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Toxicokinetics and toxicity of thioacetamide sulfoxide: a metabolite of thioacetamide

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Abstract

Thioacetamide (TA) is bioactivated by CYP2E1 to TA sulfoxide (TASO), and to the highly reactive sulfdioxide ($TASO_2$), which initiates hepatic necrosis by covalent binding. Previously, we have established that TA exhibits saturation toxicokinetics over a 12-fold dose range, which explains the lack of dose–response for bioactivation-based liver injury. In vivo and in vitro studies indicated that the second step (TASO → $TASO_2$) of TA bioactivation is less efficient than the first one (TA → TASO). The objective of the present study was to specifically test the saturation of the second step of TA bioactivation by directly administering TASO, which obviates the contribution from first step, i.e. TA → TASO. Male SD rats were injected with low (50 mg/kg, ip), medium (100 mg/kg) and high (LD_{70}, 200 mg/kg) doses of TASO. Bioactivation-mediated liver injury that occurs in the initial time points (6 and 12 h), estimated by plasma ALT, AST and liver histopathology over a time course, was not dose-proportional. Escalation of liver injury thereafter was dose dependent: low dose injury subsided; medium dose injury escalated upto 36 h before declining; high dose injury escalated from 24 h leading to 70% mortality. TASO was quantified in plasma by HPLC at various time points after administration of the three doses. With increasing dose (i.e., from 50 to 200 mg/kg), area under the curve (AUC) and C_{max} increased more than dose proportionately, indicating that TASO bioactivation exhibits saturable kinetics. Toxicokinetics and initiation of liver injury of TASO are similar to that of TA, although TASO-initiated injury occurs at lower doses. These findings indicate that bioactivation of TASO to its reactive metabolite is saturable in the rat as suggested by previous studies with TA.

Keywords: Thioacetamide; Thioacetamide sulfoxide; Liver injury; Bioactivation; Saturation

1. Introduction

Thioacetamide (TA), a model hepatotoxicant, has been shown to require metabolic activation to initiate hepatocellular necrosis. It is *S*-oxidized to thioacetamide sulfoxide (TASO) and further to thioacetamide-*S*,*S*-dioxide ($TASO_2$) (Hunter et al., 1977) via hepatic CYP2E1 (Hunter et al., 1977; Wang et al., 2000; Ramaiah et al., 2001; Chilakapati et al., 2007). Studies suggest that TASO, a relatively stable intermediate of TA metabolism, is obligatory for the hepatotoxic effects of this compound, indicating that it is the penultimate reactive metabolite (Porter and Neal, 1978; Porter

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et al., 1979). The obligate reactive metabolite of TA, presumably $TASO_2$, covalently binds to proteins with the formation of acetylimidolysine derivatives that are responsible for TA-induced hepatotoxic effects (Dyroff and Neal, 1981). TA can also form acetamide via the same reactive intermediate (Rees et al., 1966). Studies have shown, however, that acetamide cannot produce hepatic lesions like those reported for TA, even when given at doses far greater than TA (Dessau and Jackson, 1955).

The necrogenic and carcinogenic properties of TASO are similar to those of TA (Becker and Walter, 1965; Ammon et al., 1967). It is known from studies that when equimolar doses of TA and TASO are administered, TASO produces higher and earlier liver injury than TA (Hunter et al., 1977). When TA is administered, two metabolic events are required to produce the toxic metabolite, whereas only one step is required in the case of TASO, accounting for the greater toxicity of TASO as compared to TA (Hunter et al., 1977).

TA exhibits a lack of dose response for bioactivation-based initiation of liver injury in ad libitum-fed and diet restricted male Sprague–Dawley rats after administration of high doses of TA (Mangipudy et al., 1995; Ramaiah et al., 1998). A 12-fold dose range of TA (50–600 mg/kg) failed to elicit a typical dose–response relationship with respect to initial liver injury, as assessed by plasma ALT, sorbitol dehydrogenase (SDH) and histopathology (Mangipudy et al., 1995). Another study revealed a similar lack of normal dose-response of TA-initiated liver injury in moderately diet-restricted (DR) rats. Diet restriction, known to induce CYP2E1, substantially increases mechanism-based initiation of injury by TA (Ramaiah et al., 2001). Surprisingly, while initial liver injury was six-fold higher at the low dose (50 mg/kg, ip), it was only 2.5-fold higher and delayed after exposure to a lethal dose of TA (600 mg/kg, ip) in DR rats when compared with their ad libitum cohorts (Ramaiah et al., 1998).

Previous studies in this laboratory have shown that the two-step bioactivation of thioacetamide via CYP2E1 follows saturation kinetics, explaining the lack of a dose-proportional increase in TA-initiated liver injury in both AL and DR rats (Chilakapati et al., 2005; Chilakapati et al., 2007). In vivo and in vitro evidence from those studies indicated that the second step (TASO → $TASO_2$) of TA bioactivation is less efficient than the first one (TA → TASO) (Chilakapati et al., 2005). However, it is difficult to explore the reason for the TASO profile observed in the previous study when two steps are involved in the bioactivation because there stands a chance of competition between TA and TASO for the same enzyme i.e, CYP2E1. Therefore, the present studies were designed with TASO so that bioactivation of this metabolite to the reactive metabolite, TA-*S*,*S*-dioxide could be studied.

The administration of TASO bypasses the first metabolic step in TA bioactivation and therefore would allow specifically to investigate the second step (Fig. 1). The objective of the present work was to test the saturation kinetics of TASO to $TASO_2$ pathway by dosing the rats with TASO. We hypothesized that the second and more important step of TA bioactivation is saturable.

In this study, both the toxicity and tissue repair response due to TASO exposure have been examined over a dose range. These studies were followed by further investigation of the toxicokinetics of TASO. We report here that after a threshold, TASO exhibits a lack of dose-response for liver injury similar to its parent compound, TA, and that this is due to saturation of its bioactivation to $TASO_2$ via CYP2E1.

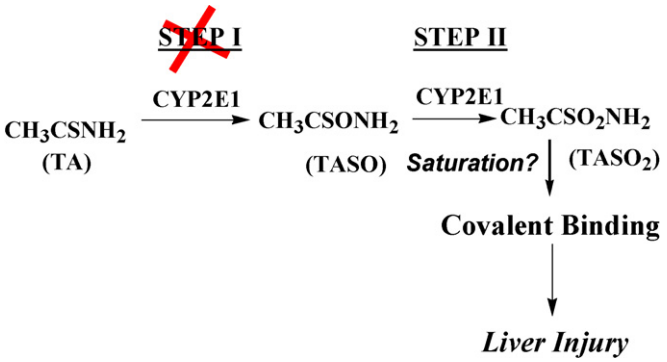

Fig. 1. Thioacetamide sulfoxide (TASO) metabolism at high doses is subject to saturation kinetics. TASO bioactivation to $TASO_2$ follows saturation kinetics even in the absence of the first step. Conversion to the reactive intermediate involves saturation, resulting in lower than expected increase in covalent binding of reactive intermediate and therefore lower than expected bioactivation-based liver injury.