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The effect of co-trimoxazole on thymidine uptake by transforming human lymphocytes *in vitro*

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Gaylarde & Sarkany (1972) have reported that co-trimoxazole (trimethoprim, TMP and sulphamethoxazole, SMX) decreases the uptake of labelled thymidine by human lymphocytes cultured in the presence of phytohaemagglutinin *in vitro*. This phenomenon was pronounced (mean suppression 84%) but only observed in 60% of a small group of subjects, prompting these authors to suggest that this could represent some pharmacogenetic difference between these two populations of subjects. During the course of a systematic study of the effects of TMP upon human lymphocytes stimulated to undergo lymphoblastic transformation with concanavalin A (con A) *in vitro* we have re-investigated these findings in this system.

Our population consisted of healthy Caucasian medical students and staff who were taking no drugs (except for one taking an oral contraceptive pill) and who had abstained from coffee or tea for 12 h before venepuncture. Most were non-smokers. Only one subject had been previously exposed to co-trimoxazole: results from this subject were not different from the others. Blood was drawn at approximately the same time each morning to obviate diurnal rhythmicity in lymphocyte responsiveness (Tavadia, Fleming & others, (1975). Heparinized venous blood from each subject was sedimented by gravity for 2 h, the leucocyte-rich plasma being periodically withdrawn into a sterile tube. After twice washing the cells with Medium TC 199 they were resuspended in TC 199 containing 10% foetal calf serum to give a concentration of 10^6 lymphocytes ml^{-1} as determined by visual cell counting. 1 ml cultures were set up in triplicate containing 40 μg ml^{-1} con A and appropriate additions of drugs made. Both TMP and SMX were dissolved in 50% ethanol and added in a total volume of 10 μl. Preliminary experiments using this vehicle showed that this concentration of ethanol did not significantly alter thymidine uptake. Cultures were incubated at 37° in 5% carbon dioxide/air mixture for 68 h when 1 μCi thymidine [methyl-^{3}H] (6·7 Ci mM^{-1}: New England Nuclear Corporation, Boston, Mass.) was added. After a further 3 h culture the incubation was terminated by two 3 ml washes with ice-cold 5% trichloroacetic acid and a 3 ml methanol wash. The acid-insoluble residue was dissolved in 0·5 ml 1M Hyamine-10-X in methanol at 70° for 15 min and washed into counting vials with ethanol. A PPO-POPOP scintillant was used to count tritium to an efficiency of 15%. The results are shown in Table 1.

Using drug concentrations similar to those employed by Gaylarde & Sarkany (SMX 2×10^{-5}M; TMP 5×10^{-6}M) we could detect no significant inhibitory effect. At a higher concentration of SMX (4×10^{-4}M which is ~normal therapeutic plasma concn) there was a small but statistically insignificant ($t = 1{\cdot}3$) inhibitory effect whilst at 5×10^{-5}M, TMP (~ normal plasma concn 10^{-5}M) clearly inhibits thymidine incorporation (Rogers & Lietman, 1975). Combining these drugs at the higher concentrations produced a slightly greater inhibition but this was not significant statistically ($t = 2{\cdot}35$). Cellular viability as assessed by the trypan blue exclusion method (Ling, 1968) was unaffected by drug treatment.

Since the incubation medium contained 10% foetal

Table 1. *Effects of TMP and SMX alone and in combination of [^{3}H]thymidine uptake by con A-stimulated lymphocytes*

No. of subjects	Mean age (range)	% incorporation [^{3}H]thymidine *vs* control (with s.d.)		
		SMX 2×10^{-5} M	TMP 5×10^{-6} M	SMX 2×10^{-5} M TMP 5×10^{-6} M
13 (9 male)	25 (20–32)	99·3 s.d. 16·0	102·7 s.d. 12·7	103·7 s.d. 10·4
		SMX 4×10^{-4} M	TMP 5×10^{-5} M	SMX 4×10^{-4} M TMP 5×10^{-5} M
10 (8 male)	26 (21–32)	90·8 s.d. 22·4	69·6 s.d. 13·2	56·4 s.d. 16·2

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calf serum the drugs would be protein-bound to the extent 42–44% for TMP (Schwartz & Zeigler, 1969) and 64% for SMX (Hall, 1961).

Why these results differ from those of other workers using an apparently similar system is not clear. Con A and phytohaemagglutinin are both T-lymphocyte mitogens although in mice at least it is known that distinct subsets of cells respond differently to these two substances (Stobo & Paul, 1973). If TMP and SMX are acting through some interference with lymphocyte folate metabolism it may be significant that Medium TC 199 contains folic acid 0·01 mg litre^{-1} whereas the Eagle's minimal medium used by Gaylarde & Sarkany (1972) contains one hundred times this amount. The observations of Bain (1975) suggest that low folate media increase thymidine uptake without affecting the degree of morphological transformation of cultures. It is possible that our population as defined above in some way differs from the 'normal subjects' of Gaylarde & Sarkany (1972). Although SMX at high concentrations produces a small inhibition we believe the major inhibitory effect of co-trimoxazole on [^{3}H]thymidine incorporation to be due to TMP at the higher concentration and that any synergistic effect must be less common than hitherto suggested.

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LETTERS TO THE EDITOR

Contraversive circling behaviour produced by unilateral electrolytic lesions of the ventral noradrenergic bundle mimicking the changes seen with unilateral electrolytic lesions of the locus coeruleus

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In investigating the possible role of central nervous system noradrenergic neurons in motor activity we have found that unilateral electrolytic lesions in the region of the noradrenaline containing locus coeruleus in rats result in circling behaviour contraversive to the side of the lesion when the animals are systemically administered either apomorphine or (+)-amphetamine (Pycock, Donaldson & Marsden, 1975). This behaviour is transient and disappears after one to two months. It is accompanied by a similar transient rise in the ipsilateral striatal dopamine content by approximately 50%, which is present at five days post-operatively, but has disappeared by one to two months. There is a persistant fall in the ipsilateral cerebral cortical noradrenaline. It was postulated that a possible explanation of these results was that a pathway between the locus coeruleus region and the nigrostriatal system existed, and that this was facilitative serving to enhance nerve impulse flow between the substantia nigra and the striatum. The interruption of the proposed link was envisaged as causing a decrease in ipsilateral nigrostriatal activity with consequent decentralization supersensitivity (Ungerstedt, 1975) in the striatal dopamine receptors and a build up of dopamine. This would explain the contraversive rotation to both apomorphine and (+)-amphetamine.

If the observed changes were due to damage to noradrenaline-containing cells of the locus coeruleus and not to adjacent structures then these results might be expected to be reproduced by lesions of the ascending noradrenergic bundle systems. Damage restricted to either the dorsal or the ventral bundle would establish which of these two pathways was involved. The locus coeruleus is known to provide noradrenergic fibres to both of these bundles (Lindvall & Bjorkland, 1974).

Unilateral electrolytic lesions were made in the rostral pons and caudal midbrain of Wistar rats, of 150 ± 10 g weight at the time of operation. The level of the lesions was chosen so as to be caudal to the level of the substantia nigra at a point where the dorsal and ventral bundle were separate from each other. The lambda-bregma line was horizontal and the skull was held in a 'Stoelting' stereotaxic frame. The anode, a stainless steel electrode with a diameter of 0·65 mm, which was

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