The importance of the intestinal microflora in nitrazepam metabolism in the rat

D.S. HEWICK & V. SHAW

Department of Pharmacology and Therapeutics, University of Dundee, Ninewells Hospital Medical School, Dundee DD1 9SY

Nitroreduction is an important route of metabolism for nitrazepam (1,3-dihydro-7-nitro-5-phenyl-2H-1,4-benzodiazepine-2-one) in rat and man (Yanagi, Haga, Endo & Kitagawa, 1975; Beyer & Sadee, 1969). Since the intestinal flora appear to play a major role in the nitroreduction of p-nitrobenzoic acid and p-nitrobenzenesulphonamide (Wheeler, Soderberg & Goldman, 1975) it was of interest to ascertain if the situation was similar with nitrazepam.

Germ-free (GF) or conventional (specific pathogen-free) female WAG rats (6–8 weeks old) were given 5-[^{14}C]-nitrazepam intraperitoneally (20 mg/kg, 1.87 μCi/kg), and water orally (25 ml/kg). Urine and faeces were collected for three days and then the GF rats were 'contaminated' by including normal rat faeces in the food. Three weeks later, the experiment was repeated with the 'contaminated' rats. After measuring total radioactivity in the urine and faeces, urinary radioactivity was examined further by extracting it from the alkalinized urine with butan-1-ol and chromatographing the extract (Yanagi *et al.*, 1975).

The rate of excretion of radioactivity in the GF animals was slower than in the conventional rats (Figure 1). During the first 8 h the GF and conventional rats excreted about 36 and 72% respectively of the urinary radioactivity eliminated in 72 hours. The percentages of total administered radioactivity eliminated in the urine and faeces respectively during 72 h were 31 and 53% for the conventional rats and 15 and 58% for the GF rats. After 'contaminating' the GF rats, the urinary (Figure 1) and faecal excretion patterns were essentially the same as in conventional rats. Urinary radioactivity comprised nitrazepam and its 7-amino and 7-acetamido-derivatives, with the reduced products accounting for 91, 55 and 85% of the urinary radioactivity excreted in 72 h in the conventional, GF and contaminated animals respectively.

When [^{14}C]-nitrazepam was incubated with rat caecal or human transverse colon contents (under nitrogen at 37°C) it was extensively reduced to the 7-amino derivative.

It is suggested that the intestinal bacteria are important in the metabolism and elimination of nitrazepam in the rat.

This work was supported by a Medical Research Council grant to Dr D. S. Hewick. The rats were purchased from the M.R.C. Laboratory Animal Centre, Carshalton, Surrey. The [^{14}C]-nitrazepam was a gift from F. Hoffman – La Roche and Co., Basel, Switzerland.

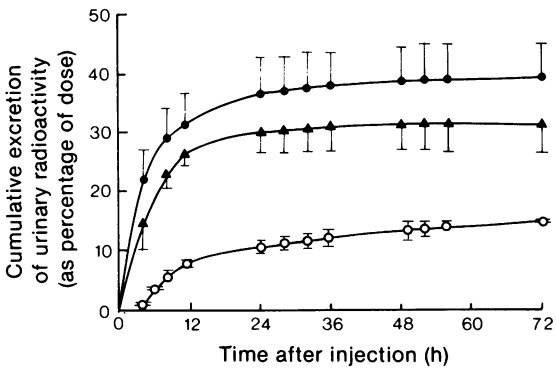

Figure 1 The elimination of urinary radioactivity by conventional (▲), germ-free (○) and 'contaminated' (●) female WAG rats after an i.p. injection of [^{14}C]-nitrazepam (20 mg/kg, 1.87 μCi/kg). The 'contaminated' rats are the germ-free rats, tested again about 3 weeks after being allowed access to normal rat faecal material. Each point is the mean of 3 groups of 2 rats. In some cases the standard deviation 'bars' have been partially omitted for the sake of clarity.

References

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