adequate linearity for concentrations in the range 0·61–4·86 μM with a correlation coefficient of 0·999.

The precision of the method was evaluated by means of intra-day (8·9% at 0·61 μM, 1·5% at 4·86 μM) and inter-day (6·1% at 0·61 μM, 1·6% at 4·86 μM) variabilities. The detection threshold was 0·05 μM.

*Animal experiments.* The results are summarized in Table 1, and indicate there is an increase in free radicals only during the reperfusion phase immediately following removal of the tourniquet.

It has been shown that cell lesions are more serious when the ischaemic tissue undergoes rapid re-oxygenation (Kloner 1993). We found that the level of free radicals rose very soon after the start of reperfusion; 1 min after release of the tourniquet, formation of free radicals was significant.

The conflicting effects of reperfusion subsequent to acute ischaemia is a subject of much debate in clinical practice (Menger et al 1991). An ischaemic area has to be reperfused since non-perfusion leads to cell hypoxia, which can cause necrosis. However, reperfusion is accompanied by the production of oxygenated free radicals, which place the cells under oxidative stress with consequences outside the site of the stress. The physiological free-radical-scavenging system is also evoked throughout the organism (Emerit et al 1991). It would be of interest to investigate whether radical scavengers (superoxide dismutase, ascorbic acid, α-tocopherol, xanthine oxidase inhibitor) could forestall effects due to reperfusion, and so be useful in clinical applications such as orthopaedic surgery.

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## References

Dargel, R. (1991) Lipid peroxidation—a common pathogenetic mechanism? Exp. Toxicol. Pathol. 44: 169–181

Emerit, J., Klein, J. M., Coutellier, A., Coney, F. (1991) Radicaux libres et peroxydation lipidique en biologie cellulaire: perspectives physiopathologiques. Path. Biol. 39: 316–327

Halliwell, B., Gutteridge, J. M. C., Cross, C. E. (1992) Free radicals, antioxidants, and human disease: where are we now? J. Lab. Clin. Med. 119: 598–620

Kloner, R. A. (1993) Does reperfusion injury exist in humans? J. Am. Coll. Cardiol. 21: 537–545

Menger, M. D., Lehr, H.-A., Messmer, K. (1991) Role of oxygen radicals in the microcirculatory manifestations of postischemic injury. Klin. Wochenschr. 69: 1050–1055

Pré, J. (1991) La lipoperoxidation. Path. Biol. 39: 716–736

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# Prolonged anti-emetic activity and 5-$HT_3$-receptor antagonism by BRL 46470 in conscious ferrets

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**Abstract**—The anti-emetic activity of oral and intravenously-administered BRL 46470 (endo-*N*-(8-methyl-8-azabicyclo[3.2.1]oct-3yl)-2,3-dihydro-3,3-dimethyl-indole-1-carboxamide HCl) has been assessed in conscious ferrets. BRL 46470 (0·05–0·5 mg $kg^{-1}$, p.o.) dose-dependently prevented emesis evoked over a 2 h period by total body X-irradiation. This anti-emetic activity occurred with oral or intravenously-administered BRL 46470 even when dosed 3–4 h before radiation. In conjunction with data obtained in other species, we conclude that BRL 46470 has a potent and long-lasting ability to antagonize actions that are mediated by the 5-$HT_3$ receptor in-vivo.

BRL 46470 is a potent, highly selective 5-$HT_3$-receptor antagonist with a long duration of action in anaesthetized rats and a demonstrable anxiolytic-like activity in conscious rats (Blackburn et al 1993). We now report on the ability of BRL 46470 to prevent emesis evoked by total body X-irradiation in ferrets, a pathological event which in this animal is wholly sensitive to 5-$HT_3$-receptor antagonism (Sanger 1993). Our results support the view that BRL 46470 has a prolonged and highly potent ability to antagonize at the 5-$HT_3$ receptor in-vivo.

## Materials and methods

The techniques have been previously described (Bermudez et al 1988). Male ferrets, either polecat or albino, 1–2 kg, were used.

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Emesis was induced by total body X-irradiation (10·4 min, at approx. 300 rads $min^{-1}$). Animals were then observed for the time of onset of emesis (the latency period) and for the number of emetic episodes within the monitoring period (2 h).

*Statistics.* The results are given as means ± s.e.m. and were analysed statistically using the Student's *t*-test for unpaired data.

*Chemicals.* BRL 46470 (endo-*N*-(8-methyl-8-azabicyclo[3.2.1]oct-3yl)-2,3-dihydro-3,3-dimethyl-indole-1-carboxamide HCl) was synthesized in-house and was dissolved in 0·9% NaCl.

## Results

Oral administration of BRL 46470 dose-dependently prevented radiation-evoked emesis (Table 1). Oral pre-dosing with BRL 46470 1 and 3 h before radiation, prevented or greatly reduced emesis throughout the subsequent 2 h observation period; pre-dosing by 4 h only partially reduced emesis (Table 2). BRL 46470 (0·5 mg $kg^{-1}$) injected intravenously 3 h before radiation also prevented emesis throughout the subsequent 2-h observation period. However, when this dose of BRL 46470 was injected 4 h before radiation, emesis was greatly reduced but not totally prevented.

Table 1. Dose-related anti-emetic action of BRL 46470 against radiation-evoked emesis in ferrets. BRL 46470 was given orally 1 h before X-irradiation. Records were made of both the number of emetic episodes and the time at which the first vomit occurred (latency period).

| Drug procedure BRL 46470 (mg $kg^{-1}$, p.o.) | n | Latency period (min) | Number of emetic episodes |
|---|---|---|---|
| Control | 7 | 19·7 ± 1·6 | 17·0 ± 1·0 |
| 0·5 | 4 | — | No vomiting*** |
| 0·1 | 4 | 42·8 ± 3·6*** | 7·0 ± 1·4*** |
| 0·05 | 4 | 28·5 ± 2·3* | 10·5 ± 1·6** |

n = Number of animals used. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 2. Duration of anti-emetic action of BRL 46470 given intravenously or orally before X-irradiation in the ferret. Records were made of both the number of emetic episodes and the time at which the first vomit occurred (latency period).

| Drug procedure BRL 46470 (mg $kg^{-1}$) | n | Dosing time before X-ray (min) | Latency period (min) | Number of emetic episodes |
|---|---|---|---|---|
| Controls | 6 | | 19·0 ± 1·4 | 19·5 ± 2·5 |
| 0·5 i.v. | 4 | 180 | — | No vomiting** |
| 0·5 i.v. | 4 | 240 | 100·8 ± 19·3 | 2·5 ± 2·5 |
| 0·5 p.o. | 4 | 60 | — | No vomiting** |
| 0·5 p.o. | 5 | 180 | 110·0 ± 10·0 | 0·6 ± 6·0* |
| 0·5 p.o. | 4 | 240 | 69·0 ± 17·2 | 6·8 ± 2·9 |

n = Number of animals used. *$P < 0.05$; **$P < 0.01$.

## Discussion

In isolated tissues, BRL 46470 antagonizes at the 5-$HT_3$ receptor in a potent and surmountable manner, suggesting a competitive interaction with 5-HT for the receptor (see Blackburn et al 1993). As anticipated (see Sanger 1993), we have now shown that BRL 46470 is a highly effective anti-emetic agent in ferrets receiving total body X-irradiation treatment. Our experiments also support the contention that BRL 46470 may have a prolonged ability to antagonize at the 5-$HT_3$ receptor in-vivo, as suggested previously by experiments with anaesthetized and conscious rats (Blackburn et al 1993) and with conscious cynomolgus monkeys (Piper et al 1991). This type of prolonged activity could be explained if BRL 46470 has a slow rate of clearance from ferret tissues, but further experiments are required to investigate this possibility. A contributing factor may also be the slow rate at which BRL 46470 dissociates from the receptor (Newberry et al 1993). If our results are applicable to man, a simple and infrequent dosing regime might be possible for BRL 46470.

## References

Bermudez, J., Boyle, E. A., Miner, W. D., Sanger, G. J. (1988) The anti-emetic potential of the 5-hydroxytryptamine$_3$ receptor antagonist BRL 43694. Br. J. Cancer 58: 644–650

Blackburn, T. P., Baxter, G. S., Kennett, G. A., King, F. D., Piper, D. C., Sanger, G. J., Thomas, D. R., Upton, N., Wood, M. D. (1993) BRL 46470A: a highly potent, selective and long acting 5-$HT_3$ receptor antagonist with anxiolytic-like properties. Psychopharmacology 110: 257–264

Newberry, N. R., Watkins, C. J., Sprosen, T. S., Blackburn, T. P., Grahame-Smith, D. G., Leslie, R. A. (1993) BRL 46470 potently antagonizes neural responses activated by 5-$HT_3$ receptors. Neuropharmacology 32: 729–735

Piper, D. C., Kenney, C. A., Jenkins, E., Blackburn, T. P., Hughes, H. C. (1991) The anxiolytic activity of BRL 46470 in the cynomolgus monkey. Biol. Psychiatr. 29: 4585

Sanger, G. J. (1993) The pharmacology of anti-emetic agents. In: Andrews, P. L. R., Sanger, G. J. (eds) Emesis in Anti-Cancer Therapy: Mechanisms and Treatment. Chapman & Hall Medical, London, pp 179–210