Effect of some nitrotoluenes on the biotransformation of xenobiotics in rats

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Summary. Male rats were treated with phenobarbital, a dinitrotoluene (2,4-DNT or 2,6-DNT), or 2,4,6-trinitrotoluene (2,4,6-TNT); and the biotransformation of model xenobiotics was determined. Phenobarbital produced a stimulation, and 2,6-DNT produced a biphasic effect in biotransformation.

Dinitrotoluenes, which include 2,4-dinitrotoluene (2,4-DNT) and 2,6-dinitrotoluene (2,6-DNT), are used industrially in the preparation of polyurethane foams. As a result, humans may be exposed to these chemicals in their environment. Many chemicals are able to alter the metabolism of xenobiotics and endogenous molecules[1-3]. The purpose of this study was to evaluate the ability of 2,4-DNT and 2,6-DNT to alter the metabolism of xenobiotics. For comparative purposes and to identify possibly structure activity relationships, 2,4,6-trinitrotoluene was included. In this study, the metabolism of xenobiotics by male rats treated with a nitrotoluene was monitored in vivo by measuring the zoxazolamine paralysis time and the hexobarbital sleeping time and in vitro by measuring hepatic nitroanisole O-demethylase activity.

Materials and methods. Male CD rats (Charles River Breeding Laboratories, Wilmington, Ms) were used in all experiments. In the 1st experiment, 0.4 mmoles/kg of each nitrotoluene (K and K Laboratories, Cleveland, Ohio) dissolved in corn oil was given orally twice a day for 3 days and once on the 4th day. In addition, 0.2 mmoles/kg of sodium phenobarbital was given orally in water according to the same treatment schedule. Therefore, a total of seven doses was given over a 4-day period. The zoxazolamine paralysis time (after 60 mg/kg i.p. of zoxazolamine) and the hexobarbital sleeping time (after 150 mg/kg i.p. of sodium hexobarbital) were determined on the 5th day of study 24 h after the last dose of the test chemical. The paralysis and sleeping times were the duration of loss of the righting reflex.

In the 2nd experiment, 0.4 mmoles/kg of each nitrotoluene or 0.3 mmoles/kg of sodium phenobarbital was given orally once a day for 5 days. Therefore, a total of 5 doses were given over a 5-day period. The zoxazolamine paralysis time and the hexobarbital sleeping time were determined on the 7th day of study 72 h after the last dose of the test chemical.

In the 3rd experiment, male rats were fed diets that contained 0.25% 2,6-DNT for 4 weeks. These diets provided average daily doses of about 0.7 mmoles/kg of 2,6-DNT. Additional rats received 50 mg/kg of sodium phenobarbital twice a day for 3 days. At the end of the treatment, the zoxazolamine paralysis time, after 45 mg/kg of zoxazolamine, and the hepatic nitroanisole O-demethylase activity[3] of the postmitochondrial supernatant were determined.

Results. Phenobarbital significantly reduced the paralysis and sleeping times and increased hepatic nitroanisole O-demethylase activity (tables 1-3). These observations reflect the ability of phenobarbital to increase the capacity of the liver to metabolize xenobiotics[2,3]. An increased sleeping time was also observed 1 day after the last dose of 2,4-DNT (table 1); however, neither the paralysis nor sleeping times were altered in other experiments (tables 1 and 2). The pretreatment with 2,4,6-TNT did not alter any of the parameters used to monitor in vivo drug metabolism (tables 1 and 2). In contrast, at 1 and 3 days after the last dose of 2,6-DNT, we observed a dramatic increase in both the sleeping and paralysis times (tables 1 and 2). However, when 2,6-DNT was incorporated into the diet for 4 weeks, the paralysis time was reduced and nitroanisole O-demethylase activity was increased (table 3).

Discussion. Treatment with a variety of chemicals will alter the metabolism of xenobiotics[1-3]. As a result, the toxicity of chemicals and the therapeutic effectiveness of drugs may be altered. Therefore, it is important to know if specific chemicals alter the metabolism of xenobiotics. In the present study, phenobarbital significantly increased the in vivo (as measured by reduced paralysis and sleeping times) and in vitro (as measured by increased hepatic nitroanisole O-demethylase activity) metabolism of xenobiotics. These effects of phenobarbital have been well characterized[2,3]. Neither 2,4-DNT nor 2,4,6-TNT produced a dramatic or consistent effect on the in vivo biotransformation of xenobiotics. In contrast, 2,6-DNT reduced biotransformation

Table 1. Zoxazolamine paralysis time and hexobarbital sleeping time in male rats 1 day after treatment with various compounds

Compound	Zoxazolamine paralysis time (min)	Hexobarbital sleeping time (min)
Peanut oil	100 ± 8[a]	37 ± 2
Phenobarbital	36 ± 1[b]	15 ± 1[b]
2,4-DNT	121 ± 14	47 ± 2[b]
2,6-DNT	154 ± 10[b]	101 ± 3[b]
2,4,6-TNT	83 ± 3	37 ± 1

[a] Mean ± SE for 8–10 rats. [b] Significantly different from control (2-sample rank test)[4].

Table 2. Zoxazolamine paralysis time and hexobarbital sleeping time in male rats 3 days after treatment with various compounds

Compound	Zoxazolamine paralysis time (min)	Hexobarbital sleeping time (min)
Peanut oil	126 ± 6[a]	37 ± 2
Phenobarbital	52 ± 3[b]	22 ± 2[b]
2,4-DNT	143 ± 7	36 ± 3
2,6-DNT[c]	250 ± 18[b]	88 ± 11[b]
2,4,6-TNT	134 ± 7	35 ± 2

[a] Mean ± SE for 8–12 rats. [b] Significantly different from control (2-sample rank test)[4]. [c] Test was terminated at 300 min for zoxazolamine paralysis time or 130 min for hexobarbital sleeping time.

Table 3. Zoxazolamine paralysis time and nitroanisole O-demethylase activity in rats receiving 2,6-DNT in their diet for 4 weeks

Compound[a]	Zoxazolamine paralysis time (min)	Nitroanisole O-demethylase activity[b]
None	111 ± 3[c]	3.8 ± 0.3
Phenobarbital	68 ± 6[d]	6.7 ± 0.6[d]
2,6-DNT	52 ± 10[d]	19.0 ± 0.5[d]

[a] Phenobarbital given i.p. and 2,6-DNT given in feed. [b] nmoles p-nitrophenol formed per mg protein. [c] Mean ± SE for 7–9 rats (paralysis time) or 4 rats. [d] Significantly different from control (2-sample rank test)[4].

when given for 5 days or less and increased biotransformation when administered for 4 weeks.

Many chemicals produce a biphasic change in biotransformation[5]. In these cases, an initial inhibition is followed by a stimulation 24–48 h after treatment. In the case of 2,6-DNT, an inhibition of biotransformation was observed 24–72 h after the last dose of 2,6-DNT. This was followed by a stimulation in metabolism when rats were fed 2,6-DNT for 4 weeks.

1 J.R. Fouts, Tox. appl. Pharmac. *17*, 804 (1970).
2 B. Testa and P. Jenner, in: Drug Metabolism Chemical and Biochemical Aspects. Marcel Dekker, New York 1976.
3 B.N. LaDu, H.G. Mandel and E.L. Way, in: Fundamentals of Drug Metabolism and Drug Disposition. The Williams and Wilkins Company, Baltimore 1971.
4 A. Goldstein, Biostatistics. Macmillan, New York 1964.
5 R. Kato, E. Chiesara and P. Vassanelli, Biochem. Pharmac. *13*, 69 (1964).

Thick filament size changes in contraction of human muscles[1]

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Summary. Measurements done on electron micrographs show that in myofibres with sarcomeres contracted to below 2.1 μm, proportional shortening of the A bands occurs. In muscles from patients with idiopathic scoliosis very short A bands are especially prominent.

In the course of a study of muscle biopsies taken from patients with scoliosis (lateral displacement and rotation of the spine) of varying etiology, several hundred human muscle specimens from various locations have been examined[3,4].

As part of the study myofilament dimensions were measured on electron micrographs greatly enlarged by projection. It was noted that while Z line and M band thickness and M band periodicity were fairly constant, the A bands and sarcomeres varied considerably. Significant differences were found in measurements of these 2 in muscle specimens from idiopathic scoliosis (adolescent disease of unknown etiology) as compared to muscles obtained from cases of secondary scoliosis (mainly paralytic) and from non-scoliotic patients (mainly spondylolysthesis) (table 1). Since this finding was unexpected and not easily explainable a more thorough examination of additional muscles in different states of contraction was undertaken.

Materials and methods. Specimen preparation for electron microscopy is known to cause many artifactual modifications. Numerous preliminary experiments, testing the effect of various modes of fixation on human muscle were therefore carried out.

In all the cases, biopsy specimens were obtained during corrective spinal surgery while the patients were under general anaesthesia using tubocurarine for respiratory control and nitroprusside to produce hypotension. Samples from the deep paraspinal, trapezius and the glutei muscles, were usually taken isometrically while those from deltoids and quadriceps by needle biopsy only. The muscle specimen (1.5×0.3 cm) was tied in situ to a sterile wooden 'matchstick', excised, and fixed while still splinted, in 2% glutaraldehyde at room temperature. The buffer was 0.1 M cacodylate (pH 7.4). In most of the samples no buffer rinsing or postosmication was done. The material was dehydrated rapidly and embedded in araldite. Postosmicated and unsplinted muscle samples were also examined.

Some muscle specimes were stretched to 130–150% of their in situ length by tying and cutting one end only at first and then pulling on it before applying the second suture and excising the specimen. Others were obtained in a stretched state by tying the muscle to the splint after flexing or extending the limb to vary the muscle length.

Longitudinal sections were cut at right angles to the fibre axis onto formvar-coated and uncoated 300 mesh grids. The sections were stained with uranyl and lead and photographed with a Philips 300 EM using both 35 mm film and 60×90 mm cut film.

3–5 blocks with good orientation were selected from each muscle sample and in each, 10–40 myofibres were measured (averaging at least 10 sarcomeres per myofibre). The enlargement was measured by repeated photography of a replica grating. Final measurements were performed in several ways. In one technique the photographic negatives were projected onto a screen in a large room at magnifications of half to one million times (table 1). This was done to assess intrafilamentous periodicities (especially the well defined ones of the M band) and to minimize the errors arising from the difficulty of defining the edges of the A band. Most negatives were examined with lower magnifications while in some a microdensitometer (Joyce-Loebl) was used to obtain photodensimetric graphs of the sarcomere structures and measurements were made on the tracing.

Results. As can be seen from tables 1 and 2 normal human muscles, splinted at slack rest length and kept splinted for glutaraldehyde fixation (whether osmified or not) has sarcomeres around 2.2 μm and A bands ranging from 1.35 to 1.60 μm. Cutting out a piece of muscle, fixing and further treating it freely, resulted in sarcomere shortening to

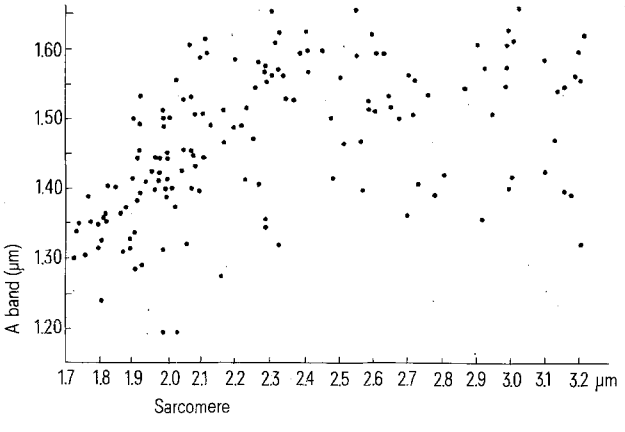

Fig. 1. Scattergram of myofibres in 3 normal muscles fixed in various ways. In sarcomeres longer than 2.1 μm the A bands remain fairly constant. At short sarcomere lengths the A bands become correspondingly shorter. Sarcomeres below 1.75 μm were very rare.