British Journal of Pharmacology (2001) 134, 449–450

www.nature.com/bjp

CORRESPONDENCE

Reply to Dodds & Rivory

[1]C. Blandizzi, [1]B. De Paolis, [1]R. Colucci, [1]G. Lazzeri, [1]F. Baschiera & *,[1]M. Del Tacca

[1]Division of Pharmacology and Chemotherapy, Department of Oncology, Transplants and Advanced Technologies in Medicine, University of Pisa, Pisa, Italy

We appreciate the interest of H.M. Dodds and L.P. Rivory in our study on the mechanisms related to the acute cholinergic effects of irinotecan (Blandizzi *et al*., *Br. J. Pharmacol*., 132: 73–84, 2001). However, despite the efforts made to reproduce our experiments on the *in vitro* acetylcholinesterase (AChE) assay, we note that these authors still fail to propose any convincing explanation accounting for the discrepancy between our data (Blandizzi *et al*., 2001) and their repeated observations (Dodds & Rivory, 1999). Therefore, it is conceivable that *in vitro* assays, carried out on purified preparations of AChE extracted from various sources, do not offer sufficient levels of reliability to allow a clear assessment of the putative anti-AChE properties of irinotecan. For instance, while in their letter Dodds and Rivory emphasize the fact that the incubation time of irinotecan with AChE must be kept as close as possible to zero, at a temperature of 25°C, to avoid spontaneous degradation of the drug, Morton *et al*. (1999) allowed an incubation time of 22 h at 37°C, which is much longer than that adopted in our study (20 min).

We wish also to point out that, beyond the debate concerning the *in vitro* effects of irinotecan on AChE activity, several lines of evidence, emerging from the variety of experimental models adopted in our study (Blandizzi *et al*., 2001), as well as from previous clinical reports (Gandia *et al*., 1993; Rowinsky *et al*., 1994), argue against the hypothesis that AChE blockade plays a significant role in the acute cholinergic syndrome evoked by irinotecan. In particular, the following points are worthy to be considered:

(1) At variance with physostigmine (0.01–0.1 μM), irinotecan (tested up to 100 μM) did not significantly affect the atropine-sensitive motor responses induced by electrical stimulation of human colonic longitudinal muscle (Blandizzi *et al*., 2001).

(2) Irinotecan moderately enhanced the cholinergic twitch contractions of guinea-pig ileum longitudinal muscle strips only when applied at very high concentrations (Blandizzi *et al*., 2001). Of interest, in this model there is no need for long pre-incubation time, and changes in motor activity of ileal smooth muscle can usually be recorded immediately after spiking the test drugs into the organ bath solution.

(3) The measurement of electrically-induced acetylcholine release, from longitudinal muscle strips of guinea-pig ileum, represents a highly suitable technique in studies aiming to characterize the influence of drugs on synaptic cholinergic neurotransmission. When tested on this model, irinotecan (up to 100 μM) slightly inhibited acetylcholine release, whereas, as expected, physostigmine (0.1 μM) caused a marked decrease through an indirect activation of muscarinic autoreceptors located on cholinergic axon terminals (Blandizzi *et al*., 2001).

(4) In the clinical setting, we could not detect any significant inhibition of AChE activity in the blood of colorectal cancer patients subjected to treatment with irinotecan (Blandizzi *et al*., 2000). In this study, irinotecan was infused at the dose of 200–350 mg/m^2 over 60 min, and whole blood samples were taken immediately before (baseline) as well as 15 and 45 min after the beginning of drug infusion. Interestingly, similar data have been reported also by Gandia *et al*. (1993), who measured AChE activity in erythrocytes isolated from cancer patients under infusion with irinotecan.

(5) When examining the features of the acute syndrome evoked by irinotecan (Gandia *et al*., 1993; Rowinsky *et al*., 1994), it can be noted that all symptoms are consistent with a picture of parasympathetic activation, leading to the recruitment of muscarinic receptors on peripheral effector organs. However, drugs causing a systemic blockade of AChE are expected also to promote a concomitant activation of nicotinic receptors, with the subsequent occurrence of symptoms, like for instance altered patterns of skeletal muscle motor activity, that have not been observed in patients treated with irinotecan (Gandia *et al*., 1993; Rowinsky *et al*., 1994).

In conclusion, much work remains to be done, at experimental level, to clarify the differences of Dodds and Rivory (1999) data on irinotecan-induced inhibition of AChE, compared with our results. However, in order to better elucidate the pathophysiological mechanisms which subserve the cholinergic side effects of irinotecan, we do not believe that *in vitro* pharmacological assays of AChE can be regarded as critical tests until they are unconfirmed on functional basis by means of more integrated methodological procedures.

*Author for correspondence at: Divisione di Farmacologia e Chemioterapia, Dipartimento di Oncologia, dei Trapianti e delle Nuove Tecnologie in Medicina, Università di Pisa, Via Roma, 55, 56126 Pisa, Italy; E-mail: m.deltacca@do.med.unipi.it

References

BLANDIZZI, C., DE PAOLIS, B., COLUCCI, R., LAZZERI, G., BASCHIERA, F. & DEL TACCA, M. (2001). Characterization of a novel mechanism accounting for the adverse effects of the anticancer drug irinotecan. *Br. J. Pharmacol.,* **132,** 73–84.

BLANDIZZI, C., DE PAOLIS, B., DANESI, R., DI PAOLO, A., FALCONE, A. & DEL TACCA, M. (2000). Assay of acetylcholinesterase activity in cancer patients treated with irinotecan. VII World Conference on Clinical Pharmacology and Therapeutics (Abs. N. 952); *Br. J. Clin. Pharmacol.,* **50** (Suppl.), 245.

DODDS, H.M. & RIVORY, L.P. (1999). The mechanism for the inhibition of acetylcholinesterase by irinotecan (CPT-11). *Mol. Pharmacol.,* **56,** 1346–1353.

GANDIA, D., ABIGERGES, D., ARMAND, J.-P., CHABOT, G., DA COSTA, L., DE FORNI, M., MATHIEU-BOUE, A. & HERAIT, P. (1993). CPT-11 induced cholinergic effects in cancer patients (letter). *J. Clin. Oncol.,* **11,** 196–197.

MORTON, C.L., WADKINS, R.M., DANKS, M.K. & POTTER, P.M. (1999). The anticancer prodrug CPT-11 is a potent inhibitor of acetylcholinesterase but is rapidly catalyzed to SN-38 by butyrylcholinesterase. *Cancer Res.,* **59,** 1458–1463.

ROWINSKY, E.K., GROCHOW, L.B., ETTINGER, D.S., SARTORIUS, S.E., LUBEJKO, B.G., CHEN, T.-L., ROCK, M.K. & DONEHOWER, R.C. (1994). Phase I and pharmacological study of the novel topoisomerase I inhibitor 7-ethyl-10-[4-(1-piperidino)-1-piperidino]-carbonyloxycamptothecin (CPT-11) administered as a ninety-minute infusion every 3 weeks. *Cancer Res.,* **54,** 427–436.