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Oral absorption of metronidazole in rabbits irradiated with cobalt-60 gamma radiation

PADMANABH P. BHATT, ANIL Y. NIMBKAR, *The Bombay College of Pharmacy, University of Bombay, Kalina, Bombay—400098, India*

Abstract—The absorption of oral metronidazole in control rabbits and in rabbits irradiated with cobalt-60 gamma radiation was studied. It was observed that the bioavailability of metronidazole was significantly reduced in irradiated animals the reduction being dependent on the dose of radiation. The maximum decrease in absorption was seen 48 h post-irradiation.

Treatment with ionizing radiation is a common method of cancer therapy and patients often receive drugs as an adjunct to this. The structure and function of the gastrointestinal system may be altered (Detrick et al 1954) by ionizing radiation thus affecting the bioavailability of orally administered drugs.

This study was undertaken to determine the effect of radiation on absorption and then to establish (a) the extent of change in absorption, (b) the duration of the effect and (c) whether the effect is dose-dependent.

Metronidazole has been reported to be a selective radiosensitizer of the hypoxic cells of tumours (Kuropteva & Zhumabaeva 1986; Hodgkiss 1987) and has been used in radiotherapy.

Materials and methods

Materials. Metronidazole was a gift from Lupin Laboratories. Heparin, pentobarbitone sodium, metoclopramide and *p*-dimethylaminobenzaldehyde were obtained from Sigma Chemical Co. NaCl, NaOH, Na_2SO_4 and Zn dust were obtained from BDH. $CHCl_3$ and HCl were supplied by SD Chemical Co. Industrial ethanol was double-distilled before use.

Colorimetric estimation of metronidazole. A modification of the method described by Populaire et al (1968) was used. Instead of using a solution of titanium chloride in ethanol as the reducing agent, we used 0·1 g zinc dust and 0·25 mL conc. HCl. The optical density was read at a wavelength of 500 nm using an Erma-EA-11 colorimeter.

Extraction of metronidazole from plasma. The heparinized blood samples containing metronidazole were extracted using the method of Populaire et al (1968) after one modification, i.e. a boiling water bath rather than one at 45°C was used to evaporate off the chloroform.

Irradiation of rabbits. Adult, healthy albino rabbits of either sex, 1·1–2·8 kg were irradiated (except the head) with cobalt-60 gamma radiation. Half the radiation dose was administered dorsally and half ventrally. One group of rabbits was administered 200 rad, another group 400 rad, and a third group 800 rad of radiation. The irradiation time was 2–8 min depending on the dose.

Oral absorption study. Rabbits were given 2 mg metoclopramide orally through a gastric catheter and were then fasted for 24 h before the experiment. On the day of the study, the animal was anaesthetized using pentobarbitone sodium (40 mg kg^{-1}, i.p.). Heparin (2500–3000 iu) was injected through the marginal ear vein. The left common carotid artery was cannulated. Metronidazole (125 mg kg^{-1}) suspension was made with Tween 80 and distilled water and given orally.

Blood samples were drawn, from the left common carotid artery, just before drug administration and at various times, up to 6 h, after drug administration. Each time, after drawing blood, an equal volume of 0·9% NaCl (saline) was passed through the marginal ear vein. At the end of the experiment, the animal was killed by intravenous administration of sodium pentobarbitone.

This oral absorption study was also carried out in a control group of non-irradiated rabbits. With irradiated rabbits, the study was done 24, 48 or 72 h post-irradiation.

Correspondence to: P. P. Bhatt, Department of Pharmaceutical Chemistry, University of Kansas, Malott Hall, Lawrence, Kansas 66045, USA.

Results

The colorimetric method of estimation of metronidazole was sensitive to a minimum concentration of 1 $\mu g\ mL^{-1}$. Beer's Law was obeyed in the concentration range of 1 to 60 $\mu g\ mL^{-1}$.

The extraction procedure of metronidazole from plasma gave a mean recovery of 93·2%.

The plasma concentrations of metronidazole achieved after oral administration, in control rabbits, are shown in Table 1.

Table 1. Plasma concentration of metronidazole after oral administration in control group of three rabbits (mean ±s.e.m.)

Time (h)	Plasma concn ($\mu g\ mL^{-1}$)
0·25	64·69 ± 2·90
0·5	43·61 ± 3·51
1	33·22 ± 2·64
2	29·93 ± 2·05
4	21·93 ± 0·90
6	16·44 ± 0·50

The highest concentration of 64·69 $\mu g\ mL^{-1}$ is seen at 15 min, after which a progressive decrease in concentration is observed over the period of 6 h. Thus, the time at which peak level was reached must be 15 min or less.

Results pertaining to the oral absorption of metronidazole after 200 rad of radiation (Table 2) show that the peak concentration of 17·76 $\mu g\ mL^{-1}$ is achieved after 30 min when the study was carried out 24 h post-irradiation. In the 48 h post-

Table 2. Effect of 200 rad of cobalt-60 gamma radiation on oral absorption of metronidazole in four rabbits (mean ±s.e.m.)

	Concn of metronidazole ($\mu g\ mL^{-1}$)		
Time (h)	24 h PR	48 h PR	72 h PR
0·25	14·48 ± 0·91*	5·59 ± 1·37*	11·84 ± 0·52*
0·5	17·76 ± 2·07*	7·24 ± 1·89*	10·45 ± 0·18*
1	14·64 ± 0·83*	6·99 ± 0·71*	9·38 ± 0·47*
2	10·45 ± 0·27*	8·31 ± 1·32*	7·98 ± 0·43*
4	8·06 ± 0·47*	6·00 ± 0·30*	5·02 ± 0·51*
6	6·58 ± 0·23*	4·77 ± 0·27*	3·29 ± 0·62*

PR: Post-irradiation
*$P < 0·005$ compared with control group (Student's *t*-test).

irradiation study, the highest concentration achieved was 8·31 $\mu g\ mL^{-1}$ after 2 h. In the 72 h post-irradiation study, slight recovery is apparent with the time to achieve peak concentration reverting back to within 15 min and the peak plasma concentration increasing to 11.84 $\mu g\ mL^{-1}$. Thus the maximum effect of 200 rad of radiation, with respect to both the time to reach peak level and the peak concentration attained, is seen 48 h post-irradiation.

Plasma concentrations achieved after 400 rad of radiation are shown in Table 3. Here too, the time to attain peak concentration was delayed (30 min) and the peak value decreased to 9·95 $\mu g\ mL^{-1}$ in the 24 h study. In the 48 h study, the highest concentration achieved was 6·17 $\mu g\ mL^{-1}$, while in the 72 h study, the peak plasma value of 11·35 $\mu g\ mL^{-1}$ was achieved within 15 min, as in control rabbits.

Table 3. Effect of 400 rad of cobalt-60 gamma radiation on oral absorption of metronidazole in four rabbits (mean ±s.e.m.)

	Concn of metronidazole ($\mu g\ mL^{-1}$)		
Time (h)	24 h PR	48 h PR	72 h PR
0·25	5·68±2·10*	3·70±1·60*	11·35±1·92*
0·5	9·95±3·11*	4·38±1·15*	10·86±1·05*
1	9·95±1·58*	3·21±0·54*	10·45±0·86*
2	8·72±2·14*	4·53±1·06*	6·99±1·12*
4	4·69±0·99*	6·17±1·39*	4·03±1·11*
6	3·95±1·41*	4·62±1·35*	3·29±1·05*

PR: Post-irradiation
*$P < 0·005$ compared with control group (Student's t-test).

Comparing Tables 2 and 3, it is seen that animals irradiated with 400 rad have plasma concentrations of metronidazole less than those given 200 rad, at 24 and 48 h post-irradiation.

The concentrations of metronidazole obtained in rabbits given 200 or 400 rad of radiation are significantly lower than those seen in control animals; $P < 0·005$ using Student's t-test.

The 800 rad dose of radiation was found to be lethal, as only one out of six rabbits survived. The drug in the survivor was measured 48 h post irradiation. Plasma concentrations of metronidazole were very low (Table 4).

Table 4. Effect of 800 rad of cobalt-60 gamma radiation on oral absorption of metronidazole in one rabbit, 48 h post-irradiation

Time (h)	Concn of metronidazole ($\mu g\ mL^{-1}$)
0·25	0·00
0·5	0·66
1	3·29
2	4·93
4	4·60
6	4·15

The maximum decrease in the extent of absorption (area under the average plasma concentration-time curve) was at 48 h post-irradiation, with a slight recovery at 72 h in groups irradiated with 200 or 400 rad. The decrease in absorption depended on the dose of radiation, being greatest with 800 and least with 200 rad.

Discussion

Anorexia, nausea and vomiting (Walsh 1960), and diarrhoea, are some of the common manifestations of radiation sickness associated with alterations in the functions of the gastrointestinal tract. While the more serious effects, depending on the dose of radiation, are changes in the tone and motility of the gut (Bond 1963; Conard 1970; Gerber & Altman 1970), changes in the gastric emptying rate (Goodman et al 1952; Swift et al 1955), inflammation and ulceration of the stomach and intestines (Detrick et al 1954; Bloom & Bloom 1960), and death within several days following extensive gastrointestinal damage.

Our study shows that the rate as well as the extent of absorption of metronidazole decreased in rabbits that were irradiated. The decrease in bioavailability was found to be dose-dependent and the maximum decrease was seen after 48 h of irradiation.

Our results could be explained with the findings of Goodman et al (1952) who reported that the inhibition of gastric emptying starts immediately following exposure to radiation with its maximum effect 48 h post-irradiation. At 72 h post-irradiation the gastric emptying rate begins to return to control values, hence the time to peak concentration was 15 min or less.

The changes in the extent of absorption following irradiation could be explained on the basis of histological changes, especially in the small intestine since the number of goblet cells (and therefore the mucous lining) increases after irradiation (Shetty 1979). Reduced bioavailability may also result from a reduction in intestinal blood flow as found by Kabal et al (1972) in dogs exposed to 1500 rad of mixed gamma-neutron radiation. Other possible mechanisms could be alteration in the transfer of the drug across the gastrointestinal epithelium due to change in permeability, a decrease in the surface area of the intestinal mucosa and inactivation of carriers associated with transport across the intestinal epithelium.

Our studies were done up to 72 h post-irradiation. Further studies are required to determine the time taken for the gastrointestinal physiology (including absorption mechanisms) to return to normal, if at all, after irradiation.

In clinical situations, radiotherapy patients often receive drugs. Our study suggests that if the drugs are administered orally to these patients their blood levels should be monitored and the dosage adjusted to account for any gastrointestinal malfunction.

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