Biochemical Pharmacology, Vol. 26, pp. 162–163. Pergamon Press, 1977. Printed in Great Britain.

A species and developmental comparison of trinitroglycerin metabolism *in vitro*

(*Received* 8 *May* 1976; *accepted* 6 *July* 1976)

Nitroglycerin (glyceryl trinitrate, trinitroglycerol, TNG) is used clinically to treat angina pectoris and industrially in the preparation of explosives. TNG is metabolized *in vivo* through a pathway which includes a stepwise denitration to glycerol [1], oxidation to carbon dioxide [1], and conjugation with glucuronic acid [2]. TNG is not metabolized as extensively *in vitro* and the isomers of dinitroglycerin (1,3-DNG and 1,2-DNG) are the major denitration products [3]. The denitration reaction, which occurs primarily in the liver [4], is catalyzed by a glutathione-dependent organic nitrate reductase [3]. The metabolism of TNG has been the subject of several recent reviews [5, 6]. Since a previous species comparison of the metabolism *in vitro* of TNG [7] did not include samples of human liver, the present study was undertaken to extend these observations to humans. In addition, livers from developing rodents and placentas from rodents and humans were included to provide information on the metabolism of TNG during development.

Male and female CD rats, Swiss Webster mice (comparative study), CD-1 mice (developmental study), New Zealand albino rabbits, beagle dogs, and rhesus monkeys were used. Animals were sacrificed by decapitation (rats), cervical dislocation (mice), injection of air (rabbits), or an overdose of magnesium sulfate (dogs and monkeys). Autopsy samples of human liver were obtained from a 65-year-old Negro male who died of a dissecting aneurism of the aorta, a 21-year-old male Caucasian who died of a gunshot wound, a 67-year-old female Caucasian who died with central nervous system seizures, and a 60-year-old female Caucasian who died with a metastatic lung carcinoma. These samples were obtained 4–13 hr after death and immediately used.

Livers and term placentas were homogenized in 3 vol. of 1.15% KCl and added to an *in vitro* system. The *in vitro* system as modified from Needleman and Krantz [8], contained either 1 mM TNG-1,3[^{14}C] or 1 mM 1-mononitroglycerin-1,3[^{14}C] (1-MNG-1,3[^{14}C]) which were both purchased from New England Nuclear (Boston, Mass.), 8 mM reduced glutathione, 8 mM potassium cyanide, homogenate equivalent to 125 mg tissue, and 75 mM phosphate buffer for a final volume of 2 ml with a pH of 7.4. The incubations were conducted at 37° for 10 min and terminated with 0.5 ml of 5% mercuric chloride and 2.5 ml of absolute ethanol. When [^{14}C]TNG was incubated, the supernatant fluid, which was obtained following centrifugation, was co-chromatographed with authentic nitroglycerin standards on Silica gel t.l.c. plates. The metabolites were resolved in a solvent system of benzene and ethyl acetate (4:1), as previously described [2]. Nitroglycerins were visualized, after spraying with 5% diphenylamine, and quantified using liquid scintillation counting. In this system, TNG, 1,3-DNG, and 1,2-DNG migrate with R_f values of 0.84, 0.59 and 0.33, respectively, while MNG isomers (1-MNG and 2-MNG), glycerol, and glucuronides remain at the origin. When [^{14}C]MNG was the substrate, MNG and glycerol were resolved with R_f values of 0.58 and 0, respectively, in ethyl acetate and *n*-heptane (9:1). Protein determinations [9] were made on the homogenates using bovine serum albumin as the standard. Control reactions, which contained 1.15% KCl instead of the homogenate, were conducted to measure non-enzymatic denitration. The enzymatic formation of metabolites was corrected for non-enzymatic denitration which did not exceed 25 per cent of the total radioactivity. The results were expressed as nmoles metabolite/mg of protein.

TNG, as reported by others [3–6], was metabolized primarily to 1,3-DNG and 1,2-DNG after a 10-min incubation. The enzyme activity was relatively stable, since livers from rats which were sacrificed and stored for 18 hr in the refrigerator produced the same amount of DNG isomers as fresh liver. The radioactivity at the origin represented 5 per cent or less of the total radioactivity and was excluded from further consideration. This radioactivity probably represented isomers of MNG, since [^{14}C]MNG was not metabolized to glycerol in any of the species tested. This conclusion was also supported by the observation that [^{14}C]glycerol, but not [^{14}C]TNG, was metabolized to [^{14}C]CO_2 [4]. As a result, it was proposed that the liver was unable to catalyze the complete denitration of TNG to glycerol.

A comparison of the metabolism of TNG by livers from various species is presented in Table 1. Since there was no apparent sex difference, the values obtained for both males and females were combined. The parameters which were used to evaluate TNG metabolism permitted the species to be divided into several groups. If the formation of 1,3-DNG was the basis for comparison, then rats produced more of this metabolite than other species. In contrast, if the comparison was made in terms of 1,2-DNG

Table 1. Metabolism of [^{14}C]TNG by livers from various species

Species	No. of determinations	1,3-DNG (nmoles/mg protein)	1,2-DNG	1,3-DNG/1,2-DNG
Rat	7	40.6 ± 1.9*	28.0 ± 1.3	1.4
Mouse	6	16.7 ± 0.9	7.0 ± 0.3	2.4
Rabbit	6	18.3 ± 0.8	51.3 ± 2.2	0.4
Dog	7	21.1 ± 0.7	46.8 ± 2.0	0.4
Monkey	5	16.3 ± 2.1	52.7 ± 5.2	0.3
Human	4	12.5 ± 2.9	32.5 ± 4.9	0.4

* Mean ± S.E. values for males and females were combined.

Table 2. Metabolism of [^{14}C]TNG during development in mice and rats

Species	Tissue	Days after birth	1,3-DNG (nmoles/mg protein)	1,2-DNG (nmoles/mg protein)
Mouse				
	Embryo	−7	0	3.8 ± 0.9
	Liver	−1	7.4 ± 0.6*	5.9 ± 0.2
	Carcass	−1	3.4 ± 1.2	4.9 ± 0.4
Rat				
	Liver	−2	12.2 ± 0.8	9.4 ± 1.0
	Liver	1	14.6 ± 0.4	9.1 ± 0.1
	Liver	7	27.0 ± 1.0	23.1 ± 0.6
	Liver	14	26.9 ± 1.2	21.4 ± 2.0
	Liver	21	29.1 ± 1.0	24.8 ± 1.8

* Mean ± S.E. for three determinations.

Table 3. Metabolism of [^{14}C]TNG by placentas from various species

Species	Gestational age	1,3-DNG (nmoles/mg protein)	1,2-DNG (nmoles/mg protein)
Mouse	Day 12	1.5 ± 0.8*	3.9 ± 0.9
	Day 18	3.1 ± 0.1	5.2 ± 0.4
Rat	Day 19	2.5 ± 0.6	3.8 ± 0.2
Human	Term	0.7 ± 0.4	4.7 ± 1.3

* Mean ± S.E. for three determinations.

formed, then the species were classified as low producers (mice), intermediate producers (rats and humans), or high producers (rabbits, dogs and monkeys). A comparison of the total amount of denitrated metabolites produced indicated that rats, rabbits, dogs and monkeys produced more isomers of DNG than did humans. Mice, in contrast, had the lowest activity for denitrating TNG. A qualitative comparison between species, in terms of the DNG isomers produced, indicated that rats and mice produced more 1,3-DNG relative to 1,2-DNG than other species. Although none of the species metabolized TNG exactly like humans, there were some similarities to humans in terms of 1,3-DNG formed (mouse, rabbit, dog and monkey), 1,2-DNG formed (rat) and ratio of 1,3-DNG to 1,2-DNG (rabbit, dog and monkey). The results of the present study are qualitatively similar to those obtained for rats, rabbits and dogs [7].

A previous measure of organic nitrate reductase activity [10], which used erythrityl tetranitrate as the substrate and the formation of inorganic nitrate as the measure of activity, concluded that livers from both humans and rats had the same capacity for denitration. The present study does not support this conclusion. There may be both qualitative and quantitative differences between species in their capacity to denitrate this class of nitrate ester containing compounds.

Since the metabolism of many compounds changes during development, studies were undertaken to measure organic nitrate reductase activity in developing tissues. Low levels of activity were found in mouse embryos on day 12 of gestation and in mouse livers and carcasses on day 18 of gestation (Table 2). The activity was also low in fetal rat livers but increased between 1 and 7 days of age. Although there were no further changes in activity between 1 and 3 weeks of age, rats at 3 weeks of age tended to produce less 1,3-DNG than adults. The ratio of 1,3-DNG to 1,2-DNG for the weanling and adult rats was 1.17 and 1.45 respectively. Placentas from mice, rats and humans had poor ability to denitrate TNG (Table 3). These developmental studies indicate that both immature livers and placentas have a poor capacity to metabolize TNG.

In summary, homogenates of livers from various species metabolized TNG primarily to 1,3-DNG and 1,2-DNG. None of the livers from the various species tested metabolized TNG exactly like human liver, although there were some similarities in the various parameters examined. Embryos, fetal liver, and placentas had poor ability to metabolize TNG. Increased ability to metabolize TNG was acquired during the postnatal period.

Acknowledgements—This research was supported by the U.S. Army Medical Research and Development Command, Washington, D.C. 20314, under Contract DAMD-17-74-C-4073. The authors thank Mr. Paul Blotkamp for competent technical assistance.

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