens[11] and is an inhibitor of post-replication repair in eucaryotic organisms[12]. The Salmonella/mammalian microsome mutagenicity test with caffeine shows negative or weak effects[13]. Using the sister chromatid exchange (SCE) test as a sensitive measure of DNA damage an increase of the SCE rate in Chinese hamsters[11] and in mice[14] by caffeine was observed at high doses. With the same test system a more pronounced effect of theobromine was established recently in our laboratory[15]. In early 1981 the US Food and Drug Administration deleted coffee from the GRAS list (substances which are generally recognized as safe).

The metabolism of methylxanthines has been studied by several authors: Caffeine is demethylated (in humans) into the 3 primary metabolites theophylline, theobromine and paraxanthine (1,7-dimethylxanthine). A small portion of theobromine and theophylline is excreted unchanged in the urine. Furthermore methyluric acids and monomethylxanthines appear. The organism does not demthylate beyond the monomethylxanthine stage. The metabolic pathways of theobromine and theophylline differ because the methyl groups in theophylline are both on the pyrimidine ring leaving the imidazole ring unsubstituted. It has been suggested that this may be the reason why oxidation without demethylation is a major pathway for theophylline but not for theobromine[16,17]. It is presumed that methylation at position 3 - combined with an other methylated position - is of greatest importance for the action on chromosomes[3-5].

In our investigations bone marrow cells of Chinese hamsters were used. The SCE test was performed applying the 'tablet method'[18,19]: A bromodeoxyuridine mini tablet implanted s.c. in the animals causes a continuous incorporation of BrdU instead of thymidine into the DNA. After 2 cell cycles a special staining technique reveals differentially stained chromatids exhibiting SCEs. After administration of the test substances (dispersed in corn oil and given by stomach tube) dose relationships for caffeine, theophylline, theobromine and paraxanthine were established. In agreement with other investigators who use the SCE test results are considered as positive when the numbers of SCEs/cell reach at least $1\frac{1}{2}$ times those of control (≥ 5.7 SCEs/cell corresponding to $p < 0.0001$, t-test). The frequency of SCEs as a function of dose (mg/kg b.wt) is shown in the figure. Equal doses of theophylline and theobromine produced the same rates of SCEs in this test system. Caffeine produced a distinctly weaker effect and increased the SCE rate only when doses above a threshold of 150 mg/kg b.wt were applied whereas paraxanthine induced only a very small increase of SCEs. The monomethylxanthines (1-methylxanthine, 3-methylxanthine and 7-methylxanthine) showed no effects.

The hypothesis that methylation at position 3 is of highest importance for the action on chromosomes seems to be confirmed by the ineffectiveness of paraxanthine as compared to the other tested compounds. The mentioned difference in the metabolism of theobromine and theophylline is not reflected by the SCE test which indicates that the substance itself generates the mutagenic effect and not its metabolites.



Induction of SCEs by methylxanthines.
Chinese hamsters, bone marrow cells; 4 animals/dose, 50 cells/animal; the variation coefficient of the SCE-values ranges from 2 to 5%.

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