Biochemical Pharmacology, 1966, Vol. 15, pp. 249–254. Pergamon Press Ltd., Printed in Great Britain.

RESEARCHES ON THE INCREASE OF LIVER WEIGHT PRODUCED BY SOME DRUGS*

B. SILVESTRINI, B. CATANESE and P. DEL BASSO

Pharmacological Department A.C.R. Angelini Francesco, Rome

(*Received* 5 *July* 1965; *accepted* 25 *August* 1965)

Abstract—Chlorpromazine, phenylbutazone, SKF 525-A, benzydamine and CCl_4 produce in mice, after a weekly treatment, a liver weight increase. At the same time CCl_4 increases the sleeping time induced by hexobarbital, and the retention of BSP, while the other drugs produce the opposite effects. These actions were generally already evident 24 hr after a single administration, while 40 min after this administration CCl_4 produced inconstant effects and the other drugs increase the BSP retention, or the sleeping time, or both. The results obtained are discussed in consideration of the available data regarding the enzymatic induction produced by drugs.

INTRODUCTION

THE EXPERIMENTS reported in this paper have been suggested by the observation that benzydamine, a recently described analgesic anti-inflammatory drug,[1] produced in the mice and rats, following chronic treatments, an increase in liver weight unconnected with degenerative nor with proliferative changes that could be demonstrated histologically.[2] The bromosulphtalein (BSP) test was used, which after successive improvements introduced in the original method proposed by Rosenthal and White[3] has now become an excellent diagnostic test in hepatic pathology both in men and animals.[4–7] The duration of sleep produced by a barbiturate was also investigated as suggested by Kutob and Plaa[8] so as to determine the entity of liver damage. Finally the body growth curve and weight variations of the liver were studied.

Benzydamine has been compared with CCl_4, a typical hepatotoxic agent and with some drugs which are known to increase the activity of several liver microsomal enzymes. This action, which has been reported for several drugs,[9–12] may be accompanied by an increase in liver weight and in total liver proteins.[13, 14] Therefore, our working hypothesis was that even liver weight increase produced by benzydamine could be interpreted in the same way.

MATERIALS AND METHODS

CF 1 mice of both sexes and weighing between 17 and 23 g, and Long Evans rats weighing between 65 and 75 g were used.

In acute experiments the compounds were administered only once via a stomach tube and the tests were carried out 40 min or 24 hr later. In chronic experiments the compounds were administered in the diet (with the exception of CCl_4 which was given by a stomach tube), for 7 or 50 days. The tests were carried out at the end of the

* A preliminary report of this research was presented at the XIIIth meeting of the Italian Society of Pharmacology (Palermo, April 22, 23 and 24, 1965).

experiments, after the animals had been left without any treatment for 16 hr. The quantity of drugs administered was calculated on the basis of daily food intake, which was approximately 15 per cent of body weight in mice and 10 per cent in rats.

The retention of BSP was investigated by administering 100 mg/kg of the product i.v. and then determining the plasma levels according to Casals and Olitsky's method.[5] A series of preliminary investigations was undertaken to measure the BSP retention 15 min after administration, since this lapse of time made it possible to demonstrate both the positive and the negative effects. Blood was taken from animals killed by severing of the jugular vein and carotid artery.

Effects on sleeping time were investigated by injecting sodium hexobarbital (100 mg/kg i.p.) to animals kept at a room temperature (23–24°). The recovery of righting reflex was considered as the end point of narcosis.

Aqueous solutions of the compounds were used, excepting CCl_4 and BSP, which were dissolved in olive oil and in a 0·9% saline solution respectively. Controls and treated animals received a quantity of liquid corresponding to 10 ml/kg. Doses reported refer to the salt used. Statistical evaluation of the results was carried out according to the Student's *t* method.

RESULTS

Benzydamine was previously shown to produce a liver weight increase following six months treatments in mice and rats.[2] This length of time appeared too long to permit an easy reconfirmation and an extention of this result to other drugs. We therefore primarily wanted to establish whether the liver weight would also increase following shorter treatments. Results obtained in mice and rats treated with benzydamine for 50 days have been summarized in Table 1.

TABLE 1. EFFECT OF BENZYDAMINE IN MICE AND RATS TREATED FOR 50 DAYS

Treatments and concentrations in the diet	Animal species and sex	Body weight			Liver weight		BSP retention		Hexobarbital sleeping time	
		No. of animals	Beginning	End	No. of animals	g%	No. of animals	mg%	No. of animals	Minutes
Benzydamine 0·1%	mice ♂	20	11·9	29·6 (1)	10	7·6 (1)	10	2·7 (1)	10	16·1 (1)
Controls	mice ♂	20	12·1	32·9	10	5·79	10	9	10	43
Benzydamine 0·1%	mice ♀	20	11·9	24·7	10	7·7 (1)	10	2·5 (1)	10	10 (1)
Controls	mice ♀	20	12·1	25·8	10	5·3	10	6·2	10	36
Benzydamine 0·5%	rats ♂	20	69·6	222 (1)	10	5·7 (1)	10	4 (2)	10	9 (1)
Controls	rats ♂	20	69·5	271	10	4·3	10	9·2	10	16
Benzydamine 0·5%	rats ♀	20	70·1	180 (1)	10	6·1 (1)	10	2·2 (1)	10	17 (1)
Controls	rats ♀	20	70·1	216	10	4·1	10	10·4	10	89·6

(1) $P < 0.01$.
(2) $P < 0.02$.

The effects on liver weight have been confirmed. Moreover the following effects have been observed: an inhibition of body growth in male mice and in rats of both sexes; a lower BSP retention; a reduced sensitivity to hexobarbital. The effective concentrations of benzydamine in the diet confirm previous observations, indicating that mice are more sensitive to this drug than rats are.

A second set of experiments was thereafter carried out to verify whether these effects occurred even following shorter treatments. Different drugs have been compared in mice. The results obtained are summarized in Table 2.

TABLE 2. EFFECTS OF DIFFERENT COMPOUNDS IN MALE MICE TREATED FOR 7 DAYS

Treatments and concentrations in the diet or daily doses (mg/kg per os)	Body weight			Liver weight		BSP retention		Hexobarbital sleeping time	
	Number of animals	Beginning	End	Number of animals	g%	Number of animals	mg%	Number of animals	min
Benzydamine 0·1%	30	17·7	23	20	8 (2)	10	2·74 (2)	20	10·6 (1)
Controls	30	17·7	24	20	6·7	10	5·2	20	36·9
Benzydamine 0·03%	20	19·5	21	20	6·3	10	7·8	10	24·1 (1)
Controls	20	19·5	22·7	20	6·4	10	7·3	10	51·8
Phenylbutazone 0·1%	15	17·9	23·3	10	7·1	5	4·6	10	23·6 (1)
Controls	15	17·8	22·4	10	7	5	4·2	10	40·7
Phenylbutazone 0·3%	30	17·6	20·7	30	7·2 (2)	15	4·1 (2)	15	16·1 (1)
Controls	30	17·4	21·5	30	6·3	15	6·25	15	42·6
Chlorpromazine 0·1%	20	21·3	18·2 (1)	10	10·3 (1)	5	1·2 (1)	10	6·25 (1)
Controls	20	21·3	24·6	10	6·7	5	8·6	10	41·8
CCl_4 25 mg/kg per os	20	17·6	18 (1)	20	7 (1)	15	10	5	83·2 (1)
Controls	20	17·6	21	20	6	15	7	5	40·6
SKF 525-A 0·05%	30	19·5	21·4	30	8·33 (1)	10	2·46 (1)	20	5·7 (1)
Controls	30	19·5	21·8	30	6·52	10	4·49	20	38

(1) $P < 0.01$.
(2) $P < 0.05$

All the products were administered with the diet, with the exception of CCl_4, which was given by means of a stomach tube. Only chlorpromazine and CCl_4 produced a significant inhibition of body growth. All the drugs increased the liver weight. BSP retention and duration of hexobarbital induced sleeping time were inhibited by all the tested compounds, with the exception of CCl_4, which caused the opposite action. Results obtained with two different dosage levels of benzydamine and phenylbutazone indicate the existence of a dose/effect relationship.

Table 3 shows the results obtained investigating BSP retention and the length of sleep induced by hexobarbital in mice treated 24 hr previously with the products under evaluation.

Under these experimental conditions, BSP retention was inhibited by phenylbutazone at a dose of 200 mg/kg, while CCl_4 increases it in a very marked manner even

TABLE 3. EFFECTS OF DIFFERENT COMPOUNDS RECORDED IN MALE MICE 24 HOURS AFTER A SINGLE ADMINISTRATION

Compounds	Doses (mg/kg)	AFTER A SINGLE ADMINISTRATION BSP retention		Hexobarbital sleeping time	
		No. of animals	mg%	No. of animals	min
Benzydamine	100	10	4·7	10	13·6 (2)
Controls	/	10	4·9	10	47·3
Benzydamine	25	10	4·2	10	35 (2)
Controls	/	10	3·9	10	65·2
Benzydamine	12·5	/	/	10	30·4
Controls	/	/	/	10	27·3
Phenylbutazone	200	30	3·25 (1)	15	15 (2)
Controls	/	30	4·77	15	47·3
Phenylbutazone	50	15	3·8	10	28·2 (2)
Controls	/	15	4·1	10	65·2
Phenylbutazone	25	5	3·56	5	47·6
Controls	/	5	4·64	5	64·6
Chlorpromazine	25	20	7·63	10	39·9
Controls	/	20	5·28	10	35·9
SKF 525-A	10	5	4·41	10	42·8 (2)
Controls	/	5	4·64	10	64·3
SKF 525-A	5	10	5·52	10	46·8 (2)
Controls	/	10	4·19	10	65·2
CCl_4	200	5	30 (1)	5	200 (1)
Controls	/	5	3·2	5	46
CCl_4	100	15	16·2 (1)	5	192 (1)
Controls	/	15	4·3	5	47·3
CCl_4	50	10	23·1 (1)	5	183 (1)
Controls	/	10	5·2 (1)	5	45·4
CCl_4	25	5	20·28 (1)	5	49·6
Controls	/	5	6·4	5	48

(1) $P < 0.01$.
(2) $P < 0.05$

at low doses. The length of sleep induced by hexobarbital was increased by CCl_4 and was shortened by benzydamine, phenylbutazone and SKF 525-A. Chlorpromazine produced inconstant effects. This last substance even shortens the length of sleep when the trial is carried out 48 hr after administration.

Table 4 shows the results obtained studying BSP retention and sleeping time 40 min after the administration of the drugs.

Benzydamine, CCl_4, large doses of phenylbutazone and even very small doses of chlorpromazine, increase BSP retention, whereas large doses of SKF 525-A had an inhibiting effect. Length of sleep was, on the other hand, considerably increased in the animals treated with phenylbutazone, chlorpromazine, and SKF 525-A, whereas CCl_4 and benzydamine proved to be inactive.

DISCUSSION

Results obtained show that short-term treatments with benzydamine produce in mice a liver weight increase quite similar to the effect previously observed in mice and rats following longer treatments. Therefore the one week experiment has been used to study this phenomenon in details and to carry out a comparative evaluation of different drugs.

TABLE 4. EFFECTS OF DIFFERENT COMPOUNDS RECORDED IN MALE MICE 40 MIN AFTER A SINGLE ADMINISTRATION

Compounds	Doses mg/kg	BSP retention			Hexobarbital sleeping time		
		No. of animals	mg%		No. of animals	minutes	
Benzydamine	100	24	15·9	(1)	30	45·9	
Controls	/	24	8·3		30	45·9	
Benzydamine	50	30	9·6		10	37·3	
Controls	/	30	10·3		10	37·3	
Phenylbutazone	200	25	19·1	(2)	10	88	(1)
Controls	/	25	14·7		10	45·3	
Phenylbutazone	100	10	6·4		15	83·6	(1)
Controls	/	10	5·3		15	43·8	
Phenylbutazone	50	10	10		10	58·8	(1)
Controls	/	10	11		10	32·2	
Phenylbutazone	25	10	5·8		10	93·4	(1)
Controls	/	10	5·8		10	57·4	
Chlorpromazine	25	15	21·3	(1)	10	180	(1)
Controls	/	15	8·4		10	59	
Chlorpromazine	12·5	5	13·4	(1)	10	147	(1)
Controls	/	5	6·9		10	49·6	
Chlorpromazine	6·25	5	9·13	(1)	5	73	(1)
Controls	/	5	4·13		5	32·2	
Chlorpromazine	3·1	10	5·8		10	73·1	(2)
Controls	/	10	5·9		10	38·5	
SKF 525-A	25	10	3·4	(3)	5	166	(1)
Controls	/	10	6		5	26·6	
SKF 525-A	10	10	9·7		10	169·6	(1)
Controls	/	10	10·3		10	38·4	
SKF 525-A	5	5	6·11		5	158·6	(1)
Controls	/	5	5·8		5	63·4	
SKF 525-A	2·5	5	7·1		5	49·2	
Controls	/	5	5·8		5	32·2	
CCl_4	200	/	/		10	66·5	
Controls	/	/	/		10	50·9	
CCl_4	100	10	20·7	(3)	15	61·6	
Controls	/	10	8·9		15	53·2	
CCl_4	50	18	10·9		5	59·6	
Controls	/	18	9·4		5	41·2	

(1) $P < 0{\cdot}01$.
(2) $P < 0{,}02$.
(3) $P < 0{\cdot}05$

It has been observed that the liver weight increase produced by benzydamine is accompanied by a more rapid BSP clearance and by a reduced sensitivity to hexobarbital. The last two effects are in evidence 24 hr after a single administration, whereas 40 min after the single administration no effects or opposite effects are observed. A similar behaviour has been recorded with phenylbutazone, chlorpromazine and SKF 525-A.

During the last few years it has been reported that the above mentioned drugs and many others as well, may produce an induction of microsomial enzymes, which in turn activates the breakdown of the "inducer" or of quite different compounds.[9, 11] This effect often follows a brief stage of enzymatic inhibition.[12]

Our experiments show that even BSP clearance may be affected in the same way by some drugs. Whether this effect is related to an involvement of the aspecific secretory

function of the liver, or to some more specific actions on enzymatic systems deputized to the BSP detoxication,[15, 16], is not quite clear. Experiments are in program aiming at verifying this point.

Anyway it may be advanced the hypothesis that even the liver weight increase produced by benzydamine is related to an enzymatic induction and not to a toxic effect.

This hypothesis is further endorsed by the results obtained while studying CCl_4, which is considered a typical hepatotoxic agent. As a matter of fact the liver weight increase produced by this compound is accompanied by quite opposite effects on BSP clearance and hexobarbital response. Results obtained suggest some general consideration.

Firstly the phenomenon of enzymatic induction which appears to be quite common among therapeutic agents, should be taken into consideration in the evaluation of chronic toxicity results. Effects on BSP retention and on sleeping time which were used by Kutob and Plaa[8] to evaluate the hepatotoxicity of some compounds, show that these tests may be affected in a quite peculiar way by some pharmacological treatments.

Secondly, it should always be kept in mind that many drugs may produce, besides the specific pharmacological effects, an enzymatic induction, which is less specific and long lasting.

A few examples show that some drugs may stimulate microsomial enzymes even in men, but the general importance of this phenomenon, in the common range of therapeutic doses, is yet to be explored. The results obtained on animals by acute and chronic experiments need therefore to be always compared and evaluated with extreme care.

Addendum—After this manuscript was prepared, we read the paper by Fujimoto *et al.* [*Biochem. Pharmac.* **14**, 515 (1965)] reporting results which are in agreement with ours.

REFERENCES

1. B. SILVESTRINI, In *Non Steroidal Anti-Inflammatory Drugs*, An International Symposium. International Congress Series n.82, p. 180. Excerpta Medica Foundation, Amsterdam.
2. B. SILVESTRINI, P. SCORZA BARCELLONA, B. CATANESE and E. A. GARAU, *Exptl. Mol. Path.* in press (1966).
3. S. M. ROSENTHAL and E. C. WHITE, *J. Am. Med. Ass.* **84**, 1112 (1925).
4. V. A. DRILL and A. C. IVY, *J. clin. Investig.* **23**, 209 (1944).
5. J. CASALS and P. K. OLITSKY, *Proc. Soc. exp. Biol.* **63**, 383 (1946).
6. C. W. WIRTZ, A. CANTOROW, W. J. SNAPE and B. DESELRONE, *Am. J. Physiol.* **165**, 680 (1951).
7. G. C. LINDER, R. G. F. PARKER and G. SELZER, *Brit. J. exp. Path.* **34**, 650 (1953).
8. S. D. KUTOB and G. L. PLAA, *Toxicol. appl. Pharmac.* **4**, 354 (1962).
9. A. H. CONNEY and J. J. BURNS, *Advances in Pharmacology*, Vol. 1, p. 31. Academic Press, New York (1962).
10. H. REMMER, Metabolic Factors Controlling Duration of Drug Action, *Proceedings First International Pharmacological Meeting*, Vol. 6, p. 235. Pergamon Press, London and MacMillan, New York (1962).
11. H. REMMER, *Some Factors Affecting Drug Toxicity*. International Congress Series n.81. 4, 57. Excerpta Medica Foundation, Amsterdam (1964).
12. R. KATO, E. CHIESARA and P. VASSANELLI, *Biochem. Pharmac.* **13**, 69 (1964).
13. A. H. CONNEY, G. DAVISON, R. GASTEL and J. J. BURNS, *J. Pharmac. exp. Ther.* **1**, 130 (1960).
14. H. REMMER and H. J. MERKER, *Klin. Wschr.* **41**, 276 (1963).
15. J. BOOTH, E. BOYLAND and P. SIMS, *Biochem. J.* **79**, 516 (1961).
16. B. COMBES and G. S. STAKELUM, *J. clin. Investig.* **40**, 981 (1961).