Coffee-mediated protective effects against directly acting genotoxins and gamma-radiation in mouse lymphoma cells

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Received 9 September 2003; accepted 16 March 2004

Keywords: apoptosis, coffee, cytokinesis block micronucleus test, gamma-radiation, genotoxic chemicals, mouse lymphoma cells, protective effects

Abstract

The cytokinesis-block micronucleus test was performed using L5178Y mouse lymphoma cells to ascertain whether or not standard (caffeinated) instant coffee, the commonly consumed polyphenolic beverage with antioxidant activity can protect against chromosomal damage induced by the directly acting agents N-methyl-N-nitro-N-nitrosoguanidine (MNNG), mitomycin C (MMC), methyl methanesulfonate (MMS) and gamma radiation. Our results demonstrated significant reductions in the in vitro genotoxic effects of MNNG, MMC, and MMS following co-treatment of mouse lymphoma cells with standard instant coffee. Subsequently, the comet assay was carried out to assess the effect of coffee co-treatment on the level of DNA damage induced by MMS in mouse lymphoma cells. The results demonstrated a significant reduction in MMS-induced DNA damage following co-treatment with standard instant coffee. Protective effects were observed in mouse lymphoma cells which were treated with coffee immediately after exposure to gamma radiation (1 and 2 Gy). Another experiment showed protection when the mammalian cells were irradiated (0.5 and 1 Gy) midway (at 2 h) during a 4 h coffee treatment. However, the protective effect against the lower dose (0.5 Gy) was not significant. In addition we assessed the modulatory effect of coffee on MNNG-induced apoptotic frequency by flow cytometry. The results revealed only a minor influence of coffee on the frequency of apoptotic cells induced by the test compounds, rendering an increase in sensitivity for apoptosis as a reason for the reduced genomic damage an unlikely or at least incomplete explanation.

Abbreviations: MN, micronucleated; BN, binucleate; GR, gamma-radiation; MMC, mitomycin C; MMS, methyl methanesulfonate; MNNG, N-methyl-N-nitro-N-nitrosoguanidine

Introduction

The health effects of coffee have been viewed with considerable concern because it is one of the two most popular beverages in the world. Apart from the extensive work on the pharmacological effects of coffee, a large number of investigations are being carried out to assess the possible influence of coffee consumption on genotoxicity and carcinogenicity. In many countries, epidemiological surveys have been carried out to ascertain the associa-
tion between coffee intake and cancer in different sites (Hartman et al., 1998; Lin et al., 2002; Michel et al., 2002; Petridou et al., 2002; Woolcett et al., 2002). To date, the information that has emerged from these studies suggest the lack of conclusive evidence for a correlation between intake of coffee and cancer in any specific organ. The findings suggest that the response can differ depending on the site under investigation. Interestingly, there are reports which suggest an inverse relationship between coffee drinking and the risk of oral, pharyngeal, esophageal and colon cancer (IARC, 1991; Tavani and La Vecchia, 2000; Tavani et al., 1997, 2003; Rodriguez, 2004). However, a recent publication has cautioned that it may be premature to arrive at a conclusion on the ‘protective effect’ of coffee intake against colon cancer (Terry et al., 2001). In contrast, studies using animal models have clearly demonstrated that coffee can exert protective effects against carcinogenesis by modulating the activity of enzymes involved in the metabolic activation/detoxification of procarcinogens (Wattenberg and Lam, 1984; Cavin et al., 2002; Huber et al., 2003).

Laboratory investigations have furnished substantial evidence for the in vivo antigenotoxicity of coffee. Abraham (1989) demonstrated for the first time the in vivo antigenotoxic effects of different coffee preparations against cyclophosphamide, mitomycin C and procarbazine in the mouse bone marrow micronucleus test. Subsequent studies using mice have shown the protective effects of coffee against a wide range of genotoxic carcinogens like aflatoxin B1, benzo(a)pyrene, urethane, diethylnitrosamine and 7,12-dimethylbenz(a)anthracene (Abraham 1991, 1995; Abraham and Singh, 1999). Aeschbacher and Jaccaud (1990) reported the in vivo inhibitory effect of coffee against DNA damage induced by nitrosation. Protection against somatic mutation and recombination (leading to loss of heterozygosity) has been observed in Drosophila following co-administration of coffee with cyclophosphamide, mitomycin C and urethane (Abraham, 1994; Abraham and Graf, 1996; Graf et al., 1998).

Since coffee is habitually consumed all over the world, the likelihood of its interaction with DNA-damaging environmental agents leading to modulatory effects cannot be ignored. At present, there is emphasis on the need for treating coffee drinking as a potential effect modifier of carcinogenic exposures (Porta et al., 2003). This calls for more studies on genotoxicity and antigenotoxicity of coffee. However, there is a need to carry out such investigations by avoiding or minimizing the use of laboratory animals. Hence it would be of interest to examine the feasibility of using in vitro mammalian test systems for assessing the antigenotoxic effects of complex mixtures like coffee. Therefore we initiated the present work with the main aim of evaluating in the cytokinesis-block micronucleus test using mouse lymphoma cells, the modulatory effects of instant coffee on chromosomal damage induced by MNNG, MMC, MMS and gamma-radiation. Coffee is already known to inhibit the genotoxicity of MMC in in vivo test systems (Abraham, 1989, 1994, 1995; Abraham and Graf, 1996). Hence this study using MMC would also enable a comparison of the response in in vivo and in vitro test systems.

In the present study, results obtained from the cytokinesis-block micronucleus test experiments revealed the protective effects of standard instant coffee against chromosomal damage induced by the directly acting genotoxins MNNG, MMS, and MMC. This observation evoked interest in performing the comet assay to assess the level of DNA damage induced by a directly acting genotoxin with or without co-treatment with standard instant coffee. The single cell gel electrophoresis/comet assay is known for its high sensitivity to detect low levels of DNA damage induced in vitro or in vivo (Tice et al., 2000). Therefore, in