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Misonidazole Toxicity and Pharmacokinetics in Mice: Dependence on Strain and Size*

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Abstract—*The toxic side-effects of misonidazole (MISO) have been studied in two strains of mice over a wide weight range. The sensitivity of the mice, determined as the* LD_{50} *within* 7 days *of administration, varied by almost a factor of two. Differences were seen in the same strain, with heavy mice being most sensitive, and from strain to strain, with CBA mice being more susceptible than WHT albino mice. The toxicity of the MISO was closely related to a change in the peak blood levels, but showed no correlation with biological half-life. The tissue volume available for drug distribution appears to be reduced in large, heavy mice at high drug doses, and cannot be explained simply on the basis of drug exclusion from adipose tissue. For radiobiological studies with MISO doses of more than* 0.5 mg/g *it is recommended that it should be administered in a dosage that will give a known blood level of the drug, rather than simply by giving a constant dose based on body weight.*

INTRODUCTION

MISONIDAZOLE was first used as a radiosensitizer of hypoxic tumour cells in 1974 [1, 2]. Since then many studies have been undertaken using this compound as an 'oxygen mimetic' radiosensitizer, both in experimental animals and in the clinic (for review see [3]). It is well recognised that the degree of sensitization is related to the drug concentration achieved in the tumour cells, and the enhancing effect of this radiosensitizer is often expressed in relation to the administered dose (mg/kg body wt or mg/m^2). Its use in the clinic is limited by the difficulty of giving an adequate dose without causing neurotoxic side-effects in man [4]. In contrast to the many publications relating to its sensitizing efficiency, relatively little has been published about its toxic side-effects. These have been shown to differ in the mouse from those seen in man. Whilst neurotoxicity is detectable by subtle behavioural or biochemical tests [5, 6], most workers have used the crude endpoint of death as the measure of toxicity, because the neurological symptoms in rodents do not closely mirror those seen in man.

In this study we have investigated the variation on toxicity that has been seen in our two strains of mice, and within each of these the variation with size of the animal. Most of the studies involved single doses of MISO, either alone or combined with veterinary Nembutal (a barbiturate anaesthetic).

MATERIALS AND METHODS

The Gray Laboratory inbred colonies of CBA/Ht and albino WHT mice have been used for these studies since these are the strains used here for studies of tumour sensitization [7]. These mice have been inbred for at least 20 yr and have been maintained as a conventional brother-sister mated colony. In 1977 the colony was upgraded to specific pathogen-free status by caesarian derivation and fostering onto SPF mothers of the BSVS strain. The animals were then designated CBA/HtGyfBSVS and WHTGyfBSVS respectively. The toxicity studies extended over the period from conventional to SPF status and the mice would therefore have a different microbial flora in the intestine.

Initially the minimum of toxicity data was obtained simply to determine the maximum tolerated dose. With the upgrading to SPF status, however, it was noticed that the mice were less

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tolerant of misonidazole, and the standard dose of 1 mg/g was found to be lethal to a proportion of the animals. In order to understand whether this related to their microbial flora or their increase in body weight after SPF derivation we undertook the following study.

Misonidazole was freshly made up and administered i.p. to mice in graded doses to determine the LD_{50} and to study the drug pharmacokinetics. The stock solution was prepared at a concentration of 30 mg/ml, so that a volume of 1 ml was given to each mouse of standard weight 30 g for an administered dose of 1 mg/g. After administering a range of drug doses (on the basis of body weight) the mice were observed for 7 days for abnormal symptoms or deaths. Mice aged 2-5 months were selected for treatment on the basis of weight, and ranged from 17 g in young CBA males to 54 g in older WHT males. Each weight group contained eight mice, with a total weight range not exceeding 3 g.

In a small sample of mice rectal temperatures were measured over a 6-hr period using a Bailey Thermocouple probe because this had been shown to be a sensitive parameter [6].

For the pharmacokinetic study mice of defined weight ranges were injected with MISO and killed either at 20 ± 5 min (for peak blood levels) or at 15-360 min, to determine the half-life. Blood was obtained from the major thoracic vessels immediately after termination by neck luxation. The blood was collected in heparinised vials and the concentrations of MISO and of DESMISO (the *O*-demethylated metabolite) were determined using high-performance liquid chromatography (HPLC) [4].

The tissue distribution of MISO was studied using (2-^{14}C)-radiolabelled MISO and liquid scintillation counting. The radioactive MISO was administered at a specific activity of 0.071 μCi/g. Mice were killed at 20 and 60 min to determine the distribution in a range of body tissues and fluids. The half-life was also determined for [^{14}C]MISO (kindly provided by Dr C. Smithen of Roche Products Ltd, Welwyn Garden City, at a specific activity of 53.6 μCi/mg) by sampling blood at intervals up to 4 hr.

RESULTS

Figure 1 shows the lethality data for WHT and CBA mice. The data have been fitted by a logit analysis to obtain LD_{50} values ± 1 S.E.M. The susceptibility of the mice of each strain increases with increasing size. Only small mice have an LD_{50} value of 1.8 mg/g, which is that commonly quoted. It is also evident that CBA mice are more sensitive than WHT mice of an equivalent size range. The LD_{50} values from these and other experiments are listed in Table 1. In each strain there are no significant differences in MISO toxicity in conventional mice and in SPF mice if they are of the same size. Female WHT mice seem to be slightly less susceptible than weight matched males from the SPF colony.

The addition of pentobarbital anaesthesia had a different effect on the two strains: in CBA mice it reduced the LD_{50}, whereas in WHT mice it allowed them to tolerate a higher MISO dose. In both strains there was a sparing effect if the MISO was given as two fractions with a 24-hr interval instead of as a single dose. The dose per fraction had to be reduced but the total dose tolerated increased by about 30% (see Table 1).

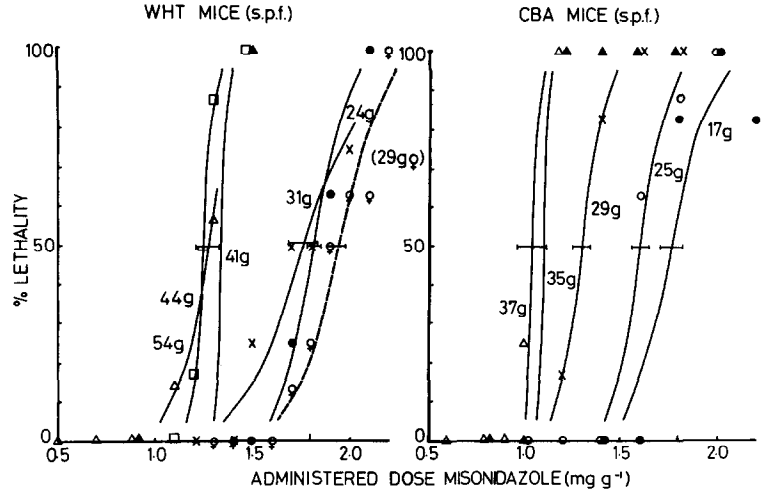

Fig. 1. Lethality within 7 days resulting from i.p. administration of MISO to mice of different weights. In both strains the drug is more toxic to the heavier mice.

Table 1. Mean lethal doses (LD_{50} 7) of misonidazole

	CBA							WHT							
Mean body weight	17 g	25 g	27.5 g*	29 g	32.5 g*	35 g	37 g	24 g	27.5 g*†	29 g†	30 g*	31 g	41 g	44 g	54 g
Single dose no anaes.	1.76 ±0.1	1.60 ±0.0	1.63 ±0.1	1.29 ±0.1	—	1.10 ±0.2	1.03 ±0.1	1.81 ±0.0	1.80 ±0.2	1.94 ±0.0	1.79 ±0.0	1.76 ±0.1	1.34 ±0.5	1.27 ±0.1	1.24 ±0.0
Single dose + anaes.	— —	— —	1.42 ±0.1	— —	1.37 ±0.1	— —	— —	— —	— —	— —	2.24 ±0.5	— —	— —	— —	— —
2F/24 hr + anaes.	— —	— —	1.98 ±0.6	— —	1.82 ±0.1	— —	— —	— —	— —	— —	2.84 ±0.1	— —	— —	— —	— —

LD_{50}, mean ± 1 S.E.M.
*Conventional mice.
†Female mice.

Rectal temperatures

The body temperatures of groups of mice of different sizes was assessed over a 4- to 6-hr period using a rectal thermocouple. In male WHT mice weighing 33.1 ± 0.9 or 48.3 ± 1.5 g the temperature fell rapidly. Within the first 2 hr it had fallen to 28°C in the heavier mice and remained low for 6 hr. In the lighter mice the reduction was smaller (to 31°C) and recovery commenced within 3–4 hr. In CBA male mice the temperature fell to 32–33°C in small- and medium-sized mice (18.4 ± 2.1 and 32.3 ± 0.7 g) but recovery began by 3–4 hr. In the largest CBA males (39.8 ± 0.7 g) the temperature fell to 31°C. Thus the extent of temperature loss was greatest in the heavier mice of both strains.

Figure 2 shows the blood concentration of MISO 20 min after graded doses given to animals in different weight ranges. After 0.5 mg/g there was relatively little difference in the peak concentrations, but after higher doses the data became more spread, with much higher peak concentrations in the heavier mice. The difference at 1.0 mg/g was by a factor of 1.6 in both strains (i.e. similar to the difference in LD_{50} values) and was still apparent if the MISO dosing was converted to a per surface area basis.

The pharmacokinetics of MISO and of (MISO and DESMISO) are listed in Table 2. For administered doses of 1 mg/g there was no significant difference in the $t^1/_2$ for male or female WHT mice or for CBA males over a wide weight range. However, the difference in the peak concentration is again apparent for the different weight ranges, and for the two strains, when total nitroimidazole at 15–20 min is considered (last column).

In Fig. 3 the lethality and pharmacokinetic data have been summarized for comparison. Panel A shows the progressive fall in the toxic dose with increasing weight for both CBA and WHT males. The curves for the two strains are displaced horizontally, with the CBA sensitivity being equal to that of WHT mice weighing 7–10 g more. Panel B illustrates the very similar shape of the curves derived by plotting the dose that would need to be administered to give a peak blood level of 1000 μg/ml. Again the curves are laterally displaced by 7–10 g. Panel C results from the ratio of the data in A to those in B. It is an indirect estimate of the peak blood level that would correspond to an LD_{50} dose. When plotted in this way it is clear that the ratio for WHT mice is constant over a wide body weight range, corresponding to a 1.6- to 1.8-fold increase above the dose needed to give 1000 μg/ml. Thus a 30-g 'standard mouse' will have 1000 μg/ml after an administered dose of 1.0 mg/g MISO and half of

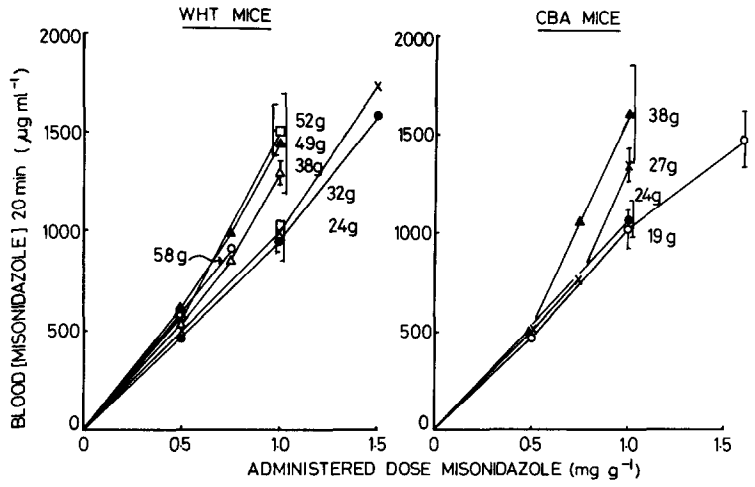

Fig. 2. Concentration of MISO in blood at 20 min after i.p. injection to mice of different weights. Higher levels are observed in the heavier mice of both strains. Each point represents mean ± 1 S.E.M. of 3 mice.

them will die if given 1.8 mg/g. The values for the CBA strain are not so constant; the change in LD_{50} is more abrupt than the variation in peak blood levels and hence this ratio falls with increasing mouse weight. Thus, in heavy CBA mice another factor seems to be operating. That this is not due to a changing half-life is shown in Table 2.

The distribution volume of the mouse could change with increasing size, i.e. the space that is freely accessible for drug diffusion. Since MISO is less soluble in lipids than in water it might be expected that mice with large fat deposits would exclude the MISO from this tissue and hence have a higher than expected concentration in the other tissues. The available space or 'apparent weight' of the mouse has been calculated from the lethality data as shown in Fig. 4. A 30-g WHT mouse has been accepted as standard for that strain and a 25-g mouse for the smaller CBA strain. All other LD_{50} values have then been normalized to this, on the assumption that in the standard mouse the total body space is available for drug diffusion. If the distribution space remained 100% the data would all fall on the 45° line. At weights below the 'standard' they do not deviate significantly from 45°, but at higher weights the 'apparent weight' diminishes progressively. The deviation of 10–15 g in the large mice suggests totally unavailable tissue of that magnitude, which is similar to the total gross fat that could be dissected (10 g) from a 60-g mouse. However, the partition coefficient (octanol:water) is only 0.43 for MISO and the fat should therefore contain a significant fraction of the MISO.

To study this further the results shown in Tables 3 and 4 were obtained, i.e. concentrations in selected tissues, measured on tissue homogenates by HPLC (Table 3) or by scintillation counting after [^{14}C]MISO was administered (Table 4). Table 3 shows that the MISO level in

Table 2. Misonidazole: pharmacokinetics in blood

Mouse weight (g)	Administered dose (mg/g)	Misonidazole half-life (min)	Total nitroimidazole: half-life (min)	peak concentration (μg/ml)
WHT ♀				
25–29	1.0	94.0 ± 9.5	—	1045
WHT ♂				
31–34	1.0	94	96	1060
CBA ♂				
16–20	1.0	108	117	1065
20–26	1.0	104 ± 4.0	110 ± 5	1097
26–29	1.0	—	—	1379
31–37	1.0	106 ± 5.0	109 ± 5	1755
39–41	1.0	108	114	1641

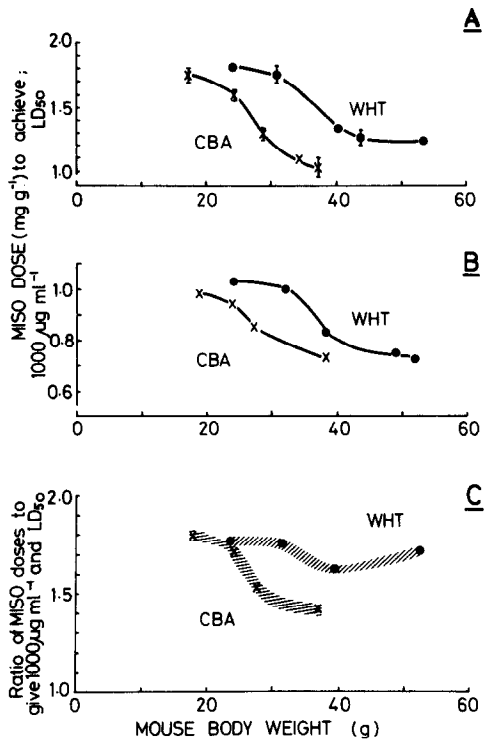

Fig. 3. Lethality (panel A) and blood concentrations (panel B) are dependent upon body weight in both CBA and WHT mice. Panel C shows the ratio of the lethality data to that for blood concentrations. Error bars (panel A, ± 1 S.E.M.) are shown except where they are smaller than the points.

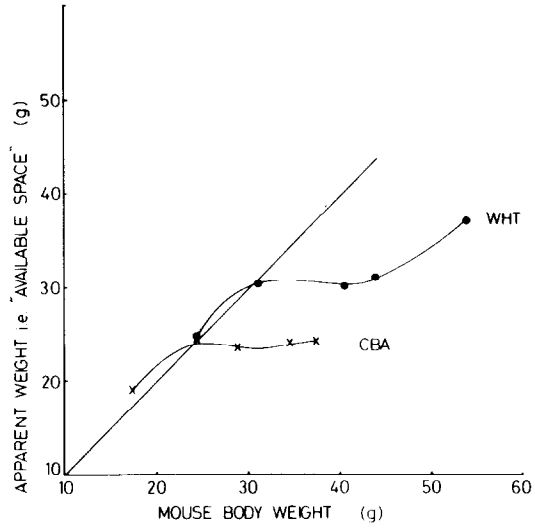

Fig. 4. Apparent weight i.e. 'available space' as a function of actual body weight. Apparent weight defined as:

$$\frac{\textit{observed } LD_{50} \times \textit{mouse weight}}{LD_{50} \textit{ at standard weight}}.$$

It has been assumed that a standard CBA male of 25 g or WHT male of 30 g has the entire body space available for drug diffusion. (Data derived from Figs 1 and 2.)

blood is lower than that in the abdominal fluid for all four cases tested, indicating incomplete diffusion into the vasculature at 20 min. The levels in liver, muscle, kidney and brain are approximately 60–70% of that in blood for male mice up to 48 g in weight and are even lower in the 60-g mouse. In the abdominal fat, or that derived from the subcutaneous region on the back of the neck, it is much lower, being only 5–30% of that in blood. The values are lowest in the very heavy WHT males and the 37-g WHT females. It is possible that the low levels in fat result from the combined effect of the low partition coefficient and the poor vascularity of adipose tissue. In some tissues, e.g. bone and muscle, difficulty in preparing homogenates for HPLC analysis led us to make radioactivity determinations, shown in Table 4, using three weight groups of WHT mice at 20 and 60 min. Once again the concentration is low in fat, at both 20 and 60 min. Furthermore, it is even lower in the large mice than in the small mice (9–16% cf. 11–43%). Most of these values are below the partition coefficient and are again consistent with poor vascularity of the adipose tissue. It is also interesting to note from Table 4 that the concentration in skin and in bone remains low even at 60 min. Tables 3 and 4 confirm that the MISO is not distributed evenly throughout the body mass. The concentration in the peritoneal fluid exceeds that in blood, even after 60 min. The level in most tissues stays below that in blood, and the tissue/blood concentrations tend to be even lower in the heavy mice than in the small mice. Radiographic evidence showed that the increase in size was not simply due to accumulation of fat, since the skeleton enlarged considerably in mice weighing 50 g rather than 30 g. However, since bone also appears to exclude MISO (Table 4), this would be expected to lead to higher blood concentrations.

DISCUSSION

The data presented here demonstrate that at relatively high MISO doses (0.5 mg/g) it is inappropriate to dose mice on a simple mg/g body wt basis. Even dosing on a mg/m^2 (i.e. surface area) basis does not obviate the problem. Both the toxic side-effects and the peak concentration in the blood change with body weight and mouse strain. In the albino mice blood MISO concentration correlates well with toxicity at all weights. For the CBA mice, however, the toxicity in heavy mice is even higher than can be explained on the basis of blood concentration alone.

The 'apparent volume' available to the drug has been shown to change with increasing mouse size. HPLC and radiolabelled MISO determinations indicate that this results from a partial

Table 3. Misonidazole distribution in body tissues (μg/g) at 20 min following 1 mg/g i.p. (HPLC determination)

	CBA ♂		WHT ♂		WHT ♀	
Weight (g)						
Mean	24	42	21	42	60	37
Range	23-25	40-44	20-23	41-43	—	—
Blood	1171 ± 62	1443 ± 20	1076 ± 19	1336 ± 198	1734	1331
Peritoneal fluid	1552 ± 125	2042 ± 222	1254 ± 27	1586 ± 690	—	—
(% blood)	132.5	141.5	116.5	118.7		
Liver	773 ± 41	922 ± 80	661 ± 48	796 ± 134	413*	415*
(% blood)	66.0	63.9	61.4	59.6	23.8	31.2
Muscle	788 ± 98	848 ± 137	653 ± 36	798 ± 140	680*	618*
(% blood)	67.3	58.8	60.7	59.7	39.2	46.4
Kidney	736 ± 18	976 ± 41	722 ± 67	915 ± 11	—	—
(% blood)	62.9	67.6	67.1	68.5		
Brain	773 ± 40	916 ± 159	715 ± 40	722 ± 130	704*	685
(% blood)	66.0	63.5	66.4	54.0	40.6	51.5
Fat (neck)	330 ± 63*	300 ± 134*	268 ± 34*	242 ± 96*	91*	77*
(% blood)	28.2	20.8	24.9	18.1	5.2	5.8
Fat (abdomen)	281 ± 125*	335 ± 187*	220 ± 21*	164 ± 41*	147*	91*
(% blood)	24.0	23.2	20.4	12.3	8.5	6.8

Errors ± 1 S.D.
*Less than 50% of MISO concentration in blood.

exclusion from most tissues, but especially from fat, bone and skin. This may partly be understood in terms of lipid:water partition coefficients, but probably also relates to the relative blood flow in the different tissues. At both 20 and 60 min equilibrium was not achieved between blood MISO concentrations and that remaining within the peritoneum.

No clear explanation has been found for the difference in sensitivity between the two strains, or the increased sensitivity with size in CBA mice even when the drug blood levels have been taken

Table 4. Distribution of radiolabelled MISO in WHT mice

Mouse body wt (g)	26.3 ± 0.6 g		36.0 ± 1.5 g		49.2 ± 1.0 g	
Blood	20 min	60 min	20 min	60 min	20 min	60 min
(mg equivalent of miso)	2.12 ± 0.3	1.46 ± 0.7	1.80 ± 0.3	1.48 ± 0.1	2.37 ± 0.7	2.19 ± 0.2
MISO concentration in tissue as a percentage of that in blood						
Peritoneal fluid	141*	135*	134*	117*	182	103
Liver	94	106	99	103	83	82
Muscle	71	88	82	91	62	73
Kidney	99	143*	129	136	119	105
Brain	68	85	76	83	55	69
Intestine	89	111	95	110	67	101
Heart	78	99	92*	97	77	82
Lung	84	97	88	95	83*	81
Bone (Skull)	44	57	46	45	46	41
Skin	50	71	53	69	38	47
Fat (neck)	29	43	27	32	13*	16*
Fat (abdominal)	20*	27*	14	11	10	9

Errors ± 1 S.E.M.
*Errors on mean value ≥ 25%.

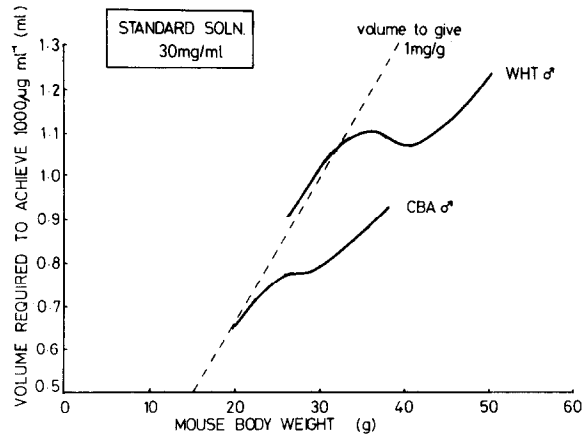

Fig. 5. Dosage curves for CBA and WHT from which the dose needed to give a constant blood concentration can be read off. This demonstrates the error of giving a volume based simply on body weight.

into account. Mice increase in body weight with age and the older (5 months) animals used in this study were heavier than those treated at 2 months of age. The possibility of some age-related change in response cannot, therefore, be discounted.

The importance of these findings for clinical application of MISO as a radiosensitizer is not clear. It has been shown in the clinic [4] that neurotoxicity in man is related to peak blood MISO concentrations rather than to dosing on a mg/kg or mg/m^2 basis. For the continued use of MISO as an experimental tool in studying hypoxia in tumours it would clearly be more appropriate to dose animals from a standard curve rather than on a simple body weight basis. Figure 5 shows the standard curves for our two strains of mice. To achieve a uniform blood peak concentration the administered dose would decrease, but differently for the two strains. Over the normal weight range (30-40 g) for WHT male mice there would be less variation in blood level if a constant volume of 1.1 ml was administered rather than a dose based on body weight. Ideally, mice of a narrow weight range should be used for all experiments. If the dose used is critical, then a curve like those in Fig. 5 should be established for each colony of mice.

The variation in serum level with weight and strain within a single species highlights the need for extensive pharmacokinetic back-up in any experiment involving drug administration [9]. This has been demonstrated here for misonidazole but may equally well apply to other cancer chemotherapy drugs in which a similar small margin exists between toxicity and a therapeutic dose.

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