Available online at www.sciencedirect.com
SCIENCE DIRECT
Toxicology and Applied Pharmacology 215 (2006) 71–82
Toxicology and Applied Pharmacology
www.elsevier.com/locate/ytaap

Study of a novel indolin-2-ketone compound Z24 induced hepatotoxicity by NMR-spectroscopy-based metabonomics of rat urine, blood plasma, and liver extracts

Quanjun Wang [a,1], Ying Jiang [b,1], Chunqi Wu [a], Jianyu Zhao [c], Shouzhong Yu [a], Benli Yuan [a], Xianzhong Yan [c,*], Mingyang Liao [a,*]

[a] *Beijing Institute of Pharmacology and Toxicology, 27 Taiping Road, Beijing 100850, PR China*
[b] *Beijing Institute of Radiation Medicine, 27 Taiping Road, Beijing 100850, PR China*
[c] *National Center of Biomedical Analysis, 27 Taiping Road, Beijing 100850, PR China*

Received 26 November 2005; revised 2 February 2006; accepted 5 February 2006
Available online 3 April 2006

Abstract

Antiangiogenic compound has been believed to be an ideal drug in the current cancer biological therapy, but the angiogenesis inhibitors suffer setback for unknown toxicity now. A novel synthetic indolin-s-ketone small molecular compound, 3Z-3-[(1*H*-pyrrol-2-yl)-methylidene]-1-(1-piperidinylmethyl)-1,3-2*H*-indol-2-one (Z24) can inhibit angiogenesis in new blood vessels. The hepatotoxicity effects of Z24 oral administration (dosed at 60, 130 and 200 mg/kg) have been investigated in female Wistar rats by using metabonomic analysis of ^{1}H NMR spectra of urine, plasma and liver extracts, as well as by clinical chemistry analysis, liver histopathology and electron micrographs examination. The ^{1}H NMR spectra of the biofluids were analyzed visually and via pattern recognition by using principal component analysis. The metabonomic trajectory analysis on the time-related hepatotoxicity of Z24 was carried out based on the ^{1}H NMR spectra of urine samples, which were collected daily predose and postdose over an 8-day period. Urinary excretion of citrate, lactate, 2-oxo-glutarate and succinate increased following Z24 dosing. Increased plasma levels of lactate, TMAO and lipid were observed, with concomitant decrease in the level of glucose and phosphatidylcholine. Metabolic profiling on aqueous soluble extracts of liver tissues with the high dose level of Z24 showed an increase in lactate and glutamine, together with a decrease in glucose, glycogen and choline. On the other hand, studies on lipid soluble extracts of liver tissues with the high dose level of Z24 showed increased level in lipid triglycerides and decreased level in unsaturated fatty acids and phosphatidylcholine. Moreover, the most notable effect of Z24 on the metabolism was the reduction in the urinary levels of creatinine and TMAO and the increase in acetate, citrate, succinate and 2-oxo-glutamate with time dependence. The results indicate that in rats Z24 inhibits mitochondrial function through altering the energy and lipid metabolism, which results in the accumulation of free fatty acids and lactate because of the lack of aerobic respiration. These data show that the metabonomic approach represents a promising new technology for the toxicological mechanism study.

Keywords: Metabonomics; Pattern recognition; Z24; Hepatotoxic mechanism research

Abbreviations: ^{1}H NMR, ^{1}H nuclear magnetic resonance; RH, relative humidity; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, urea nitrogen; CK, creatinine kinase; ALP, alkaline phosphatase; Crn, creatinine; Tbil, total bilirubin; Alb, albumin; Glu, blood glucose; TSP, 2,2′,3,3′, -deuterotrimethylsilylproprionic acid; FIDs, free induction decays; FT, Fourier transformation; PCA, principal components analysis; PC, principal components; TI, one vector for components 1; CPMG, Carr–Purcell–Meiboom–Gill; p.d., postdose; TCA, citric acid cycle; Gln, glutamine; Glu, glutamate; GSH, glutathione; DMA, dimethylamine; TMAO, trimethylamine-*N*-oxide; Cr, creatine; pCHo, phosphatidylcholine; 2-OG, 2-oxo-glutarate; DMG, dimethylglycine; Glc, glucose; Cho, choline.

* Corresponding authors. X. Yan is to be contacted at National Center of Biomedical Analysis, 27 Taiping Road, Beijing 100850, PR China. M. Liao, Beijing Institute of Pharmacology and Toxicology, 27 Taiping Road, Beijing 100850, PR China. Fax: +8610 68159974.
E-mail addresses: yanxz@nic.bmi.ac.cn (X. Yan), liaomingy@hotmail.com (M. Liao).
[1] Contributed equally to this work.

0041-008X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.taap.2006.02.012

Introduction

Angiogenesis is the process of sprouting of capillaries from preexisting blood vessels, which is essential for the sustained growth and metastasis of primary solid tumors (Folkman, 1972; Holash et al., 1999). Antiangiogenic therapy has been believed to be an ideal strategy in the current cancer biological therapy. By the end of 2002, at least 10,000 cancer patients worldwide have received some form of experimental anti-angiogenic therapy, and more than 300 angiogenesis inhibitors have been discovered, of which 80 are in clinical trials (Madhusudan and Harris, 2002). SU5416, a model antiangiogenesis compound, had been developed as an effective drug for antiangiogenesis. But, it was withdrawn from a Phase III trial in advanced colon cancer for the significant incidence of toxicity and the lack of target, although it exhibited good efficiency in preclinical tests (Cristofanilli et al., 2002; Gerber, 2003). The angiogenesis inhibitors suffer setback. But it is too early to proclaim the antiangiogenesis strategy a failure (Ken et al., 2002). The urgent work that toxicologists have to do is to learn about why it fails and its toxicological mechanism so as to reduce the high attrition rates of drugs entering clinical trials (Cui et al., 1995). Obviously, it is of importance to systematically study the toxicological mechanism of rejected compounds for next better drug. 3Z-3-[(^{1}*H*-pyrrol-2-yl)-methylidene]-1-(1-piperidinylmethyl)-1,3-2*H*-indol-2-one (Z24, Fig. 1), a novel synthetic indolin-2-ketone small molecular compound, is an inhibitor of angiogenesis in new blood vessels that inhibits the growth of multiple tumor types in vivo (Lu et al., 2003; Wang et al., 2004). Z24 is a structural analog of SU5416, which belongs to indolin-2-ketones. It is speculated that the antitumor activity of Z24 is similar to that of SU5416 (Lu et al., 2003).

Repeated doses (long-term toxicity test) and single dose tests have been used widely on drug toxicological mechanism research in the preclinical drug safety evaluation phase. But, these methods are labor-intensive and time-consuming and cannot be used to get a systematic result. High resolution ^{1}H NMR spectroscopic analysis of biofluids, in particular, urine and blood plasma, has been more and more widely used in the drug safety assessment process in pharmaceutical industry as a method for identifying target organ toxicity through urinary biomarkers (Nicholson et al., 1999, 2002; Lindon et al., 2000, 2003). When coupled with pattern recognition methods, a thorough analysis of the resulting complex multiparametric spectroscopic data sets can be obtained (Beckwith-Hall et al., 1998; Holmes et al., 2000; Lindon et al., 2001). This approach to study the metabolic processes in biological systems has been termed metabonomics (Nicholson et al., 1999, 2002). Metabonomics combines the techniques of high resolution NMR with the pattern recognition technology to rapidly evaluate the metabolic status of an animal, such that the onset, duration, severity and target organ localization can all be determined from peripheral samples such as urine, blood plasma and so on. The information obtained from metabonomics is complementary to that from proteomics and genomics and is applicable to a wide range of problems in diverse biomedical research areas. Metabonomics is now recognized as an independent and widely used technique for evaluating the toxicities of drug candidate compounds (Waterfield et al., 1993; Robertson et al., 2000; Nicholson et al., 2002).

In previous study, it was proved that Z24 had hepatotoxicity to rodent and some genes related with metabolic pathways (like Glycolysis) were changed with Z24 treatment (unpublished data). However, its hepatotoxic mechanism, especially the changes in metabolic profiling, is unclear. In this study, the hepatotoxic mechanism of Z24 was investigated by using an integrated metabonomic approach, and the feasibility of metabonomic method for drug toxicological mechanism research and screening in vivo was also evaluated.

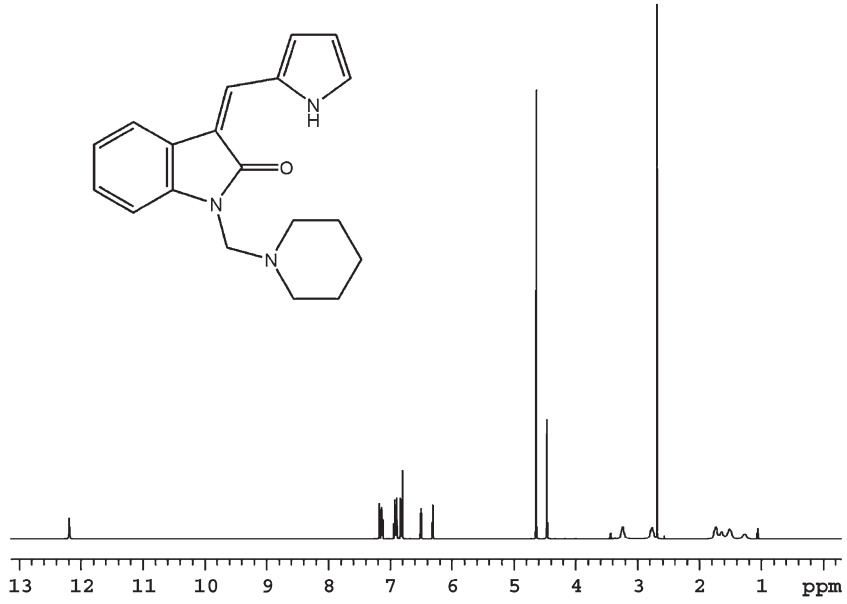

Fig. 1. Chemical structure of Z24 and its ^{1}H NMR spectrum in methylsulfonate form.