Available online at www.sciencedirect.com
SCIENCE DIRECT®
Toxicology 211 (2005) 70–76
TOXICOLOGY
www.elsevier.com/locate/toxicol

# Acrylamide tissue distribution and genotoxic effects in a common viral infection in mice

Lilianne Abramsson-Zetterberg [a], Jennie Wong [a], Nils-Gunnar Ilbäck [a,b,*]

[a] *Toxicology Division, National Food Administration, Uppsala, Sweden*
[b] *Section of Infectious Diseases, Department of Medical Sciences, Uppsala University Hospital, S-751 85 Uppsala, Sweden*

Received 4 February 2005; accepted 26 February 2005
Available online 29 March 2005

**Abstract**

Acrylamide (AA) has been shown to cause neurotoxic effects in humans and neurotoxic, genotoxic, reproductive, and carcinogenic effects in laboratory animals. Infection with the human coxsackievirus B3 (CB3) in the murine model results in changed uptake and tissue distribution of several environmental pollutants, which may result in aggravated disease. In the present study female Balb/c mice were infected with CB3, and on day 1 of the infection, dosed orally with approximately 50 μg/kg bw of [$^{14}$C]acrylamide ($^{14}$AA) and subsequently sacrificed on day 3 of the infection for studies of the distribution of radioactivity and genotoxic effects in terms of the frequency of micronucleated erythrocytes. Infected mice developed an expected clinical signs of disease. The infection decreased the radioactivity by 45% ($p < 0.05$) in the pancreas but increased it by 70% ($p < 0.05$) in the blood and more than two-fold in the thymus ($p < 0.01$). However, the infection caused no changes in the radioactivity in the brain, heart, liver, lungs, spleen, or kidneys. As a response to the infection the proportion of young red blood cells (PCE) decreased to about a third ($p < 0.001$) of that in the control mice, but no genotoxic effects were observed. Thus, the tissue radioactivity after $^{14}$AA administration indicated an infection-induced change in the metabolism of AA, the exact pathogenic interpretation of which warrants further studies.


*Keywords:* Acrylamide; Genotoxicity; Red blood cells; Tissue distribution; Virus infection

## 1. Introduction

After absorption, acrylamide (AA) is either conjugated with glutathione and excreted or metabolised by the cytochrome P450 (CYP450) pathway and mainly excreted in the urine (Sumner et al., 1992). One of the metabolites, epoxide glycidamide, is reactive both to DNA and to haemoglobin adducts (Bergmark et al., 1991; Segerbäck et al., 1995; Perez et al., 1999; Paulsson et al., 2003). A clear dose–response in the frequency of micronucleated erythrocytes has been shown even at comparatively low doses of AA

* Corresponding author. Tel.: +46 18 17 57 50; fax: +46 18 17 14 33.
*E-mail address:* nils-gunnar.ilback@slv.se (N.-G. Ilbäck).

0300-483X/$ – see front matter © 2005 Published by Elsevier Ireland Ltd.
doi:10.1016/j.tox.2005.02.008

(Abramsson-Zetterberg, 2003). The tissue distribution and many toxicological effects of AA have been extensively studied in healthy animals. However, it is generally not known whether diseases, such as infection involving metabolic processes and the immune system affect the detoxification and distribution of AA.

Infections, in general, are associated with an "acute-phase" reaction that involves the immune system, the metabolic pathways and the changes in the nutritional requirements of the host (Beisel, 1998). One consequence of this response is the inhibited muscle protein synthesis and the accelerated proteolysis leading to protein degradation and tissue wasting (Friman and Ilbäck, 1998). The purpose of this costly, though essential, process for host survival is to mobilise amino acids for de novo synthesis of acute-phase proteins that participate in the combat of the infection (Beisel, 1998).

Virtually all humans contract several enterovirus infections during their lifetime, including the presently used coxsackievirus B3 (CB3). Although the majority of these infections pass without any apparent symptoms, several disease manifestations can occur, including pancreatitis, myocarditis or meningoencephalitis (Woodruff, 1980). Mouse models of CB type 3 infections have been developed that closely resemble the disease in humans (Woodruff, 1980; Huber, 1993).

Aggravated disease, including complications, can occur when the infected experimental animals are exposed to environmental pollutants, such as dioxin and heavy metals, even at such low doses that may be present in human food (Ilbäck et al., 1994, 1996; Funseth et al., 2000, 2002). Earlier studies using the present CB3 infection have shown that dioxin is further accumulated in the thymus as well as in the pancreas, and that the infection becomes aggravated (Funseth et al., 2000). The changed tissue distribution and toxicity of contaminants in infection may be regarded as a part of the "acute-phase reaction", which involves down-regulation of the detoxifying P450 system (Darnerud et al., 2005; Funseth et al., 2002).

In the present study, the gastrointestinal uptake and tissue distribution after a low dose of radioactively labelled AA ($^{14}$AA) were studied during early coxsackievirus infection in normally fed animals. The used dose of about 50 μg/kg bw is low as compared to other published studies but several times higher than the calculated daily intake in human adults (Dybing and Sanner, 2003; Svensson et al., 2003, WHO, 2003). We used a murine mouse model of human CB3 infection that is characterised by a short-lasting viremic phase with a pathogenesis resembling that in humans (Woodruff, 1980). The aim was to study whether this common infection changes the tissue distribution of $^{14}$AA. Furthermore, using the flow cytometer-based micronucleus assay the genotoxic potential of AA in virus-infected mice was studied.

## 2. Materials and methods

### 2.1. Animals

Adult female Balb/c mice were purchased from Charles River and maintained at the Animal Department, Biomedical Centre, Uppsala, Sweden. The mice were randomly assigned to groups of similar initial mean body weight and housed individually at 23 ± 1 °C on a 12 h light/dark cycle behind the hygienic barriers with free access to food (R3, Ewos, Södertälje, Sweden) and water. Control and infected mice were studied simultaneously.

The experiment described in this report had been approved by the Ethical Committee for Experimental animals, Uppsala, Sweden.

### 2.2. Virus

Coxsackievirus type B3 was propagated in HeLa cells, which were grown in Eagle's minimal essential medium supplemented with 5% fetal calf serum and antibiotics (Ilbäck et al., 1994; Funseth et al., 2000). Virus titres were determined on HeLa cells as plaque-forming units (pfu) and a stock solution was stored at −20 °C until use. The stock solution of $10^7$ to $10^8$ pfu/ml was diluted with phosphate buffered saline to get $10^5$ pfu/ml.

### 2.3. Compound

Radioactively labelled [1-$^{14}$C]acrylamide, obtained from Cambridge Isotope Laboratories (Cambridge, MA, USA) with a specific activity of 5 mCi/mmol was dissolved in distilled water.