J. Pharm. Pharmacol. 1992, 44: 687–690
Communicated November 28, 1991

Dispositional study of opioids in mice pretreated with sympathomimetic agents

YOSWA M. DAMBISYA, KELVIN CHAN, CHAK-LAM WONG*, *Department of Pharmacology, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong and *School of Pharmacy, Portsmouth Polytechnic, Portsmouth PO1 2DT, UK*

Abstract—Brain and plasma levels of morphine and codeine were determined by an assay method involving solid-phase extraction and ion-pair reversed phase HPLC. Detection was by a variable wavelength UV-detector (for codeine) and an amperometric electrochemical detector (for morphine) coupled in series. Ephedrine or phenylpropanolamine pretreatment did not interfere with the plasma disposition of morphine, evidenced by overlapping plasma concentration-time profiles. Brain opioid levels were equally unaffected by sympathomimetic pretreatment. The relative ratios of brain to plasma concentrations at the time corresponding to the respective peak anti-nociceptive activity for morphine and codeine revealed no significant differences. It is concluded that single doses of ephedrine and phenylpropanolamine do not affect the disposition of morphine and codeine in mice.

The increasing abuse of cough and cold medicines is a subject of growing concern in Hong Kong (CRDA 1990). Chan et al (1990) noted a high abuse rate of cough mixtures, especially those containing codeine and ephedrine, among teenagers. In view of the wide-spread availability of proprietary preparations containing opioids and sympathomimetics, a series of studies was undertaken to investigate the interactions between ephedrine or phenylpropanolamine (PPA), both of which are common ingredients of these preparations, with codeine and morphine. It has been shown that ephedrine or PPA potentiates the acute anti-nociceptive effects of morphine and codeine in mice (Dambisya et al 1990). In addition, the acute lethality of these opioids is enhanced by pretreatment with moderate doses of the sympathomimetics (Dambisya et al 1991a). Chronic interaction studies have indicated that ephedrine and PPA enhance the development of opioid tolerance with no effect on that of physical dependence, while in the expression phase, tolerance was unaffected but the withdrawal signs were suppressed by sympathomimetic pretreatment (Dambisya et al 1991c).

There are reports suggesting that ephedrine and PPA may alter the pharmacokinetic profiles of caffeine, another commonly used drug (Weinberger et al 1975; Lake et al 1990). This is thought to be through metabolic impairment, and probably explains the high incidence of adverse effects when caffeine and ephedrine or PPA are co-administered (Lake et al 1990). Leza et al (1990) have advanced the possibility that some histamine receptor blockers (also common ingredients of cough and cold preparations) may facilitate opioid passage across the blood-brain barrier. The metabolism of codeine, especially its *O*-demethylation to morphine, has gained much interest recently (Yue et al 1989a, b; Sjöqvist 1991).

The objective of the present study was to establish whether or not ephedrine and PPA altered the plasma or brain levels of codeine and morphine after single dose treatment. Evidence for the possible conversion of codeine to morphine was also sought.

Materials and methods

Animals. Male ICR mice, 30–35 g, were kept under air-conditioning (20 ± 2°C) and artificial light for 12 h each day, with free access to standard laboratory diet and tap water.

Correspondence: Y. M. Dambisya, Department of Pharmacology, Faculty of Medicine, Basic Medical Sciences Building, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong.

Treatment protocol and sample collection. Drugs. Morphine sulphate and codeine phosphate (Macfarlan Smith Ltd, UK) and ephedrine HCl (May and Baker, UK) and phenylpropanolamine HCl (Sigma, St Louis, MO) were dissolved in physiological saline (0·9% NaCl) and administered in a volume of 10 mL kg^{-1} intraperitoneally or subcutaneously as applicable. The doses refer to the weight of the salts used. The controls received saline injections at the corresponding times. As in our previous studies (Dambisya et al 1990, 1991a, c), the sympathomimetics were administered intraperitoneally 10 min before the subcutaneous dose of opioid. The pretreatment dose of ephedrine or PPA in this study was 10 mg kg^{-1} while morphine and codeine were administered in doses of 8 and 60 mg kg^{-1}, respectively. Codeine 60 mg kg^{-1} is approximately the (anti-nociceptive) ED50 of codeine using the tail-flick test, while morphine 8 mg kg^{-1} is the highest dose level of morphine used in our earlier studies. The higher dose level of morphine was preferred to the ED50 as the former resulted in higher plasma and brain levels. This limitation was not applicable in the case of codeine due to its high ED50. At these dose levels, the sympathomimetics potentiate the opioid (anti-nociceptive) effects (Dambisya et al 1990). Mice were randomly assigned to the treatment groups.

Sample collection. After the opioid dose, mice were killed at 15 and 30 min, and at 1, 2, and 3 h under light ether anaesthesia by exsanguination and/or decapitation. Blood from 3 to 4 animals was pooled in tubes with EDTA and centrifuged at 2000 *g* for 30 min. The plasma was stored frozen at −20°C until assay. After decapitation, the brain was rapidly removed, wrapped in aluminium foil and kept at −20°C. The whole brain was later homogenized, the homogenate centrifuged at 2000 *g* for 30 min and the supernatant assayed for opioids.

Opioid assay. The assay method used was a modification of that described by Svensson et al (1982) and a full account of the local adaptations involved has been presented separately (Chan et al 1992).

Materials. Methanol and acetonitrile were HPLC grade, water was glass distilled, while other chemicals, sodium dodecyl sulphate, naloxone HCl (internal standard), sodium dihydrogen phosphate and ammonium sulphate, were of analytical grade.

Plasma samples were processed through solid-phase extraction using Sep-Pak C_{18} cartridges (Waters) (Svensson et al 1982), but the eluent was 3·0 mL of 15% acetonitrile in 10 mM phosphate buffer, pH 2·1 (Yue et al 1989a). A sample (100–300 μL) was injected into the HPLC system. Brain was homogenized in 3 mL of saline and centrifuged as above. The supernatant was then treated as for plasma. By using a vacuum manifold (Visipress, Supelco), it was possible to process the samples in batches of 12 at a time.

The mobile phase consisted of 45% methanol in 10 mM phosphate buffer pH 2·1 (v/v) containing sodium dodecyl sulphate (1 mM). The eluent was delivered at 1 mL min^{-1}

through an RP8 precolumn (guard column) linked to a μBondpak C_{18} stainless steel analytical column (Waters). Detection was with a UV-variable wavelength detector set at 210 nm for codeine, and an amperometric electrochemical detector, for morphine, coupled in series. The mobile phase was recycled during the assay. The calibration graphs of peak height ratios to concentration were linear over the ranges 0·1–5 μg mL^{-1} for plasma morphine (r = 0·9985), 0·5–15 μg mL^{-1} for plasma codeine (r = 0·9989), 0·1–5 μg g^{-1} for brain morphine (r = 0·9993) and 0·5–30 μg g^{-1} for brain codeine (r = 0·9978). Intra-assay variations were less than 10% and often less than 5%. A more detailed account of the methodology has been presented separately (Chan et al 1992).

Statistical calculations. Plasma and brain levels of morphine and codeine are presented as mean ± s.e.m. for the various time points. Data for corresponding concentration-time points were analysed and compared by Student's *t*-test with the level of significance set at $P < 0·05$ and where indicated, differences among the concentration-time curves were tested by one-way analysis of variance (Bowman & Rand 1980).

Results

Effects of ephedrine and PPA on plasma levels of morphine and codeine. The plasma concentration-time profiles of morphine with or without sympathomimetic pretreatment are presented in Table 1a. The values are similar with no significant differences among the various treatment groups, except for PPA + morphine at 2 h where the morphine concentration is significantly lower than the corresponding value in the morphine only group ($P < 0·05$). Analysis of variance for the time-concentration profiles, however, show no differences (F-ratio less than unity). It is noteworthy that at 30 min, corresponding to the peak anti-nociceptive effects of morphine, the concentrations are the same.

The plasma concentration-time profiles for codeine are shown in Table 1b. As is the case for morphine, there are no significant differences between the codeine only group levels and those from the sympathomimetic pretreated ones. No significant amounts of either morphine or codeine were detected in the 3 h plasma samples.

Effects of ephedrine and PPA on brain opioid levels. Table 2a shows brain morphine levels between 15 min and 3 h. There are no discernible differences between the morphine only and sympathomimetic groups.

Table 2b shows the brain codeine profiles between 15 min and 2 h. There are no differences between the codeine only group and the PPA + codeine concentration-time profiles, while that for ephedrine + codeine shows some disparity on account of the markedly lower terminal concentration (2 h). No significant amounts of codeine were detectable in the brain at 3 h.

A comparison was made of the brain opioid concentrations (μg g^{-1}) expressed as a ratio of the corresponding plasma concentration (μg mL^{-1}) at 15 min for codeine and 30 min for morphine, the respective times for peak (anti-nociceptive) effects. The ratios were 0·48 for morphine alone, 0·42 for morphine + ephedrine and 0·57 for morphine + PPA; while those for the codeine group were 2·69 for codeine alone, 2·52 for codeine + ephedrine and 2·56 for codeine + PPA, showing that the ratios were not significantly different with or without ephedrine or PPA for either morphine or codeine. That codeine crosses the blood-brain barrier more readily than does morphine is evident from the high brain to plasma concentration ratios for codeine compared with the values for morphine.

Morphine levels after single dose of codeine. Morphine was detected in significant amounts in plasma of mice that had received codeine (60 mg kg^{-1}) 2 h before (Table 3). There was no consistent concentration-time pattern observed. Comparison of corresponding time points showed statistical significance for ephedrine + codeine at 30 min and 1 h and for PPA + codeine at 1 h, but analysis of variance of the three sets of data revealed no significant differences.

Discussion

Unlike earlier reports (Weinberger et al 1975; Lake et al 1990; Leza et al 1990), the present study has provided no evidence that ephedrine or PPA increases opioid levels in plasma or facilitates their penetration into the brain.

The finding of measurable levels of morphine after single dose codeine was interesting for a number of reasons. Codeine is *O*-demethylated to morphine (Adler et al 1955) and is increasingly being considered as a pro-drug that exerts analgesic and addictive effects (Sjöqvist 1991). After oral administration, with a first pass effect, this may occur early and to a greater extent (Rogers et al 1982), so as to be of clinical significance in the mediation of pain relief, but, even then, the extent to which this is the case is unresolved (Findlay et al 1978). Shah & Mason (1990) have suggested that demethylation occurs more rapidly in rats, with morphine detectable in a 2 min post-dosing sample. In the present study, however, quantifiable morphine levels were only notable at 30 min, a time when the anti-nociceptive effects of codeine have tapered off (Dambisya et al 1990, 1991c). The peak effects of codeine after subcutaneous administration, using the tail-flick test (D'Armour & Smith 1941), occur at 15 min when morphine is barely detectable. That morphine levels are negli-

Table 1. Opioid levels in plasma after single dose treatment.

Time	Mean concentrations ± s.e.m. (n[a]) (μg mL^{-1})		
a	Morphine alone	Ephedrine + morphine	PPA + morphine
15 min	1·01 ± 0·04 (16)	1·12 ± 0·10 (19)	1·04 ± 0·11 (20)
30 min	0·92 ± 0·04 (21)	0·98 ± 0·04 (20)	0·95 ± 0·03 (21)
1 h	0·44 ± 0·11 (23)	0·54 ± 0·10 (21)	0·36 ± 0·07 (18)
2 h	0·35 ± 0·09 (17)	0·29 ± 0·08 (20)	0·12 ± 0·06* (17)
b	Codeine alone	Ephedrine + codeine	PPA + codeine
15 min	9·06 ± 0·41 (19)	9·45 ± 0·28 (18)	9·06 ± 0·31 (22)
30 min	5·45 ± 0·16 (20)	5·25 ± 0·19 (20)	5·84 ± 0·25 (21)
1 h	2·07 ± 0·06 (22)	2·39 ± 0·07 (20)	2·05 ± 0·08 (18)
2 h	0·66 ± 0·08 (20)	0·78 ± 0·09 (19)	0·65 ± 0·03 (20)

[a] n = no. of samples. * $P < 0·05$ compared with the corresponding value in the non-pretreated group.

Table 2. Brain levels of morphine and codeine after single dose treatment.

Time	Mean concentrations ± s.e.m. (n[a]) (μg g^{-1})		
a	Morphine alone	Ephedrine + morphine	PPA + morphine
15 min	0·72 ± 0·05 (19)	0·68 ± 0·09 (20)	0·64 ± 0·03 (22)
30 min	0·44 ± 0·03 (24)	0·41 ± 0·03 (16)	0·54 ± 0·08 (24)
1 h	0·38 ± 0·12 (18)	0·31 ± 0·08 (19)	0·35 ± 0·13 (18)
2 h	0·26 ± 0·06 (18)	0·26 ± 0·07 (20)	0·21 ± 0·09 (19)
3 h	0·12 ± 0·06 (16)	0·16 ± 0·02 (16)	0·14 ± 0·02 (16)
b	Codeine alone	Ephedrine + codeine	PPA + codeine
15 min	24·38 ± 0·52 (18)	23·38 ± 0·81 (21)	23·19 ± 1·47 (22)
30 min	17·59 ± 0·48 (22)	18·32 ± 0·78 (22)	16·35 ± 1·23 (17)
1 h	7·32 ± 0·56 (16)	5·04 ± 0·38* (19)	6·43 ± 0·81 (19)
2 h	2·84 ± 0·54 (14)	1·37 ± 0·65* (17)	1·98 ± 0·81 (16)

[a] n = no. of samples. *$P < 0·05$ compared with the corresponding value in the non-pretreated group.

Table 3. Plasma levels of morphine after single dose codeine.

	Mean concentrations ± s.e.m. (n[a]) (μg mL^{-1})		
Time	Codeine alone	Ephedrine + codeine	PPA + codeine
15 min	— (19)	— (18)	— (22)
30 min	0·96 ± 0·24 (20)	0·59 ± 0·03* (20)	1·21 ± 0·35 (21)
1 h	1·65 ± 0·34 (22)	1·01 ± 0·04* (20)	0·80 ± 0·04* (18)
2 h	0·73 ± 0·15 (20)	0·59 ± 0·08 (19)	0·57 ± 0·06 (20)

[a] n = no. of samples. — trace amounts. *$P < 0·05$ compared with the corresponding value in the non-pretreated group.

gibly low while codeine levels are high at the time of peak effect suggests that codeine alone may exert anti-nociceptive effects. More so, there were no significant morphine levels detected in the brain following single dose codeine. Our data are consistent with those of other workers who have suggested that the morphine metabolite may have only a minor analgesic influence after single dose codeine (Shah & Mason 1990). It should be noted, however, that plasma and brain levels of morphine after single dose morphine were higher at 15 min than at 30 min, and yet peak effects of morphine occur at 30 min. It appears there is no obvious relationship between morphine levels in plasma or brain and peak anti-nociceptive effects.

Single dose treatment with ephedrine or PPA had no effect on the metabolism of codeine to morphine. Quantifiable levels still occurred at 30 min, and although the concentration-time profiles showed no consistent pattern for the various treatment regimens, there seem to be no major differences attributable to the sympathomimetics. The significance of the discrepancies observed becomes even more difficult to appreciate in view of the large number of animals involved, each of which may have handled codeine metabolism differently.

Brain levels of morphine and codeine were also not significantly affected by ephedrine and PPA pretreatment. It should be noted, however, that whole brain morphine/codeine content was measured and this cannot exclude the possibility that the sympathomimetics may affect opioid levels in discrete brain areas, especially in those involved in the processing of nociceptive information.

The present study was constrained by the fact that sample collections were end-point procedures by themselves. Consequently, a more frequent schedule was not adopted, and there were no follow up samples from single mice. Samples were pooled, and so the results are representative mean values from groups of 60–80 mice. Inter-individual variations in drug handling is a well-known phenomenon in all species (Sjöqvist & Von Bahr 1973). These limitations notwithstanding, it was possible to find fairly consistent results, suggesting that ephedrine and PPA had no significant effect on the pharmacokinetics of morphine and codeine in mice after single dose treatment.

In our previous reports the time of peak anti-nociceptive effects of the opioids was unaltered by sympathomimetic pretreatment in both naive (Dambisya et al 1990) and opioid tolerant/dependent mice (Dambisya et al 1991c). Coupled with the findings now reported, there is a strong indication that the observed potentiation of acute opioid anti-nociception (Dambisya et al 1990) and lethal toxicity (Dambisya et al 1991a) by ephedrine and PPA may not be due to pharmacokinetic interactions. In a recent report (Dambisya et al 1991b) it has been shown that the effects of these sympathomimetics on opioid activity are mediated via α_2-adrenoceptor activation. These results are in close agreement with the general conclusions of Monasky et al (1990) who found that intrathecally administered α_2 agonist ST-91, 2(2,6-diethyldiphenyl-amino-2-imidazoline), did not affect the spinal clearance of co-administered morphine in the rat.

Dr Y. M. Dambisya is supported by the Commonwealth Scholarship and Fellowship Plan (Hong Kong Awards).

References

Adler, T. K., Fujimoto, J. M., Way, E. L., Baker, E. M. (1955) The metabolic fate of codeine in man. J. Pharmacol. Exp. Ther. 114: 251–262

Bowman, W. C., Rand, M. J. (1980) Textbook of Pharmacology Blackwell Scientific Publications. London P41.1–48

Central Registry of Drug Abuse (CRDA) (1990) Narcotics Division, Government Secretariat, Hong Kong Government, Twenty-seventh report (1981–1990)

Chan, K., Dambisya, Y. M., Wong, C. L. (1992) Determination of morphine and codeine in mouse plasma and brain by high

performance liquid chromatography (HPLC). Neurosci. Lett. In press
Chan, K., Lai, B., Chu, S. M. (1990) A pilot study on the dependent consumption of cough and cold remedies in Hong Kong youths. Ibid. 40 (Suppl.): S18
Dambisya, Y. M., Chan, K., Wong, C. L. (1991a) Ephedrine and phenylpropanolamine potentiate the lethal toxicity of morphine and codeine in naive mice. Asia Pacific J. Pharmacol. 6: 255–258
Dambisya, Y. M., Chan, K., Wong, C. L. (1991b) Role of adrenoceptors in the potentiation of opioid antinociception by ephedrine and phenylpropanolamine in mice. Psychopharmacology 105: 563–567
Dambisya, Y. M., Wong, C. L., Chan, K. (1990) Effects of ephedrine and phenylpropanolamine on the antinociceptive effects of morphine and codeine in mice. Arch. Int. Pharmacodyn. 308: 5–12
Dambisya, Y. M., Wong, C. L., Chan, K. (1991c) Effects of sympathomimetic agents on opiate analgesia, tolerance and dependence in mice. Meth. Find. Exp. Clin. Pharmacol. 13: 239–248
D'Armour, F. E., Smith, D. L. (1941) A method for determining loss of pain sensation. J. Pharmacol. Exp. Ther. 72: 74–79
Findlay, J., Jones, E. C., Butz, R., Welch, R. (1978) Plasma codeine and morphine concentrations after therapeutic oral doses of codeine-containing analgesics. Clin. Pharmacol. Ther. 24: 60–68
Lake, C. R., Rosenberg, D. R., Gallant, S., Zaloga, G., Chernow, B. (1990) Phenylpropanolamine increases plasma caffeine levels. Ibid. 47: 675–685
Leza, J. C., Lizasoain, I., Lorezo, P. (1990) H_1- and H_2-histamine receptor blockers and opiate analgesia in mice. Meth. Find. Exp. Clin. Pharmacol. 12: 671–678
Monasky, M. S., Zinsmeister, A. R., Stevens, C. W., Yaksh, T. L. (1990) Interaction of intrathecal morphine and ST-91 on antinociception in the rat: dose-response analysis, antagonism and clearance. J. Pharmacol. Exp. Ther. 254: 383–392
Rogers, J. F., Findlay, J. W., Hull, J. H., Butz, R. F., Jones, E. C., Burstrack, J. A., Welch, R. M. (1982) Codeine disposition in smokers and nonsmokers. Clin. Pharmacol. Ther. 32: 218–227
Shah, J. C., Mason, W. D. (1990) Plasma codeine and morphine concentrations after a single oral dose of codeine phosphate. J. Clin. Pharmacol. 30: 764–766
Sjöqvist, F. (1991) Interethnic differences in polymorphic drug oxidation—clinical implications. Proceedings of the Sixth South East Asian/Western Pacific Regional Meeting of Pharmacologists, Hong Kong. Abstract S.6-2
Sjöqvist, F., Von Bahr, C. (1973) Interindividual differences in drug oxidation: clinical importance. Drug Metab. Dispos. 1: 469–482
Svensson, J. O., Rane, A., Sawe, J., Sjöqvist, F. (1982) Determination of morphine, morphine-3-glucuronide and (tentatively) morphine-6-glucuronide in plasma and urine using ion-pair high performance liquid chromatography. J. Chromatogr. 230: 427–432
Weinberger, M., Bronsky, E., Bensch, G. W., Bock, G. N., Yecies, J. J. (1975) Interaction of ephedrine and theophylline. Clin. Pharmacol. Ther. 17: 585–592
Yue, Q. Y., Svensson, J. O., Alm, C., Sjöqvist, F., Sawe, J. (1989a) Interindividual and interethnic differences in the demethylation and glucuronidation of codeine. Br. J. Clin. Pharmacol. 28: 629–637
Yue, Q. Y., Svensson, J. O., Alm, C., Sjöqvist, F., Sawe, J. (1989b) Codeine *O*-demethylation co-segregates with polymorphic debrisoquine hydroxylation. Ibid. 28: 639–645

J. Pharm. Pharmacol. 1992, 44: 690–692
Communicated December 5, 1991

HI-6 pharmacokinetics in rabbits after intravenous and intramuscular administration[1]

B. J. LUKEY, C. L. WOODARD, C. R. CLARK, M. P. MCCLUSKEY, *United States Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, Maryland, USA*

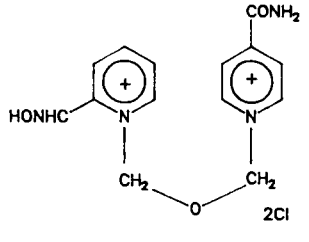
Abstract—The pharmacokinetics of HI-6 ((4-carboxamidopyridinium (1) methyl)-(2′-hydroxyiminomethyl-pyridinium (1′) methyl) ether dichloride) have been studied in rabbits receiving an intramuscular (50 $\mu g\ kg^{-1}$) or intravenous (12·5 $\mu g\ kg^{-1}$) dose. The plasma concentration-time profile for the intramuscular dose (n = 8) fits a one-compartment open model with first-order absorption and elimination. The absorption half-life was 2 min and maximum concentration (51 $\mu g\ mL^{-1}$) was reached in 9 min. The pharmacokinetics for the intravenous dose (n = 8) was described by a two-compartment open model with first-order distribution and elimination. The apparent volume of distribution was 0·1 $L\ kg^{-1}$. Half-lives of distribution and elimination were 5 and 38 min, respectively. The results indicate HI-6 is rapidly absorbed, distributed and eliminated in rabbits receiving an intramuscular dose.

Correspondence: Commander, USAMRICD, SGRD-UV-PA/CPT B. J. Lukey, Aberdeen Proving Ground, MD 21010-54245, USA.

[1]The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Army or the Department of Defense. In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985). The use of trade names does not constitute an official endorsement or approval of the use of such commercial hardware or software. This document may not be cited for purposes of advertisement.

The oxime, 2-pralidoxime chloride is the standard antidote against organophosphate insecticides due to its ability to reactivate inhibited acetylcholinesterase (Harris et al 1969). However, 2-pralidoxime chloride is ineffective in the treatment of soman poisoning (Loomis & Salafsky 1963). Hagedorn et al (1976, 1978) synthesized a number of bispyridinium oximes in an effort to obtain an effective antidote for soman poisoning. Of the many tested, HI-6 (I) proved to be extremely effective in reactivating acetylcholinesterase, before ageing inhibited by soman (DeJong & Wolring 1980) and protecting against soman lethality (Boskovic 1981, 1985).

Our organization has used rabbits as an animal model in testing HI-6 efficacy for organophosphate poisoning. However,



I. (4-Carboxamidopyridinium (1)methyl)-(2′hydroxyiminomethyl-pyridinium (1′) methyl) ether dichloride.