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Some observations on the toxicology of morphine-*N*-oxide

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The intravenous and subcutaneous acute toxicities of morphine-*N*-oxide (MNO) in mice were respectively 3·2 and 8 times less than that of morphine. Amiphenazole or tacrine reduced the acute toxicity of MNO but not that of morphine in mice. The chronic toxicity of MNO was examined in mice and rats. Daily oral doses of 100 mg/kg did not significantly affect growth or condition, or produce gross or microscopic lesions in mice treated for 3 weeks or rats treated for 3 months. No teratogenic effect of MNO or of bromolysergic acid diethylamide was observed in rats.

Woo, Gaff & Fennessy (1968) identified morphine-*N*-oxide (MNO) in the urine of patients treated with morphine in combination with either amiphenazole (2,4-diamino-5-phenylthiazole) or tacrine (1,2,3,4-tetrahydro-9-aminoacridine) but not after morphine alone. They suggested that MNO was an intermediate metabolite in the breakdown of morphine. Earlier reports suggested that MNO was without analgesic activity (Freund & Speyer, 1910; Rosenthäler, 1933; Keil, Schmidt & Günther, 1933; Anton, Theiss & Weissig, 1935; Braenden, Eddy & Halbach, 1955). However, Fennessy (1968) found it to have a weak analgesic activity which was markedly potentiated when it was administered with either amiphenazole or tacrine to rats and mice. The potentiation of the analgesic action of MNO and its presence in urine after morphine was suggested to be due to impairment of metabolism of MNO in the liver by amiphenazole and tacrine.

MNO may be of clinical interest as a metabolite of morphine or as an analgesic in its own right. We have examined its toxicology.

EXPERIMENTAL

Acute toxicity

Swiss mice of either sex, about 25 g were randomly assigned to groups of 10; various doses of morphine, MNO, amiphenazole, tacrine or nalorphine, given subcutaneously, were randomly assigned to these groups and the mice dead in each group after 24 h were recorded. The LD50 values with 95% confidence limits were calculated (Litchfield & Wilcoxon, 1949).

The intravenous LD50 values for morphine and MNO were also determined in normal mice and in mice pretreated 30 min before with amiphenazole, tacrine or nalorphine injected subcutaneously in doses corresponding to one-third and two-thirds of their subcutaneous LD50 doses.

Subacute toxicity

Female Swiss mice, 4 weeks old, about 15 g, were randomly assigned to 3 equal

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groups of 10 animals. One group received 100, another 1000 mg/kg of MNO by stomach tube in 0·2 ml of a suspension in 1% gum tragacanth. The third group were given the suspending agent. Food and water were allowed *ad libitum*. Body weights were recorded every 5 days. On the 22nd day, drug treatment was discontinued and body weight was recorded for a further 75 days.

Chronic toxicity

Female Sprague-Dawley rats, mean weight 154 g, were randomly assigned to 3 equal groups of 8 animals. The first group were given a daily dose of 25 mg/kg of morphine, the second group 100 mg/kg of MNO, and the remaining group were untreated. Each rat was given the required dose dissolved in water and mixed with 15 g of diet (Barostoc Growers Pellets). This amount of food was always consumed by the rats. The daily dose of 100 mg/kg of MNO was the maximum the animals would consume, even at the point of starvation. Water was given *ad libitum*. Body weights were recorded for 124 days and mean differences between the groups were tested for significance using the *t*-test. After 124 days the rats were killed and the organs examined histologically.

In another experiment 5 groups, each of 8 female Sprague-Dawley rats, mean weight 255 g, were used. The first group, controls, received no drug treatment; the second received daily a mixture of MNO (50 mg/kg) and amiphenazole (50 mg/kg); the third a mixture of MNO (50 mg/kg) and tacrine (5 mg/kg); the fourth amiphenazole (50 mg/kg), and the fifth group tacrine (5 mg/kg). Each animal received the required dose of drug dissolved in water and mixed with 19 g of food (Barostoc Growers Pellets). Drinking water was allowed *ad libitum*. Weights were recorded weekly for 13 weeks. The animals were then killed, inspected for gross pathological changes and organs were removed for histological examination.

Teratogenic activity

Healthy Sprague-Dawley female rats were mated with healthy males; 33 of 45 becoming pregnant and these were randomly assigned to 3 groups. The rats were individually housed. On the 3rd, 4th and 5th days of gestation, rats in one group received 50 μg/kg of 2-bromo-D-lysergic acid diethylamide (BOL) (an agent reported to have teratogenic activity, Geber, 1967) those in the second group received MNO 50 mg/kg) and those in the third, normal saline. On the 20th day of gestation the rats were killed with chloroform and the uterus and contents removed. Each foetus was examined to determine its viability and development and preserved for study of skeletal structure using the alizarin technique (Mahoney, 1966).

Drugs used were: Morphine sulphate (D.H.A.), Morphine-*N*-oxide, amiphenazole hydrochloride (Nicholas Pty. Ltd.), tacrine (H. W. Woods Pty. Ltd.), nalorphine hydrobromide (Burroughs Wellcome) and 2-bromo-D-lysergic acid diethylamide (Sandoz, Aust.). The concentration of all drugs has been expressed in terms of the base. Morphine-*N*-oxide was prepared according to the method of Freund & Speyer (1910). Solutions for injection were freshly prepared each day by dissolving the MNO in 0·1 N HCl and adjusting to pH 5 with NaOH.

RESULTS

Acute toxicities in mice

The LD50 values and their 95% confidence limits obtained with intravenous and subcutaneous injections of morphine and MNO, and with subcutaneous injections of

Table 1. *Acute toxicities in mice*

Compound	LD50 mg/kg (95% confidence limits) Subcutaneous injection	Intravenous injection
Morphine	675 (527–864)	250 (211–308)
MNO	>5300	820 (773–869)
Nalorphine	731 (594–899)	—
Amiphenazole	260 (226–299)	—
Tacrine	34 (29–39·8)	—

nalorphine, amiphenazole and tacrine are in Table 1. MNO was much less toxic than morphine, the extent depending on the route of administration. The intravenous LD50 of MNO was 3·2 times greater than that of morphine and the subcutaneous LD50 could not be determined as no mice died after 5300 mg/kg, the highest dose given because of the low solubility of MNO.

Changes in the LD50 values for morphine and MNO in mice pretreated with one-third and two-thirds the LD50 doses of nalorphine, amiphenazole and tacrine are shown in Fig. 1. The toxicity of MNO was clearly antagonized by tacrine, but only slightly antagnoized by amiphenazole or nalorphine. Tacrine, however, had no effect on the toxicity of morphine, amiphenazole caused a slight potentiation, and nalorphine produced a slight antagonism.

Subacute toxicity in mice

MNO given orally for 22 days to mice impaired growth compared to controls (Fig. 2).

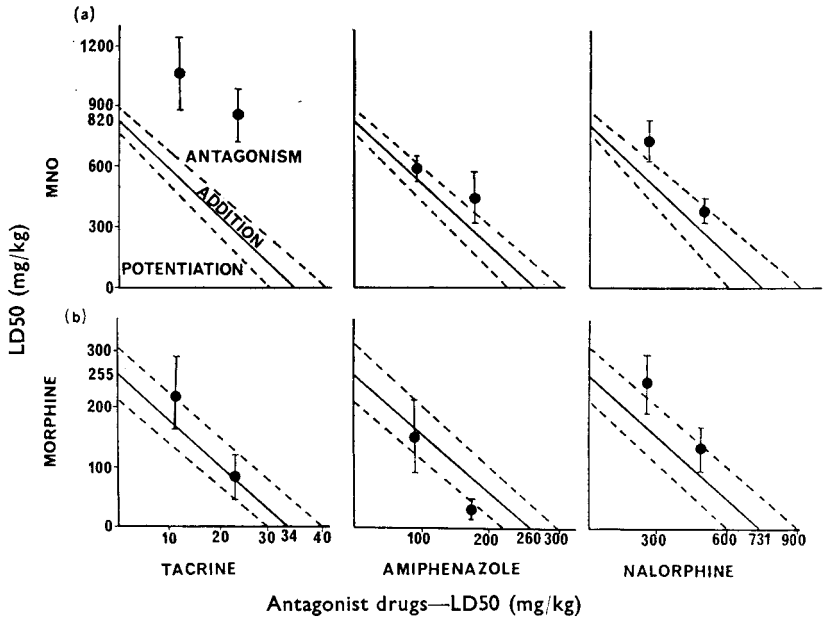

FIG. 1. Interaction between the toxic effects of analgesic drugs and narcotic antagonists in mice. The unbroken lines join the LD50 of (a) MNO or (b) morphine and the LD50 of tacrine, amiphenazole and nalorphine respectively. The broken lines join the 95% confidence limits of the corresponding LD50 of each drug. The vertical lines indicate the confidence limits for the LD50 of (a) MNO or (b) morphine in the presence of one-third or two-thirds LD50 of tacrine, amiphenazole or nalorphine given subcutaneously 30 min before the intravenous administration of either morphine or MNO.

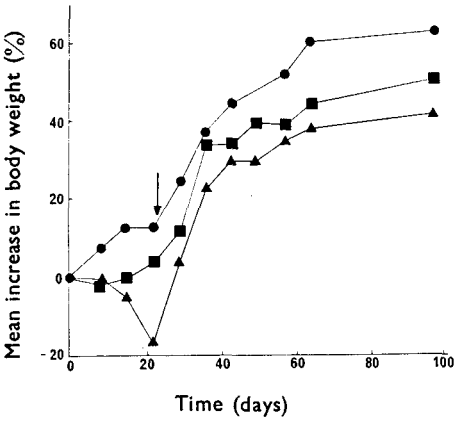

FIG. 2. The effect of MNO on body weight of mice. MNO was administered orally at dose levels of 100 mg/kg (■–■) and 1000 mg/kg (▲–▲). Control mice (●–●) received daily oral administrations of saline. Treatment was discontinued on the 22nd day (arrow). Each point is the mean weight of ten mice.

At 100 mg/kg of MNO daily the mean body weight of mice decreased slightly for the first 10 days then increased, but the weights of mice receiving 1000 mg/kg, were significantly decreased after 22 days compared to the other two groups (*t*-test, $P < 0{\cdot}05$). Discontinuation of the drug was followed by a rapid increase in body weight. After 100 days the mean weight of the mice on the higher dose of MNO was slightly less than the control value, while animals on the lower dose of MNO had a higher mean weight.

Chronic toxicity in rats

The growth rate of rats was slightly increased by MNO (100 mg/kg daily) but was significantly reduced by morphine (25 mg/kg) (Table 2). The general condition of the rats in all three groups remained good. No morphological or histological abnormalities were found in liver, kidneys, brain, bone-marrow, spleen, heart or gastrointestinal tract.

Amiphenazole (50 mg/kg) or tacrine (5 mg/kg) included in the diets of rats receiving MNO (50 mg/kg) daily caused a significant decrease in body weight after 91 days (Table 3), similar to that of rats receiving the same dose of amiphenazole or tacrine alone. No morphological abnormalities were found except that rats receiving

Table 2. *Effect of morphine and* MNO *on body weight in rats.* Morphine (25 mg/kg) or MNO (100 mg/kg) was given orally, mixed with the diet, daily for 124 days. There were 8 rats in each group.

	Mean body weight g ± s.e.		Mean percentage increase in weight ± s.e.
	Before test	After test	
Controls	150·4 ± 8·3	189·7 ± 6·8	26·1 ± 1·3
Morphine	154·1 ± 6·2	162·3 ± 5·9	5·3 ± 2·6*
MNO	158·2 ± 3·1	205·5 ± 4·7	29·9 ± 4·7†

* Significantly different from control (*t*-test, $0{\cdot}01 < P < 0{\cdot}05$).
† Not significantly different from control (*t*-test, $0{\cdot}05 < P < 0{\cdot}1$).

Table 3. *Effects of amiphenazole, tacrine and combinations of amiphenazole* + MNO *and tacrine* + MNO *on body weight in rats. Drugs were given orally, mixed with the diet, to each rat for* 91 *days. There ware* 8 *rats in each group.*

	Mean body weight in g ± s.e.		Mean percentage change in weight
	Before test	After 91 days	
Control	225·2 ± 13·0	229·0 ± 10·0	+1·7
Amiphenazole (50 mg/kg)	224·6 ± 10·3	212·5 ± 9·2	−5·4
Tacrine (5 mg/kg)	225·5 ± 8·5	192·2 ± 7·4*	−14·7
MNO (50 mg/kg) + amiphenazole (50 mg/kg)	227·0 ± 6·6	210·4 ± 4·2*	− 7·5
MNO (50 mg/kg) + tacrine (5 mg/kg)	222·8 ± 9·2	196·3 ± 8·1*	−11·7

* Significantly different from controls, $P < 0{\cdot}01$.

either tacrine or a mixture of MNO and tacrine had distended stomachs and reduced spleens.

Chronic toxicity studies of MNO given subcutaneously, intraperitoneally or orally were abandoned because the treated rats became autocannibalistic; also, animals receiving MNO subcutaneously developed necrotic lesions at the site of injection within 24 h.

Teratogenic activity

When MNO (50 mg/kg), BOL (50 μg/kg) or saline were injected subcutaneously into pregnant rats on the 3rd, 4th and 5th days of gestation, and foetuses examined on the 20th day, i.e., one day before full term, there was no significant difference between the litter sizes or the mean foetal weights of animals in any of the groups (t-test, $P > 0{\cdot}1$). No skeletal abnormalities were observed after fixing and staining the foetuses. Foetal resorptions were observed in all three groups of rats, the highest being in the control group.

DISCUSSION

The acute toxicity of MNO was much lower than that of morphine. It appears probable that *N*-oxide derivatives of opium alkaloids have lower toxicities than the parent compounds, since the LD50 of codeine-*N*-oxide was found by Tagaki & Fukuda (1960) to be 1500 mg/kg when given subcutaneoulsy to mice compared with 356 mg/kg for codeine. The chronic toxicity of MNO was also lower than that of morphine. Thus MNO did not affect growth rate in rats whereas a smaller dose of morphine significantly inhibited growth. However, in mice, large daily concentrations of MNO (1 g/kg) did decrease body weight.

Although both amiphenazole and tacrine potentiate the analgesic action of MNO (Fennessy, 1968), both drugs antagonize the acute toxicity of MNO, tacrine being the better antagonist. However, in rats, tacrine caused greater inhibition of growth than amiphenazole, it also produced definite morphological abnormalities. The evidence indicates that the decreased growth rate produced by combinations of MNO with tacrine and or with amiphenazole is due to the actions of the antagonists and not to MNO.

MNO had no teratogenic activity in rats, but it is difficult to draw conclusions from the negative results obtained.

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