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Biochemical Pharmacology, Vol. 24, pp. 648–650. Pergamon Press, 1975. Printed in Great Britain.

Reduction *in vivo* of (−)-nicotine-1′-*N*-oxide by germ-free and conventional rats

(*Received* 30 *May* 1974; *accepted* 25 *September* 1974)

To date, only a few studies on the reduction *in vivo* of *N*-oxides have been reported [1–4]. These investigations are at variance with regard to the site, extent and/or mechanism(s) of reduction of the limited range of compounds studied. Recently, Beckett *et al.* [5] reported reduction of (−)-nicotine-1′-*N*-oxide to (−)nicotine after oral administration of the oxide to human subjects. However, following intravenous administration of the compound no appreciable reduction could be observed. This was interpreted as being due to reduction by gut content, although no direct evidence was given to support this contention. In the present work we have re-examined the reduction of the oxide using germ-free as well as conventional rats in order to gain more insight into the problem.

Male Wistar Albino rats of nearly the same age and weighing approx. 200 g were housed in separate metabolic cages and had free access to a standard diet (Dixon's diet 86) and water. A single oral dose of (−)-nicotine-1′-*N*- oxide (1·5 mg base/kg body wt) or (−)-nicotine tartrate (1 mg base/kg body wt) was administered by gavage to each rat. Urine, free from faeces, was then collected in tubes surrounded by carbon dioxide ice for a period of 20 hr.

The germ-free rats of the Agus strain weighing approx. 150 g were transferred individually to metabolic cages immediately after arrival from the supplier. One millilitre sterile solution of (−)-nicotine-1′-*N*-oxide (0·4 mg base/ml) was given by gavage to each rat, and the urine was collected as described above for the conventional rats. The animals were then recontaminated with the microflora of the animal house by keeping them in cages containing faecal material from conventional rats for 3–4 weeks; the experiment was then repeated with a second dose of the *N*-oxide and the urine was similarly collected. The recontaminated animals, as well as the conventional rats, were then treated with antibiotics, and the administration of (−)-nicotine-1′-*N*-oxide was repeated 3 weeks after the last dose of the oxide. These animals thus served as their own control.

Antibiotic treatment consisted of 1 ml of the following mixture given orally twice daily for three consecutive days: 100 mg Tetracycline HCl, 100 mg Neomycin sulfate and 100,000 units Nystatin, suspended in 1% aqueous methyl cellulose solution. On the 4th day a similar dose of the antibiotic mixture was administered, followed after 4 hr by the administration of (−)-nicotine-1′-*N*-oxide. Four hours later an additional dose of the antibiotic mixture (1·5 ml) was given.

In an attempt to by-pass any possible intestinal reduction of the *N*-oxide, the drug was also administered intraperitoneally to the same conventional group of rats (0·8 mg base/kg body wt dissolved in 8·5 ml sterile normal saline) 3 weeks after the last oral dose of the drug. Urine samples were collected as described in the above-mentioned experiments. Each of the frozen urine samples from all the foregoing experiments was thawed and analysed for (−)-nicotine, nicotine-1′-*N*-oxide and cotinine by gas liquid chromatography as described by Beckett *et al.* [6].

It is clear from Table 1 that considerable reduction of (−)-nicotine-1′-*N*-oxide occurred following its oral or intraperitoneal administration (1·5-fold more by the oral route). The formation of (−)-nicotine following the i.p. administration is of particular interest as it strongly suggests the occurrence of hepatic and other tissue reduction of the *N*-oxide. In fact, Dajani *et al.* [7] have recently reported a profound hepatic and intestinal tissue reduction of (−)-nicotine-1′-*N*-oxide *in vitro* in the rat. It is worth noting that the foregoing experiments are in conflict with the studies reported earlier [5], probably because of species variation.

Contrary to expectation from the known effects of antibiotics on the gut flora, the amounts of (−)-nicotine formed by each rat as well as by the whole group after treatment with antibiotics was significantly more than the controls ($P < 0{\cdot}05$). It would seem that following the administration of antibiotics, changes in the number and composition of the gut flora must have occurred. These could have led to alterations in the intestinal motility and, in turn, to changes in the pH of the gut contents. Such changes would alter the rate and site of absorption of the administered drug as well as the rate and routes of biotransformation and excretion [8]. Alternatively, the increased excretion of (−)-nicotine could be due to an excessive over-growth of some reducing organisms not sensitive to the antibiotic mixture used. Despite the fact that the bacteriology of the gastro-intestinal tract content is known, a bacteriological study after the antibiotic treatment is needed to confirm this speculation.

Table 1. Percentage* urinary excretion† during 20 hr of (−)-nicotine and its major metabolites by conventional rats after a single oral or intraperitoneal dose‡ of (−)-nicotine-1′-*N*-oxide

	(−)-Nicotine			(−)-Nicotine-1′-*N*-oxide			Cotinine		
	CON		ANT	CON		ANT	CON		ANT
Rat	Oral	I.p.	Oral	Oral	I.p.	Oral	Oral	I.p.	Oral
1	11·3	12·7	26·3	46·6	88·2	54·4	6·4	0·7	4·3
2	20·0	15·4	30·7	61·7	82·5	71·9	10·2	0·8	2·1
3	26·7	13·2	27·5	66·7	85·9	61·9	13·9	0·8	3·9
4	11·3	11·8	22·8	48·0	90·0	49·1	8·2	0·8	2·9
5	27·5	14·7	21·9	38·0	79·8	70·2	10·1	1·4	3·3
6	12·1	10·9	23·7	69·3	82·8	57·8	4·9	1·0	3·2
7	28·8	10·5	27·8	62·7	86·0	63·1	4·9	0·9	2·9
8	13·0	12·3	23·7	51·6	87·5	61·2	4·4	0·7	
Average	18·8	12·7	25·6	55·6	85·3	61·2	7·8	0·9	3·2
± S.D.§	7·31	1·61	1·20	11·79	3·17	3·62	3·59	0·20	0·69

CON = Conventional rats; ANT = Antibiotic-treated rats; I.p. = Intraperitoneally injected rats.

* Per cent of administered dose calculated as nicotine base.

† Volume of urine ranged from 8 to 12 ml and had a pH of 5·9–6·5.

‡ Orally 1·5 mg or 0·8 mg in 1 ml saline i.p./kg body wt of (−)-nicotine-1′-*N*-oxide (mixture of *trans*- and *cis*-isomers 7:3). Rats initially weighed 200 ± 10 g.

§ P-values [12] for CON vs ANT group: nicotine, 0·04; nicotine-1′-*N*-oxide, 0·085; cotinine, 0·005. CON vs I.p.: nicotine, 0·015; nicotine-1′-*N*-oxide, 0·005; cotinine, 0·001.

The higher level of the unchanged *N*-oxide excreted after i.p. administration of the drug could be explained by the premise that absorption was more rapid than that following oral administration, thereby resulting in more rapid elimination before the drug had time to be biotransformed, or bind in sufficient quantities to storage sites. Similarly, smaller quantities of continine were formed after i.p. administration, possibly because of the smaller quantities of (−)-nicotine formed from the *N*-oxide and the rapid elimination of the compound by the kidney.

It is clear from Table 2 that substantial reduction occurred after the oral administration of the *N*-oxide to germ-free rats as reflected by the excretion of 13 per cent (−)-nicotine plus 4·5 per cent cotinine. The latter compound

Table 2. Percentage* urinary excretion† during 20 hr of (−)-nicotine and its metabolites by germ-free rats after a single oral dose‡ of (−)-nicotine-1′-*N*-oxide

	(−)-Nicotine			(−)-Nicotine-1′-*N*-oxide			Cotinine		
Rat	GF	GFC	GFC–ANT	GF	GFC	GFC–ANT	GF	GFC	GFC–ANT
1	12·5	17·3	12·7	74·6	71·8	73·2	4·2	4·9	4·5
2	14·6	14·2	13·9	72·8	70·7	71·0	3·6	4·1	4·0
3	11·3	13·4	11·8	69·9	66·3	70·1	5·3	5·0	4·9
4	15·4	16·2	16·0	73·7	71·2	74·0	3·8	4·2	5·2
5	13·8	18·4	15·6	66·5	67·1	72·3	3·9	3·9	4·8
6	10·9	15·8	13·2	80·2	76·4	76·2	4·3	5·7	4·6
7	12·7	13·5	11·7	77·1	75·3	77·5	6·1	5·3	3·9
8§	11·8	—	—	79·3	—	—	5·0	—	—
Average	12·9	15·5	13·6	73·0	71·3	73·5	4·5	4·7	4·6
± S.D.‖	0·60	4·18	1·58	4·33	9·89	2·47	0·80	1·36	0·43

GF = Germ-free; GFC = Recontaminated; GFC–ANT = Recontaminated, treated with antibiotics; CON = Conventional.

* Per cent of administered dose calculated as nicotine base.

† Volume of urine in ml before contamination ranged from 7–12 (pH 5·3–6·2); volume after contamination with laboratory flora ranged from 7 to 13 ml (pH 6·1–6·5).

‡ Body wt (1·5 mg/kg) (−)-nicotine-1′-*N*-oxide (mixture of *trans*- and *cis*-isomers, 7:3) in 0·8 ml water. GF rats weighed 150 g, GFC and GFC–ANT weighed 240–320 g (comparable intragroup weights).

§ Died during the recontamination period.

‖ P-values [12] for CON vs GF rats: nicotine, 0·15; nicotine-1′-*N*-oxide, 0·04; cotinine, 0·16 (see Table 1). GF vs GFC: nicotine, 0·075; nicotine-1′-*N*-oxide, 0·23; cotinine, 0·36. GF vs GFC–ANT: nicotine, 0·28; nicotine-1′-*N*-oxide, 0·40; cotinine, 0·36.

is believed to have originated from an extra-intestinal oxidation of (−)-nicotine which, presumably, was formed by the reduction of the ingested *N*-oxide. In contrast to the amounts excreted by the conventional rats, the extent of excretion of (−)-nicotine and continine by germ-free rats was not significant (see Table 2, footnote 5). Furthermore, the amounts of unchanged *N*-oxide excreted by the germ-free rats were significantly less than those eliminated by the conventional animals. This is not unexpected since these amounts are believed to be net results of oxidation and reduction reactions functioning simultaneously in the animal body. Indeed, oxidation of (−)-nicotine to the *N*-oxide and continine is well documented [6, 10]. Likewise, hepatic reduction of (−)-nicotine-1′-oxide has recently been reported [5, 7, 11].

The data in Table 2 further show that contamination of the germ-free rats with the micro-organisms usually harboured by the conventional rats did not cause substantial alteration in the quantities of (−)-nicotine and its metabolites excreted by these rats ($P > 0.05$). Even the administration of the antibiotics to the contaminated group did not appreciably change the pattern of excretion of these substances (Table 2, footnote 5). It is to be recalled that conventional rats excreted, after antibiotic treatment, significantly more (−)-nicotine than did the controls (Table 1). The discrepencies between the results of the conventional and germ-free rats could be due partly to strain and age differences. Undoubtedly, other factors that have not been measured in the present study could also be involved.

When (−)-nicotine-1′-*N*-oxide was incubated anaerobically for 6 hr with a suspension (1:50, in a nutrient medium) of the gastro-intestinal content (stomach to colon), a considerable reduction to (−)-nicotine (25 per cent) was observed. The recovery was practically quantitative (97 per cent). Previous results indicated that the caecal content was the most active in this respect and that of the stomach the least.

Apart from the established role played by the gut flora in the reduction of *N*-oxide drugs, the present findings strongly suggest the involvement of liver and/or other tissues in the reduction *in vivo* of (−)-nicotine-1′-*N*-oxide and probably other *N*-oxide compounds. Indeed, our recent studies *in vitro* [7] on the reduction of (−)-nicotine-1′-oxide as well as those of others [11] support this contention.

Acknowledgements—The authors are thankful to Messrs. Glaxo Laboratories, Ltd., Greenford, Middlesex, England, and E. R. Squibb and Sons, Liverpool, England, for the generous supply of Neomycin sulphate and Mysteclin, respectively. The supply of the germ-free rats by the Laboratory Animals Center, Carshalton, Surrey, England, is gratefully acknowledged.

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