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The Effect of Drugs on the Mortality of Mice Inoculated With Friend Leukaemia Virus or Toxoplasma gondii

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Summary

Infection and tumors provoke substantial changes accompanied with the disbalance of many neuroendocrine factors which in their summarizing effects influence the life span of animals. Our previous results showed enhanced mortality after one injection of morphine in association with Friend leukaemia virus infection. The aim of this study was to examine the effects of some other opioids (pethidine and pentazocine) and an acetylcholine esterase inhibitor neostigmine on the survival of animals under two conditions: (1) Friend leukaemia virus infection which mostly depressed immune functions, and (2) *Toxoplasma gondii* infection which in general enhanced the immune status. In contrast to our previous observation with morphine, the mortality induced by single doses of pethidine (150 mg/kg) or pentazocine (50–75 mg/kg) was unchanged during the Friend leukaemia virus infection. A single injection of neostigmine (0.42 or 0.56 mg/kg) was significantly more lethal in DBA-2 mice infected with Friend leukaemia virus. Neostigmine in doses of 0.33 and 0.4 mg/kg caused death in 46 % and 57 %, respectively, of animals infected with *Toxoplasma gondii* which was significantly higher in comparison with only 8 % and 12.5 % in control groups. Pethidine (150 mg/kg) killed 70 % of *Toxoplasma gondii* infected animals and even 90 % of non-infected mice. Thus, the Friend leukaemia virus and *Toxoplasma gondii* infections increased toxicity only of some drugs which may, at least partly, be associated with altered immune status during infection and involvement of the cholinergic system.

Key words
Friend leukaemia virus – *Toxoplasma gondii* – Neostigmine – Pethidine – Lethality

Introduction

Similarities between Friend leukaemia virus infection and human leukaemia have been extensively studied for a long time. The early phase of the disease is characterized by rapid splenomegaly, thrombocytopenia, lymphocytosis, granulocytosis and erythroblastosis are always present. Moreover, Friend virus infection induces immunodepression and it is one of the best murine models of AIDS. Neoplastic cells influence survival of animals in mutual interaction with many factors including neuroimmunological aspects. An interaction between immune status induced by infection and toxicity of drug of abuse has been proposed. For example, opiates modify host defence against infection, Friend virus or *Toxoplasma gondii* infection increase morphine toxicity (Starec *et al.* 1991, Veyries *et al.* 1995, Chao *et al.* 1990), *Legionella pneumophila* infection increases the toxicity of delta9-tetrahydrocannabinol (Klein *et al.* 1993).

Results were preliminary presented at "CNS – Advance in Research of Normal and Neoplastic Cells" which was held in Brno (April 25, 1996) as the satellite minisymposium of the 42nd International Congress of the European Tissue Culture Society (Starec et al. 1996).

In our previous experiments, we found much higher morphine lethality in mice infected with the Friend leukaemia virus than in non-infected controls (Starec *et al.* 1991). Veyries *et al.* (1995) showed that opioid receptor antagonist naloxone reduced morphine lethality rate and postponed death, but the glucocorticoid receptor antagonist mifepristone, H_1 receptor antagonist terfenadine, alpha-adrenergic blocker phentolamine or beta-adrenergic blocker propranolol did not modify the mortality to morphine in infected animals. However, they did not examine the possible involvement of the cholinergic system. In the present study, we investigated the toxicity of some other opioids (pethidine and pentazocine) during Friend virus or *Toxoplasma gondii* infection (a currently used infection model which changes the immune status and is a frequent and mortal complication of AIDS). Because the mechanisms involving cholinergic pathways have not been examined, an acetylcholine esterase inhibitor neostigmine was chosen to assess whether a drug acting on the cholinergic system would also change mortality during changed immune response to infection as well.

Methods

Male 6- to 7-week-old DBA-2 mice (Friend virus infection experiments) and F-1 generation Balb/c x C10J (*Toxoplasma gondii* infection experiments) mice, weighing 18–22 g, were used. The mice were kept in cages containing 10 animals and had free access to food and water. They were housed under natural day/night conditions for at least two weeks before the experiments.

Pentazocine (Fortral Winthrop amp. 30 mg in 1 ml), pethidine hydrochloride (Dolsin amp. Spofa 50 mg/ml) and neostigmine monomethylsulphate (Syntostigmin amp. 0.5 mg/ml) were diluted in saline and injected intraperitoneally.

The polycythaemia-inducing Friend leukaemia virus (FLV) was a generous gift from Dr. P. Tambourin (Paris, France). This FLV complex consists of a mixture of helper Friend murine leukaemia virus and defective spleen focus-forming virus. High titre virus stocks of FLV were made from mouse spleen homogenates prepared four weeks after infection and aliquots of cell-free virus suspension were stored at −80 °C and titrated for infectivity *in vivo* using the spleen focus assay (Axelrad and Steeves 1964). Mice were inoculated intravenously with 0.2 ml of the virus suspension (approximately 50 focus-forming units).

Toxoplasma gondii – strain 01529/38 was isolated by Mgr. Zástěra in 1975 from cat excrements. Each mouse was inoculated perorally with one cyst in 0.5 ml of brain homogenate from infected mice. Control mice were fed with the brain homogenate from non-infected mice. The dose used was not lethal for mice.

Animals received a single i.p. injection of pentazocine, pethidine or neostigmine 21 days after inoculation with Friend virus or *Toxoplasma gondii*. The same single doses were given to non-infected control animals and the number of deaths were compared using Fischer's exact test.

Results

In contrast to our previous results with a single morphine injection in Friend virus infected animals (Starec *et al.* 1991), pethidine or pentazocine-induced lethality was not changed in infected mice on day 21 post-infection (with Friend virus) in comparison with drug-induced lethality in control (non-infected) animals. On the other hand, an acetylcholine esterase inhibitor neostigmine in the doses 0.42 or 0.56 mg/kg killed significantly more infected than non-infected animals (Table 1). Similar results were observed in mice infected with *Toxoplasma gondii*. In contrast to the previous results with morphine (Chao *et al.* 1990), pethidine in our experiments in the dose used in the Friend virus experiment (150 mg/kg) killed 70 % of infected animals, but even 90 % of non-infected mice. Neostigmine, however, in the doses of 0.33 and 0.4 mg/kg caused death of infected animals in 46 % and 57 %, respectively, which was significantly more in comparison with only 8 % and 12.5 % in the control groups. Death mostly occurred within 2 hours, the rest within 24 hours (Table 2). The neostigmine-induced lethality in animals six weeks after infection was approximately the same as in control mice (results not shown).

Table 1
Drug-induced lethality in mice infected with Friend virus in comparison with non-infected controls

	Dose (mg/kg)	Friend virus infected mice	Non-infected controls
Pethidine	150	40 %	50 %
Pentazocine	50	0 %	0 %
	62.5	14 %	10 %
	75	50 %	50 %
Neostigmine	0.42	60 %**	0 %
	0.56	90 %**	20 %

8–30 animals in each group

Table 2
Neostigmine- or pethidine-induced lethality in mice infected with *Toxoplasma gondii* in comparison with non-infected controls

	Dose (mg/kg)	Toxoplasma infected mice	Non-infected controls
Pethidine	150.0	70 %	90 %
Neostigmine	0.1	0 %	0 %
	0.33	46 %*	8 %
	0.4	57 %*	12.5 %
	0.5	100 %	83 %
	1.0	100 %	100 %

6 – 13 animals in each group

Discussion

The major finding of this study is that, in contrast to morphine, other opioids used in our experiments – pethidine (mu-opioid receptor agonist as morphine) or pentazocine (kappa-opioid receptor agonist and mu-opioid receptor antagonist) did not change the mortality rate during infection.

On the other hand, a drug with a completely different pharmacodynamic activity – neostigmine – was more toxic during both Friend virus and *Toxoplasma gondii* infection. The exact mechanism of enhanced toxicity of morphine and neostigmine by infection with murine leukaemia virus or *Toxoplasma gondii* is not yet clear. There may be a complex relationship between immune mediators released during infection and the effects of drugs. Relatively little attention is paid to this phenomenon. Chao *et al.* (1990) proposed that immune activation during *Toxoplasma gondii* infection plays a critical role in morphine-induced mortality because it occurred only at the time of enhanced immune reactivity to the parasite. This is also in agreement with our results. After 4 weeks post-infection, the differences in neostigmine mortality between non-infected and infected mice were minimal (data not shown). In another experimental model Klein *et al.* (1993) showed that the toxicity of tetrahydrocannabinol (THC) injection in mice infected with *Legionella pneumophila* was increased in comparison with non-infected animals. This again suggests a drug-induced toxicity associated with the development of microbial immunity. The enhanced mortality was at least partly due to an elevation of acute phase cytokines, especially IL6 and tumour necrosis factor (TNF), because mice could be protected by a prior injection of anticytokine antibodies and a THC injection significantly increased the blood levels of TNF and IL6 compared with the levels induced by infection only. Thus the results of Klein *et al.* (1993) with THC suggest that cytokines might participate in these immune mechanisms.

Cytokines also play an important role in the development of Friend leukaemia virus infection. It was found (Soldaini *et al.* 1994) that there was a 100-fold increase of TNF-alpha levels induced by bacterial lipopolysacharide in Friend leukaemia virus infected mice than in non-infected animals. Friend leukaemia virus infection markedly reduced the levels of IL 6 and treatment with IL7 restored NK activity and prolonged survival time (Lu *et al.* 1992).

While little is known about neostigmine-induced immune changes, drugs of abuse, especially morphine, heroine and delta 9 tetrahydrocanabinol have been extensively studied in this respect. Their effect on immunity is mostly depressive (for review see Rouveix 1992, Donahoe 1993), the levels of cytokines, however, are sometimes enhanced. It was found that a microinjection of morphine into the periaqueductal gray suppressed many immune functions but enhanced IL2 and TNF-beta (Bian and Li 1995).

Chao *et al.* (1994) reported that morphine primes the microglia *in vitro* for enhanced production of TNF-alpha which could alter several functional activities of these cells within the brain. Morphine suppresses the release of bioactive TNF from peripheral blood mononuclear cells and the tumour growth factor beta plays a modulatory role in this inhibitory process (Chao *et al.* 1993).

Both neostigmine and morphine influence cholinergic pathways. Morphine mostly reduces the release of acetylcholine but, for instance, its analgesic effect is in part caused by spinally released acetylcholine and an intrathecally administered cholinesterase inhibitor potentiates this effect. The importance of infection for acetylcholine sensitivity could be demonstrated by the following results: *Dirofilaria immitis* infection (canine heartworm) increases acetylcholine-induced contractions of the rat trachea ring (Collins *et al.* 1994). Respiratory tract virus infection enhances airway responsiveness (mean pulmonary insufflation pressure) in anaesthetized guinea-pigs in response to inhaled acetylcholine (Elwood *et al.* 1993). Taken together, we can speculate that changes in cytokine levels (but also other immune factors) may be important for sensibilization of mice and death induced by morphine and neostigmine and that cholinergic pathways may be involved. The reason for the higher mortality in infected animals could also be caused by changed pharmacokinetics of drugs (morphine) as was proposed by Poet *et al.* (1992). They demonstrated significantly higher serum concentrations of cocaine and morphine in retrovirus-infected animals. Veyries *et al.* (1995) further analysed the mechanism of morphine-induced lethality. They showed that only the opioid antagonist naloxone decreased morphine

toxicity, thus suggesting a direct effect of morphine on its specific receptors. Our results, however, indicate that the lethality of other opioid agonists (pethidine, pentazocine) was not increased during Friend leukaemia virus infection. Pentazocine has a different opioid profile (kappa agonist and mu antagonist). Therefore, it is not so surprising that it has not the same effect as morphine in this respect. Pethidine, on the other hand, is also a mu-opioid agonist as morphine. The fact that pethidine does not increase lethality during infection could be explained by some other differences in the properties between pethidine and morphine. For example, in contrast to morphine which increases the isolated rabbit trachea contraction, pethidine decreases the contraction and relaxes the trachea (Shen *et al.* 1994). Pethidine has also a different affinity for muscarinic receptors in rat brain homogenate (Hustveit 1994) and a different effect on cytochrome P 450 expression in rats (Rane *et al.* 1995).

Further studies are needed to clarify the exact nature of different drug-induced lethality during infection.

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References

AXELRAD A.A., STEEVES R.A.: Assay for Friend leukaemia virus: rapid quantitative method based on enumeration of macroscopic spleen foci in mice. *Virology* **24:** 513–518, 1964.

BIAN T.H., LI X.Y.: Immunomodulation effects of morphine microinjected into periaqueductal gray (in Chinese), *Chung-Kuo-Yao-Li-Hsueh-Pao* **16:** 121–124, 1995.

CHAO C.C., SHARP B.M., POMEROY C., FILICE G.A., PETERSON P.K.: Lethality of morphine in mice infected with Toxoplasma gondii. *J. Pharmacol. Exp. Ther.* **252:** 605–609, 1990.

CHAO C.C., MOLITOR T.W., CLOSE K., HU S., PETERSON P.K.: Morphine inhibits the release of tumor necrosis factor in human peripheral blood mononuclear cell cultures. *Int. J. Immunopharmacol.* **15:** 447–453, 1993.

CHAO C.C., GEKKER G., SHENG W.S. HU S., TSANG M., PETERSON P.K.: Priming effect of morphine on the production of tumor necrosis factor-alpha by microglia: implications in respiratory burst activity and human immunodeficiency virus-1 expression. *J. Pharmacol. Exp. Ther.* **269:** 198–203, 1994.

COLLINS J.M., WILLIAMS J.F., KAISER L.: Dirofilaria immitis: heartworm products contract rat trachea in vitro. *Exp. Parasitol.* **78:** 76–84, 1994.

DONAHOE R.M.: Neuroimmunomodulation by opiates: relationship to HIV-1 infection and AIDS. *Adv. Neuroimmunol.* **3:** 31–46, 1993.

EISENACH J.C., GEBHART G.F.: Intrathecal amitriptyline. Antinociceptive interactions with intravenous morphine and intrathecal clonidine, neostigmine and carbamylcholine in rats. *Anesthesiology* **83:** 1036–1045, 1995.

ELWOOD W., LOTVALL J.O., BARNES P.J., CHUNG K.F.: Airway hyperresponsiveness to acetylcholine and to tachykinins after respiratory virus infection in the guinea pig. *Ann. Allergy* **70:** 231–236, 1993.

HUSTVEIT O.: Binding of fentanyl and pethidine to muscarinic receptors in rat brain. *Jpn. J. Pharmacol.* **64:** 57–59, 1994.

KLEIN T.W., NEWTON C., WIDEN R., FRIEDMAN H.: Delta 9-tetrahydrocannabinol injection induces cytokine-mediated mortality of mice infected with Legionella pneumophila. *J. Pharmacol. Exp. Ther.* **267:** 635–40, 1993.

LU L., ZHOU, Z., WU B., XIAO M., SHEN R.N., WILLIAMS D.E., KIM Y.J., KWON B.S., RUSCETTI S., BROXMEYER H.E.: Influence of recombinant human interleukin (IL)-7 on disease progression in mice infected with Friend virus complex. *Int. J. Cancer* **52:** 261–265, 1992

POET T.S. MARTINEZ F., WATSON R.R.: Effect of murine retroviral infection on hair and serum levels of cocaine and morphine. *Forensic. Sci. Int.* **54:** 29–38, 1992.

RANE A., LIU Z., HENDERSON C.J., WOLF C.R.: Divergent regulation of cytochrome P450 enzymes by morphine and pethidine: a neuroendocrine mechanism? *Mol. Pharmacol.* **47:** 57–64, 1995.

ROUVEIX B.: Opiates and immune function. Consequences on infectious diseases with special reference to AIDS. *Therapie* **47:** 503–12, 1992.

SHEN J.F., LIN Y.F., WU Q.P.: Effect of morphine, pethidine and fentanyl on isolated rabbit trachea (in Chinese). *Chung Kuo Yao Li Hsueh Pao* **15:** 456–458, 1994.

SOLDAINI E, BALDINOTTI F., MATTEUCCI D., SPECTER S., BENDINELLI, M.: Enhancement of plasma levels of tumor necrosis factor alpha but not interleukin-6 following endotoxin injection of Friend leukaemia virus infected mice. *J. Biol. Regul. Homeost. Agents* **8:** 25–31, 1994.

STAREC M., ROUVEIX B., SINET M., CHAU F., DESFORGES B., POCIDALO J.J., LECHAT P.: Immune status and survival of opiate- and cocaine-treated mice infected with Friend virus. *J. Pharmacol. Exp. Ther.* **259:** 745–50, 1991.

STAREC M., ROSINA J., FIŠEROVÁ A., SINET M., DESFORGES B.: The effect of drugs and ultraviolet blood irradiation on the mortality of DBA-2 mice inoculated with friend leukemia virus or P815 mastocytoma. *Physiol. Res.* **45:** 6P, 1996.

VEYRIES M.L., SINET M., DESFORGES B., ROUVEIX B.: Effects of morphine on the pathogenesis of murine Friend retrovirus infection. *J. Pharmacol. Exp. Ther.* **272:** 498–504, 1995.

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