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RECENT TOPICS OF PHARMACOLOGY IN ORIENTAL MEDICINE : DETOXIFICATION OF MORPHINE ADDICTION IN THE MOUSE NARCOTIC DEPENDENCE MODEL USING EXTRACTS FROM *PSIDIUM GUAJAVA LEAVES*

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SPECIAL LECTURE

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DETOXIFICATION OF MORPHINE ADDICTION IN THE MOUSE NARCOTIC DEPENDENCE MODEL USING EXTRACTS FROM PSIDIUM GUAJAVA LEAVES.

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Summary

The effects of a polar fraction of an extract from the leaf of the guava plant, Psidium guajava Linn. (Myrtaceae), including the effects of two isolated flavonoid compounds, quercetin and quercetin-3-arabinoside, on withdrawal symptoms in morphine-dependent mouse models were studied. Two types of mouse narcotic-dependence models were employed:-

A. Mouse chronic narcotic-dependence model was prepared by thrice daily i.p. injection of morphine sulphate, with an initial dose of 4 mg/kg and increasing each successive dose by 4 mg/kg, up to day-9, when the dose of 108 mg/kg reached was maintained through day-11 and the morning of day-12.

B. Mouse acutely narcotic-dependence model was prepared by a s.c. bolus injection of 50 mg/kg morphine sulphate, followed 4 h later by s.c. injection of 10 mg/kg naloxone.

Manifestations of abstinence syndrome, characterised by repetitive vertical jumping, rearing, paw-shaking, head-shaking, teeth chattering, etc., occurred about 14½ h after the last dose in group A, and 45 - 60 min after naloxone injection in group B. A s.c. dose of 0.5 mg/kg of the extract, 10 h after the last dose in group A or 1 h before the naloxone injection in group B, prevented the repeated vertical jumps and greatly reduced the other symptoms of withdrawal. Parallel experiments on satiety to the extract in mice showed no development of dependence on the extract. Quercetin may be the main active compound in the extract.

Introduction

The chance discovery of the effects of opium on the body and mind in early history of man has led to the continuous search for opiate-like compounds that bring cures for our ailments, impart a feeling of well-being and eliminate pain, anxiety and discomfort. Unfortunately, many of these drugs produce a powerful, seemingly endless, psychological state of discomfort which overwhelms the individual and induces an urgent need for relief or satisfaction. This opioid-induced satisfaction leads invariably to marked tolerance and serious physical dependence which develop rather rapidly with repeated doses of opiates like morphine or heroin.

Morphine addicts may develop extreme tolerance, making them require daily doses of up to about 60 times the normal clinical dose (300 - 600 mg, injected in divided doses). Death frequently occurs with overdose. Withdrawal of the drug from a physically dependent individual produces a characteristic and extremely unpleasant withdrawal or abstinence syndrome, which may be life-threatening. The severity of the withdrawal symptoms in a seriously dependent individual depends on the level of tolerance developed in the addict and on the duration of abstinence, the most serious symptoms occurring about 36 hr into abstinence. These include fasciculation and twitching of skeletal muscle, development of severe and painful cramps in the legs and abdomen, intense and uncontrollable vomiting, salivation, diarrhoea and urination. The addict is unable to sleep and there are increases in blood pressure, body temperature, blood sugar level and the basal metabolic rate. All these symptoms peak within 48 - 72 h, gradually subsiding subsequently over the next week or two. It may take up to 6 months without the drug for complete recovery, but psychotherapy and rehabilitation are necessary if relapse is to be prevented. Even then a very high percentage return to the drug culture and habit.

Drug therapy in narcotic-dependent individuals is by substituting an opioid compound with less dependence liability than morphine, such as Methadone. Barbiturates, chlorpromazine and the B-adrenoceptor blocker, propranolol, have all been used to control the anxiety, parasympathetic and euphoric effects of withdrawal, respectively. All these drugs have serious drawbacks and the search for drugs that have useful effects on the physical effects of the withdrawal symptoms, with little or no dependence liability, goes on.

In the search for useful morphine antidotes, many animal narcotic-dependence models have been developed, including schematic testing methods for new narcotic compounds(1).

Knowledge of the traditional uses of the leaf of the guava plant (Fig. 1), Psidium guajava Linn. (Myrtaceae), for the treatment of neurological conditions, such as toothache, epilepsy, chorea and convulsions(2,3), Table 1 , as well as its use in the treatment of acute toxic effects of alcohol(3), prompted our investigations into the pharmacological effects of extracts and isolated compounds from the leaf on the central and peripheral nervous systems of experimental animals. The neuroleptic effects of a polar fraction of the leaf extract, as well as those of the contained flavonoid compounds, quercetin and quercetin-3-arabinoside, on exploratory and spontaneous locomotor activities in the mouse have been reported by this author(4). Another report on the inhibition of neurotransmitter release in the electrically stimulated guinea-pig isolated ileum for morphine-like activity (the inhibition not reversible by naloxone) has been published(5). Other parallel studies in our laboratories show that the extract has properties of a non-narcotic, non-addictive anti-diarrhoeal(6) with potent antinociceptive actions(7), properties which are the hallmarks of agents that are likely candidates to be tested for possible usefulness in the treatment of narcotic dependence(1).

Methods

Preparation of Extract

Pulverized oven-dried leaves (40 g) were sequentially extracted with 400 ml each of petroleum ether and methanol (75%) for a total of 48 h. The extractive was centrifuged for 15 min at 2,000 rev./min and the supernatant was dried by rotary evaporation, yielding 7.88 ± 0.36 g (N=5). This was reconstituted in 200 ml of 0.05 M ammonium carbonate at 55°C and the pH adjusted to 7.2 by adding few drops of 0.01 N HCL. The resultant flocculent solution was centrifuged again at 2,000 rev./min for 15 min and the supernatant was decanted off. Dilutions of this stock solution were used for biological work.

Animals

The mice used were in-bred male Sprague Dawleys from the Animal House of the School of Medical Sciences, Universiti Sains Malaysia. Mice weighing 25 - 28 g were used in all experiments.

Mouse

Chronic Narcotic-Dependence Model

Mice were housed in groups of 5 and fed the normal mouse chow. Water was freely available in the cages. They each received i.p. injection of morphine sulphate thrice a day, the initial dose being 4 mg/kg increasing with each successive injection by 4 mg/kg up to day-9, resulting in a final dose (i.e. injection no. 27) of 108 mg/kg. This dose was maintained as the once daily dose through day-11 and the morning of day-12.

Table 2 Pre-treatment (s.c. injections) in mouse narcotic-dependence models before estimation of severity of withdrawal symptoms

MOUSE NARCOTIC DEPENDENCE MODEL	Extract (0.5 mg/kg)	Quercetin ** (2.5 mg/kg)	Quercetin-arabineside ** (10 mg/kg)	Naloxone (10 mg/kg)
A. CHRONIC (3x daily s.c. injection of morphine sulphate *				
(i) Sub-group (n=20)	ditto	-	-	-
(ii) Sub-group (n=20)	ditto	-	-	ditto
(iii) Sub-group (n=10)	-	-	-	ditto
(iv) Sub-group (n=8) (10 ml/kg saline, control	-	-	-	-
B. ACUTE (s.c. bolus injection of 50 mg/kg'				
(i) Sub-group (n=10)	-	-	-	ditto
(ii) Sub-group (n=10)	ditto	-	-	ditto
(iii) Sub-group (n=10)	-	ditto	-	ditto
(iv) Sub-group (n=10)	-	-	ditto	ditto
(v) Sub-group (n=8 (10 ml/kg saline, control	-	-	-	-

* [illegible] for acute model

** Both quercetin and quercetin-arabineside were dissolved in 1% 1-glutamic-pyrrolidone. Naloxone was dissolved in distilled water and 4 ml/kg in absolute alcohol with rotary evaporation. In group A, extract injection was [illegible] after morphine pre-treatment, and 4 hours later by naloxone injection. In group B, extract, quercetin and quercetin-3-arabinoside injections [illegible]

Mouse

Acutely Narcotic-Dependence Model

Each mouse received a s.c. bolus injection below the loose skin behind the head and neck region. The dose administered was 50 mg/kg of morphine sulphate.

Satiety and Dependence Liability Studies

Naive mice caged in groups of 5 were fed the normal mouse chow but were provided with the extract at a water-diluted concentration of 0.125 mg/ml in a watering bottle with a leak-proof nozzle. Measurements of the amount of fluid drank by the group was taken every 24 h in the morning for 12 days. Control groups on ordinary water were similarly studied.

Measurement of Severity of Withdrawal

The severity of withdrawal was quantified by the number of jumps made by mice in treatment groups of 5 in a chamber (20 x 30 cm^2 base, height = 60 cm) fitted with photo cells mounted 12.5 cm above the floor and connected to an electro-mechanical counter. The equipment was manufactured by the workshop of the School of Physics, Universiti Sains Malaysia, Penang, Malaysia. The jumps, head shaking, rearing and other withdrawal symptoms were also directly observed through the perspex front of the chamber.

The pre-treatment of the various groups, prior to the manifestation of withdrawal symptoms, were as shown in Table 2.

Results

In sub-group A (iv) (control) of the mouse chronic narcotic-dependence model, the withdrawal symptoms occurred about 14½ h after the last morphine dose and consisted of short convulsive leaps, episodes of repetitive vertical jumps, teeth chattering, rapid alternate paw-leaking, rearing, repetitive snout cleaning with paws, burrowing, rump scratching, preening, urination and defaecation. Repetitive jumping occurred in only 75% of the animals, but all animals showed most of the other symptoms. In group A (i), in which the mice were injected with the extract 2 h before the onset of the withdrawal symptoms, none of the animals convulsed or jumped throughout the period of observation, i.e. for 4 h past the time of onset of symptoms. Rearing occurred in 16% of animals but none of the other symptoms emerged in any animal. In group A (ii), where the extract-protected morphine-dependent mice were challenged with naloxone, jumping occurred only after 1 h and in only 20% of animals, but rearing, also occuring after 1 h, involved 70% in the group, whereas, in group A (iii), where no extract was injected before naloxone injection, 90% of the animals underwent increased repetitive vertical jumping within 5 minutes of the injection, and showed all the symptoms of withdrawal.

Injection of saline in group A (iv) mice produced jumping and rearing in 75% of animals. This was attributed to the normal onset time of withdrawal. The extract had a calming effect in the mice in all experiments where it was administered, causing the mice to go to sleep for about 45 min, huddled together, becoming active again in about 1 h.

In the mouse acutely narcotic-dependent model, s.c. injection of 10 mg/kg naloxone (group B (i)) evoked very severe withdrawal symptoms of the kind described above, with increased repetitive vertical jumping, the onset of the symptoms being only 5 min after naloxone administration. In

sub-group B (ii), injection of the extract 1 h before naloxone administration, prevented jumping, rearing, and all the other withdrawal symptoms. The mice went to sleep for 2 h and, by 2½ h, 40% were actively munching on mouse chow, the rest taking a longer time to emerge from the cataleptic state. Both quercetin and quercetin-3-arabinoside, which are constituents of the extract[5], in groups B (iii) and B (iv), showed effects very similar to the extract in preventing or attenuating the development of the withdrawal symptoms. Quercetin appeared to have a higher activity in this respect, whereas the arabinoside appeared to enhance rearing.

Because of the variability in the vertical jumping in animals in the same group undergoing similar drug treatment, especially when mice in the group are not from the same litter, quantification and analysis of the counted number of jumps per group presented difficulties. A narcotic-dependent mouse undergoing withdrawal jumping has an average count rate of 100 - 120 jumps in 15 h, according to East and Potts[1]. Our experiments showed that naloxone-induced vertical jumping was more consistent in both narcotic-dependent models studied than in animals undergoing normal abstinence withdrawal. In the acutely narcotic-dependent mouse, the average number of jumps counted over the 90 minutes observation period was 26 ± 9. In the chronic narcotic-dependent model the average count for 150 minutes observation time, under similar circumstances, was 57 ± 19.

The effect of the extract on the withdrawal jumping and the other symptoms of abstinence is unequivocally clear. There is inhibition in the expression of these symptoms. Naive mice with free access to the extract as the only source of drinking showed an initial increase of fluid intake which was about twice the volume of plain water drank by a control group but, by the 15th day, the daily volume of extract drank came back to normal levels and stayed at that level for the next observed 20 days. The initial increase must be due to novelty of the taste (Fig. 2). If the phenomena of tolerance and dependence were operational, the daily volume of extract drank would not revert to baseline values.

Discussion

The profound pharmacological effects of extracts from the leaf of Psidium guajava on the central and peripheral nervous systems of experimental animals give credence to the traditional beliefs in its medicinal properties.

The many identified constituents of guava leaf[8.2], coupled with the usual difficulty in the interpretation of findings in experiments involving the central nervous system, makes it a no mean task attempting to explain what the possible model(s) of action in bringing about the observed neurological effects might be.

The effects of the identified flavonoid compounds isolated from the extract, as reported here and in previous reports[2,3], strongly suggest that the flavonoids might play a major role in the neurological effects of the extract. In a previous report[9], we showed by UV spectral analyses and biological work that calcium ion chelation might be one of the major mechanisms of action of the flavonoids and, by extrapolation, the guava leaf extract.

Despite the complexity of opioid receptor subtypes[10] and the many theories on the biochemical bases of morphine action, the simplest and most useful for our purposes is the inhibition of neurotransmitter release. Release of neurotransmitter depends on the availability of free calcium. Thus, ability of the extract to interfere with biomolecular levels of free calcium might reinforce the state of adequate brain levels of morphine being available and allay the triggering mechanisms of the withdrawal syndrome.

Whether the guava leaf or an extractive from it would one day become a useful therapeutic tool for the treatment and management of narcotic dependence remains for now for the future. The potential is there, but more research need to be carried out before extending the studies to humans. But, then again, humans already use it in traditional medical practices for neurological problems, such as alcohol abuse.

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