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Metabolic activation of herbicide products by *Vicia faba* detected in human peripheral lymphocytes using alkaline single cell gel electrophoresis

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Abstract

Ametryn and metribuzin *S*-triazines derivatives and EPTC thiocarbamate are herbicides used extensively in Mexican agriculture, for example in crops such as corn, sugar cane, tomato, wheat, and beans. The present study evaluated the DNA damage and cytotoxic effects of three herbicides after metabolism by *Vicia faba* roots in human peripheral lymphocytes using akaline single cell gel electrophoresis. Three parameters were scored as indicators of DNA damage: tail length, percentage of cells with DNA damage (with comet), and level DNA damage. The lymphocytes were treated for 2 h with 0.5–5.0 μg/ml ametryn or metribuzin and 1.5–10 μg/ml EPTC. Lymphocytes also were coincubated for 2 h with 20 μl *V. faba* roots extracts that had been treated for 4 h with 50–500 mg/l of the two triazines or with the thiocarbamate herbicide or with ethanol (3600 mg/l), as positive control. The lymphocytes treated with three pesticides without *in vivo* metabolic activation by *V. faba* root did not show significant differences in the mean values bewteen genotoxic parameters compared with negative control. But when human cells were exposed to three herbicides after they had been metabolized the frequency of cell comet, tail length and level DNA damage all increased. At highest concentrations of the three herbicides produced severe DNA damage compared with S10 fraction and negative control. The linear regression analysis of the tail length values of three herbicides indicated that there was genotoxic effect concentration-response relationship with ametryn and ametribuzin but no EPTC. The ethanol induced major increase DNA damage compared with S10 fraction and the three pesticides. There were not effects in cell viability with treatment EPTC and metribuzin wherther or not it had been metabolized. High concentrations of ametryn alone and after it had been metabolized decreased cell viability compared with the negative control. The results demonstrated that the three herbicides needed to be activated by the *V. faba* root metabolism to produce DNA damage in human peripheral lymphocyte. The alkaline comet technique is a rapid and sensitive assay, to quickly evaluate DNA damage the metabolic activation of herbicide products by *V. faba* root in human cells *in vitro*.

Keywords: Ametryn; Metribuzin; EPTC herbicides; Human peripheral lymphocytes; *In vivo Vicia faba* metabolic activation; Comet tail length; DNA damaged cells

Abbreviations: EPTC (eptam), *S*-ethyl-*N*,*N*-diproylthiocarbamate; EDTA, ethylenediaminetetraacetic acid; PBS, Dulbecco's phosphate-buffered saline, pH 7.4; S10, 10,000*g* supernatant fraction *Vicia faba* root microsomal enzymes.

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1. Introduction

Many pesticides are not mutagens by themselves but become active by metabolic transformations. Thus, a promutagen may be converted into a mutagen through metabolic activation (Plewa, 1978; Plewa and Gentile, 1982). Plant activation is the process by which a promutagen is activated to a mutagen by plant enzymatic systems (Plewa, 1978; Plewa and Gentile, 1982). Many food plants are exposed to pesticides and other chemicals used in agriculture (Plewa, 1978; Plewa and Gentile, 1982).

It has been shown that the enzymatic system S10 of *Vicia faba* roots can activate pesticides *in vivo* and *in vitro*, and the genotoxic and cytotoxic action of the metabolites contained in the extracts, through sister chromatid exchanges, have been shown in human peripheral lymphocytes in culture (Calderón-Segura et al., 1999; Gómez-Arroyo et al., 1995, 2000; Flores-Maya et al., 2005) and in Chinese hamster ovary cell (Takehisa et al., 1988).

S-triazines herbicides are widely used in Mexican agricultural. Their principal mode of action is the inhibition of plant photosynthesis. *S*-triazines derivatives, including ametryn, cyanazine, metribuzin, prometryn, terbuthylazine, terbutryne, and some metabolites have been found in eggs, fruit and vegetables (Tadeo et al., 2000). Thiocarbamate herbicides belong to the group of *S*-thiocarbamate esters. They are widely used in Mexico for pre-emergent and post-emergent control of broadleaf weeds and annual grasses. Molinate, butytale and EPTC are applied to crops such as corn, rice, wheat, sugar cane, and beans (Bayer de México, 1994). The compounds in this family are considered to be meristematic inhibitors (Barret and Harwood, 1998).

Ametryn is used in México for the pre and post-emergence control of annual grasses and broad-leaved weeds in crops of pineapples, sugarcane, bananas, citrus, maize, and coffee. It is absorbed by leaves and roots, translocated acropetally in xylem and accumulates in the apical meristems (Edwards and Owen, 1989). It is metabolized in plants by hydroxylation and dealkylation reactions. The toxicity of Ametryn is Class III, which means that it is slightly toxic to humans (IARC, 2000). It is relatively nontoxic to mammals and fish (Davies et al., 1994), but highly toxic to crustaceans and mollusks (IARC, 2000), and it is embryotoxic in rats (Asongalem and Akintonwa, 1997). Ametryn inhibits bioluminescence of *Vibrio fischeri* after biodegradation (Farré et al., 2002).

Metribuzin is used as a photosynthesis inhibitor in corn, sugar soybeans, and carrot crops (Bayer de México, 1994; Frear et al., 1983, 1985). It is absorbed mainly by roots but also by leaves and is translocated in the xylem (Frear et al., 1983, 1985). The metabolism of metribuzin has been studied in several crop plants (Frear et al., 1983, 1985). The major primary metabolic reactions are deamination, sulfoxidation and demethylation (Frear et al., 1983, 1985). It is nonmutagenic in bacterial reversion assays (Moriya et al., 1983); it is mutagenic in *Escherichia coli* (Pauli et al., 1990); and has moderate genotoxic activity using SOS microplate assays (Venkat et al., 1995).

EPTC is used to control of annual and perennial grasses, and is used, as a selective herbicide in corn, sorghum, and tomato crops (Bayer de México, 1994). EPTC has been shown to undergo metabolic bioactivation via oxidation to form the reactive metabolites EPTC-sulfoxide or EPTC-sulfone, which are excellent carbamoylating agents for tissue thiols. Both of these electrophilic species are capable to interact with proteins (Lamoureux and Rusness, 1987). EPTC-sulfoxide inhibits aldehyde dehydrogenase (ALDH), one of the key enzymes involved in ethanol metabolism in humans, and to increase their hepatotoxic potential in mammals and fish (Staub et al., 1999; Coleman et al., 2000). It is a potent neurotoxicant in rats (Smulders et al., 2003).

The alkaline comet assay is a rapid, simple, and sensitive procedure to quantify DNA lesions in individual cells, and is used in environmental genotoxic monitoring both *in vivo* and *in vitro* (Tice et al., 2000). The alkaline comet assay was specially developed to detect DNA single-strand breaks and alkali–labile sites (Singh et al., 1988). It is also used to evaluate *in vivo* genotoxicity induced by the exposure to carcinogens (Tice et al., 2000).

The use of the comet assay to detect the potential genotoxic effects of pesticides is particularly relevant in the evaluation of potential health risks to humans and animals, since pesticides are applied to food crops, and can be present in the air, soil and aquatic systems. Original compounds and metabolites may pass through animals' digestive tracts and be activated and when the animals are used as food. Thus, these compounds could represent a health risk (Sandermann, 1992). When agrochemicals get into food plants, they may remain unaltered; they may undergo further transformations, or be reactivated by digestive enzymes and produce, perhaps, adverse physiological effects in organisms (Sandermann, 1992).

The present study was carried out to study the *in vivo* metabolic capability of *Vicia faba* roots to bioactivate three herbicides, using the alkaline comet assay to measure DNA damage plant promutagens effects on peripheral blood lymphocytes *in vitro*.

2. Material and methods

2.1. Chemicals and reagents

Fresh stock solutions of ametryn (Gesapax 49% CAS number 014-69-3 kindly donated by CIBA-GEIGY México), metribuzin (Sencor 48% provided by Bayer of México, CAS number 21087-64-9) and EPTC (eptam, 79% CAS number 759-94-4 kindly donated by Quimica Lucava México) were prepared in deionized water and immediately used to treat human peripheral lymphocytes and *V. faba* roots.