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GENOTOXIC PROPERTIES OF 2-DODECYLCYCLOBUTANONE, A COMPOUND FORMED ON IRRADIATION OF FOOD CONTAINING FAT

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ABSTRACT

When food containing fat is treated by ionizing radiation, a group of 2-alkylcyclobutanones is formed. These components contain the same number of carbon atoms as their precursor fatty acids and the alkyl group is located in ring position 2. Thus, from palmitic acid 2-dodecylcyclobutanone is derived. To date, there is no evidence that the cyclobutanones occur in unirradiated food. Therefore, these components cannot be considered inherent to food, and for questions pertaining to risk assessment of irradiated food it would be advisable to determine the genotoxic and toxic potentials of cyclobutanones. Measurements of DNA damage in cells exposed to 2-dodecylcyclobutanone, employing the single cell microgel electrophoresis technique, have been carried out. *In vitro* experiments using rat and human colon cells indicate that 2-dodecylcyclobutanone in the concentration range of about 0.30 - 1.25 mg/ml induces DNA strand breaks in the cells. Simultaneously, a concentration related cytotoxic effect is observed as was determined by trypan blue exclusion. To which extent these *in vitro* findings are of relevancy for the *in vivo* human exposure situation needs to be investigated in further studies. *In vivo* tests in rats are in progress.

KEYWORDS

Food irradiation; cyclobutanone; genotoxicity; comet assay

INTRODUCTION

Food irradiation is a thoroughly tested technique and numerous studies have led to the conclusion "that irradiated food produced in accordance with established good manufacturing practice can be considered safe because the process of irradiation will not lead to changes in the composition of the food that, from a toxicological point of view, would have an adverse effect to human health" [WHO, 1994]. It is well-known that some radiolytic products are formed in very low quantities, which may cause some health hazards only if consumed in amounts much higher than actually present in irradiated food. Nevertheless, it is desirable to gain knowledge on the toxic potential of the individual radiolytic products formed. Since the very great majority of radiolytic products also are found in native or otherwise processed food, many toxicological evaluations of these radiolytic products have been carried out in the past, and are set in perspective to other levels of human exposure.

Twenty-five years ago, it was reported that on irradiation of triglycerides, a cyclic compound is formed of the same carbon number as the esterified fatty acid. This compound was identified as the 2-alkylcyclobutanone [LeTellier and Nawar, 1972]. Recently, these compounds have also been identified in irradiated food, and they were proposed to be a marker of the irradiation treatment [Stevenson *et al.*, 1990; Stevenson, 1996]. In fact, an analytical detection method for irradiated food based on the formation of 2-alkylcyclobutanones in fat-containing food has now been standardized on a European level (EN 1785 : 1996). It is claimed that e.g. 2-dodecylcyclobutanone (2-DCB) derived from palmitic acid is radiation-specific and has never been detected in any non-irradiated or microbiologically spoiled food [Stevenson, 1996]. Maybe that improved analytical techniques in future will find 2-alkylcyclobutanones also in otherwise treated foodstuffs at extremely low levels. However, at present it is known that these compounds are especially produced in a dose-dependent manner in irradiated food, and therefore an assessment of the health hazard of these 2-alkylcyclobutanones would be advisable.

In this paper, the genotoxic potential of 2-DCB was assessed using the "comet assay", which measures DNA strandbreaks in cells [McKelvey *et al.*, 1993; Fairbairn *et al.*, 1995]. According to a parallelogram approach [Pool-Zobel *et al.*, 1994], the test compound 2-DCB will be subjected to *in vitro* studies using rat and human colon cells, and subsequently an *in vivo* study with rats will be carried out. We used primary rat and human colon cells since the colon is an important target tissue for many food-related cancers. In this paper, the first *in vitro* estimations of the genotoxic potential of 2-DCB are reported.

EXPERIMENTAL

2-DCB was obtained, synthesized as described by Boyd *et al.*(1991), from Dr. C.H. McMurray (The Department of Agriculture for Northern Ireland, Belfast, UK). Rat colon cells were freshly isolated from rat colon using an *in situ / ex vivo* digestion procedure [Brendler-Schwaab *et al.*, 1994]. Human colon cells were isolated from biopsies [Pool-Zobel and Leucht, 1997].
Rat and human colon cells were incubated with 2-DCB in the concentration range of 0.30-1.25 mg/ml for 30 minutes at 37°C. Both the cytotoxicity test using the method of trypan blue exclusion and the DNA comet assay were performed as described by Pool-Zobel *et al.* (1994) and Pool-Zobel and Leucht (1997).

RESULTS AND DISCUSSION

A novel technique to detect genotoxic effects of chemicals is the comet assay, a micro gel electrophoresis of single cells to measure DNA damage. Freshly isolated colon cells were chosen since they are metabolically competent and expected to convert chemicals as in *in vivo*-like conditions.

Cytotoxicity of 2-DCB in rat colon cells is observed at increasing concentrations as shown in Fig. 1a. Toxicity was apparent at 1.25 mg/ml as a reduction in the percentage of viable cells (absolute viability below 80 %). Relative viability (based on 100 % viable cells in the untreated control at 0 mg 2-DCB) gave similar results.

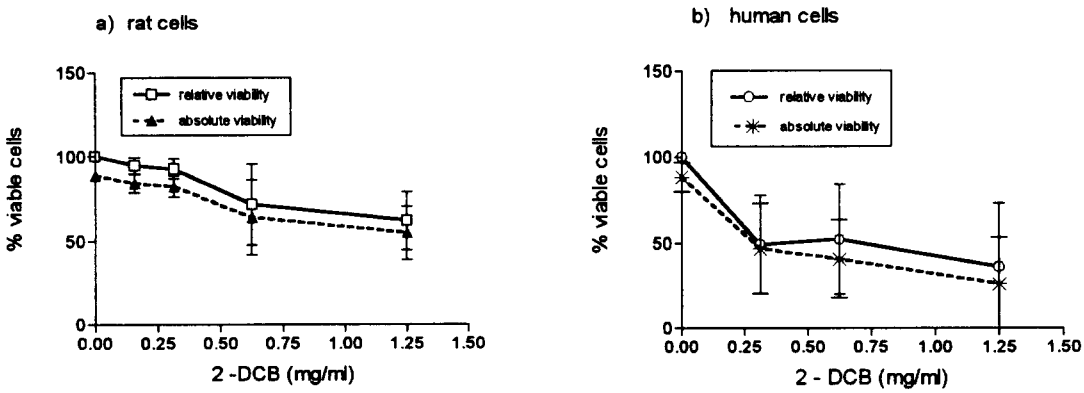

Fig. 1 a,b Viability of colon cells incubated with 2-DCB at various concentrations for 30 minutes at 37°C (means ± SEM of 3 independent experiments).

In further experiments the DNA damage under the given experimental conditions - DNA single strand breaks - were measured in rat colon cells (Fig.2). The tail moment of the comets, which is a function of "tail length" and "intensity of fluorescence in the tail", was chosen as the parameter for DNA damage [Fairbairn *et al.*, 1995].

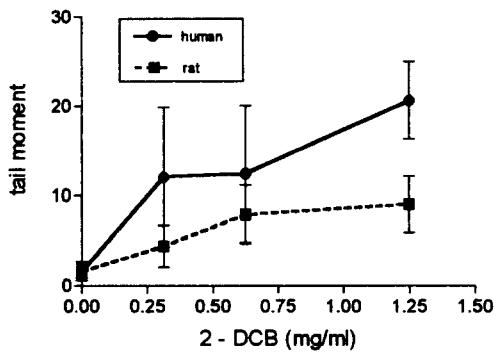

Fig. 2 DNA single strand breaks in rat and human colon cells induced by 2-DCB at various concentrations for 30 minutes at 37° C.
(means ± SEM of 4 rats and 3 separate human colons, 50 cells for each point)

Fig.1b shows the viability of 2-DCB on human colon cells (from biopsies), and a cytotoxic effect with increasing dosage is clearly demonstrated. Human colon cells seem to be more sensitive than rat colon cells, since the viability is decreased to less than 50 % at the highest concentration of 2-DCB (i.e. 1.25 mg/ml) tested. The higher sensitivity of human colon cells is also found in response to DNA damage, higher tail moments being measured (Fig.2).

These in *vitro* results clearly indicate a genotoxic effect of 2-DCB. However, concentrations tested are very high compared with actual human intake. Amounts of 2-DCB in irradiated food will vary dependent on radiation dose and other irradiation parameters, storage and storage conditions, and of course on the amount and kind of fat in the food. For chicken, amounts of about 0.3 - 0.6 µg 2-DCB / g lipid / kGy have been reported. In highly irradiated chicken meat from the Raltech study (mean radiation dose about 58 kGy), amounts of 17 µg/g lipid were still

found after 12 years of storage [Crone *et al.*, 1992]. In the Raltech study no adverse effects attributable to the irradiation treatment were observed [Thayer *et al.*,1987]. Thus, a possible risk from 2-DCB must be at a very low level. In order to assess and quantitate this minimal risk from the intake of 2-DCB with irradiated food, more experiments than these preliminary ones are required. An *in vivo* test in rats is in progress.

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