Nitric Oxide Antagonist N-nitro-L-arginine Decreases the Lethality of TCDD in Mice, but Increases It in Rats

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1. Introduction

Nitric oxide (NO) is a highly reactive secretory product of mammalian cells exerting a variety of important regulatory functions at least in vascular tone, neurotransmission, platelet function, host defense mechanisms and hepatic oxidative metabolism [1-3]. These functions are related to oxidation by NO of heme and nonheme iron and iron-sulfur complexes in active sites of key metabolic enzymes. NO is synthesized from L-arginine by nitric oxide synthase (NOS), which has been shown to occur at least in three isoforms. NOS substrate analogs are effective inhibitors of the enzyme, and one of them, N-nitro-L-arginine (NO-Arg), was shown to be an irreversible inhibitor of NOS in rat brain both *in vitro* and *in vivo* [4]. It can be therefore utilized to study the role of NO in mediating effects of toxic chemicals.

Cytokines, such as tumor necrosis factor alpha (TNF-α), have been suggested to play a role in mediating toxic effects of TCDD [5-7]. Since these cytokines play a role in regulation of NO production [1, 2, 8], the present study was carried out to examine the effect of NOS inhibition by NO-Arg to short-term lethality of TCDD.

2. Materials and Methods

Chemicals. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD, purity >99%) was dissolved in corn oil. Nω-nitro-L-arginine (NO-Arg) was obtained from Sigma (St. Louis, MO, USA). For injection experiments it was dissolved (dose of 10 mg/kg) or suspended in sterile 0.9% NaCl. NO-Arg slow release pellets were purchased from Innovative Research of America (Toledo, OH, USA). Each pellet contained 200 mg of NO-Arg and they were designed to accurately and continuously release the contents within 21 days. Matching placebo pellets were used to treat the placebo control groups.

Animals. SPF barrier maintained female NIH mice, male inbred Long-Evans (Turku/AB; L-E) and male outbred Han/Wistar (Kuopio; H/W) rats were obtained from the National Public Health Institute (Kuopio, Finland). Mice were 7 weeks old and weighed on average 21 g. They were kept in stainless steel cages, 6 mice per cage and given pelleted R36 feed (Ewos, Södertälje, Sweden), and tap water *ad libitum*. Rats were 14-17 weeks old and their mean body weights were 332 g (L-E) and 375 g (H/W). Rats were kept individually in stainless steel wire-bottom cages and given powdered R36 feed and tap water *ad libitum*. Animal room was artificially illuminated from 6 am to 6 pm, and the ambient temperature was 21.5 ± 1°C and relative humidity 55 ± 10%. Animals were acclimated to experimental conditions for at least two weeks before dosing.

Experimental Design. Two mouse studies were carried out. In the first one subcutaneously implanted pellets were used. Mice were anesthetized with ketamine (Ketalar, Parke Davis; 50 mg/kg i.p.) and

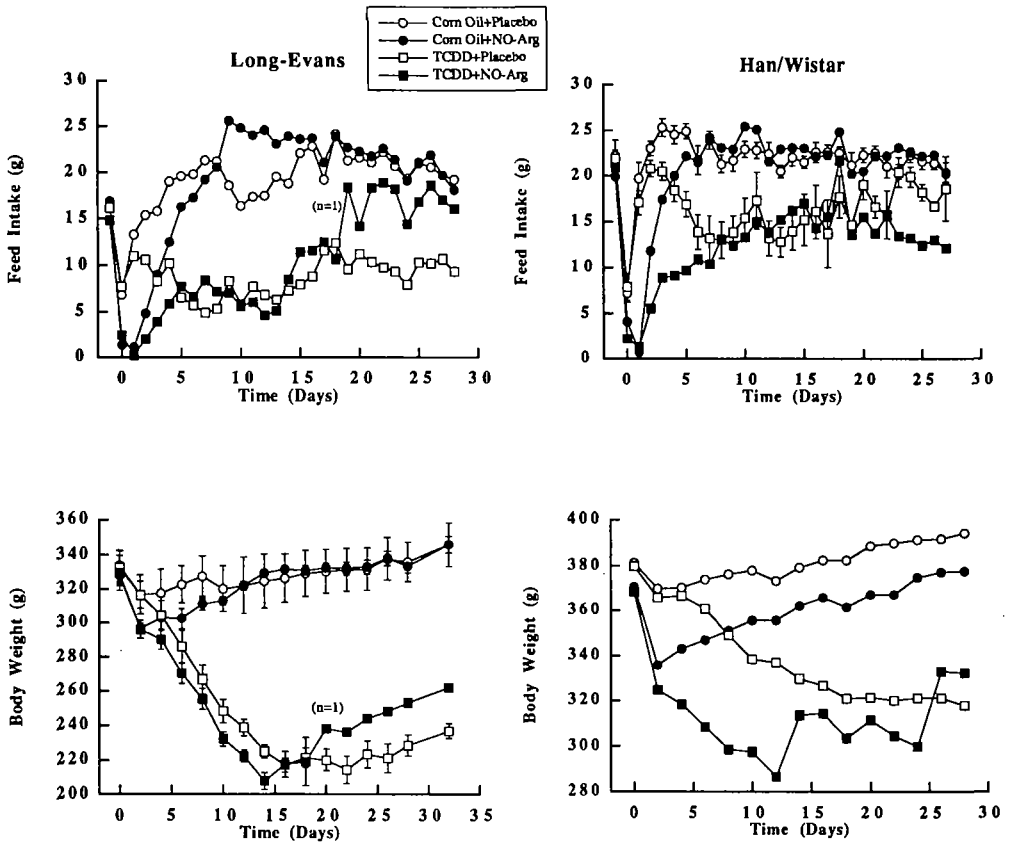

Fig. 1. Effect of NO-Arg slow release pellets or placebo on feed intake (upper graph) and body weight (lower graph) of corn oil and TCDD -treated L-E (left panel) and H/W (right panel) rats. The doses of TCDD were 25 μg/kg for L-E rats and 1000 μg/kg for H/W rats. Means ± SE (for clarity not shown for all data points), n=6 (less mortality, see Fig. 2).

medetomidine (Domitor, Orion-Farmos; 3.5 mg/kg s.c.), and either NO-Arg (200 mg/pellet, ≈450 mg/kg/day) or matching placebo pellets were implanted under the neck skin, after which the anesthesia was reversed with atipamezole (Antisedan, Orion-Farmos; 2.5mg/kg i.m.). One hour later the mice were given a single oral dose of corn oil or TCDD (500 μg/kg, 5 ml/kg; representing LD50-LD100 in NIH mice). The 24 mice were divided in 4 groups of 6. Two groups received corn oil and two TCDD and one of each placebo pellets and the other one NO-Arg pellets. In the other mouse study NO-Arg was given as subcutaneous injections 3 times per week. 36 mice were divided in 6 groups and given a single oral dose of either corn oil (2 groups) or TCDD (500 μg/kg, 5 ml/kg; 4 groups). The corn oil-treated groups received either placebo (0.9% NaCl, 10 ml/kg) or NO-Arg (200 mg/kg, 10 ml/kg) and the TCDD-treated mice either placebo or 10, 100 or 200 mg/kg of NO-A. The first dose was given 1 h before corn oil/TCDD and 3 times per week (on Mondays, Wednesdays and Fridays) thereafter. Rat studies were carried out using L-E rats, the most TCDD sensitive rat strain (LD50 17.7 μg/kg), and in

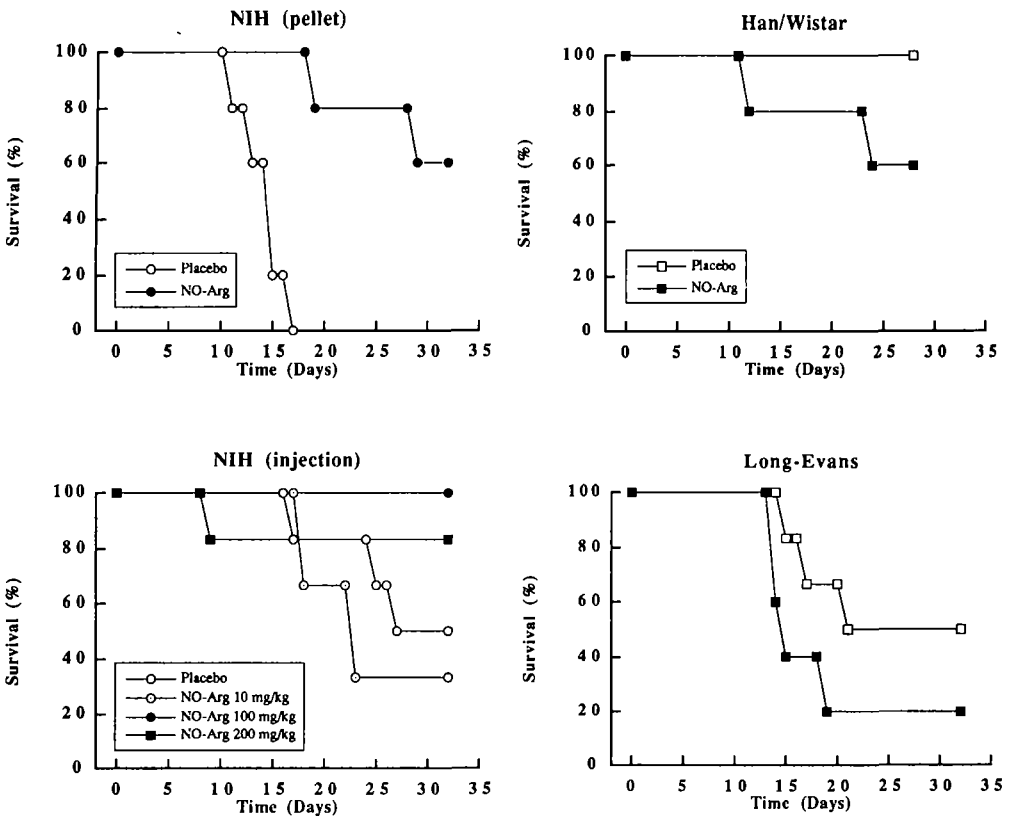

Fig. 2. Effect of NO-Arg or placebo treatment on survival of mice (left panel) and rats (right panel) after a single oral dose of TCDD.

H/W rats, which is the most TCDD resistant rat strain (LD50 >9600 μg/kg) [9]. The rats were anesthetized with medetomidine (1 mg/kg s.c.) for implantation of the slow release pellets (placebo or NO-Arg 200 mg/pellet ≈30 mg/kg/day for L-E rats and ≈26 mg/kg/day for H/W rats), and the anesthesia reversed with atipamezole (2.5 mg/kg i.m.). A single oral dose of corn oil or TCDD (25 μg/kg for L-E rats, 1000 μg/kg for H/W rats, 4 ml/kg) was given 1 h after implantation of the pellet. Doses of TCDD represent nearly LD100 for L-E rats and minimally lethal dose for H/W rats. The animals were weighed every second day and observed for 32 days (H/W rats for 28 days). Feed intake was measured daily for rats only.

3. Results

There were no substantial changes in body weights of mice (data not shown). In both rat strains TCDD treatment markedly decreased the feed intake and caused a persistent and progressive decrease in body weight throughout the observation period (Fig. 1.). In NO-Arg -treated rats there was an abrupt and

profound decrease in feed intake, which returned to the level of their placebo-treated counterparts within about a week. This decrease was seen both in corn oil and TCDD groups.

Survival of mice and rats after TCDD-treatment is depicted in Fig. 2. In mice NO-Arg treatment protected from lethality when given either as slow release pellets or as subcutaneous injections at dose-levels of 100 mg/kg or above 3 times per week. In both rat strains, however, mortality was clearly increased by NO-Arg released from the pellets during 21 days. A few rats of both strains (both corn oil and TCDD -treated) were found dead 1-2 days after implantation of NO-Arg pellets. The pellets of these rats were found defective, as they were dispersed and apparently released their contents in a very short time resulting in immediate lethality. These rats were therefore excluded from the study.

4. Discussion

This study showed an opposite response of rats and mice to lethality of TCDD when treated with NO synthesis inhibitor NO-Arg. The results suggest differences in pathways leading to lethality in these species and possibly an involvement of NO in mediating toxicity of TCDD. It remains to be clarified whether the observed effect of NO-Arg is specifically attributed to inhibition of NOS and which cells are involved. However, identically opposite responses in TCDD-treated mice [6] and rats [10] have been described after the administration of dexamethasone, which together with other corticosteroids is a well-known inhibitor of NOS [1, 11]. Dexamethasone was shown to inhibit lethality and other toxic effects in TCDD-treated C57Bl/6J mice [6], while it aggravated the TCDD-induced reduction of feed intake and body weight loss in Long-Evans rats [10]. It seems possible that the responses caused by NO-Arg and dexamethasone would have a common mechanism, but there is currently no indication about the basis of the species difference.

Similarly to our findings in the two rat strains NO-Arg has been reported to result in temporary reduction of feed intake in lean Zucker rats [12]. There was, however, no tolerance for this effect in genetically obese litter mates of the Zucker rats. This anorectic effect of NO-Arg was shown to be related with increased brain serotonin metabolism. NO seems to play a role in physiological regulation of feed intake by modulating the central serotonergic system [13]. Because wasting and lethality caused by TCDD was also associated with increased brain serotonin turnover in rats [14], it can be speculated that the aggravation of lethality of TCDD by NO-Arg would represent a synergistic effect with TCDD on the central serotonergic system.

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5. References

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