ORT COMMUNICATION

HEPATOMEGALY : PARAMETER OF CHRONIC EXPERIMENTAL DRUG TOXICITY

NIRMAL SETHI, VIJAYA TALWALKAR AND S. K. MUKHERJEE

Central Drug Research Institute, Chattar Manzil Palace, Lucknow

Summary : Gross and microscopic study of livers of rats of either sex which received 10 different compounds, was undertaken. Eight of these compounds (naphthofuron; diphenyl sulphone-4: iso-thio-cyanate; 3-p-chlorphenoxy l-n-(3,4 di-methoxyphenyl piperazino-propanol-2); 1 (m-trifluoromethyl)-4-(3-nitro-4-pyridyl) piperazine; phenaquinn hydrochloride; 4′ [2-hydroxy-3 [1-(4-phenyl piperazine)-propoxy] propiophenone; *Paspalum scrobiculatum* and (phenacetin) induced hepatomegaly. Many of them induced significant alterations in liver and endothelial cell counts. With three compounds (naphthofuron; 1 (m-tri-fluromethyl)-4-(3-nitro-4-pyridyl)-piperazine and buciridin) the ratio of parenchymal to endothelial cells increased indicating a decreased vascularity. Prolonged administration of 3 compounds did cause hepatomegaly without gross microscopic pathological change.

Key words : hepatomegaly drugs chronic toxicity liver morphology

INTRODUCTION

In chronic toxicity study of new drugs structural and functional changes in the liver of importance because liver subserves many important metabolic functions including g biotransformation.

In such studies, hepatomegaly is often interpreted as a manifestation of drug icity (7) though it can occur without alteration of the liver histology (1, 2, 3, 4). periments on rats with phenobarbitone (6) and a hypolipidaemic agent (5) suggest that r enlargement as such cannot be, in every case, due to direct drug toxicity. In this nnection, we report here our findings on 10 compounds.

MATERIALS AND METHODS

Ten compounds were used. Groups of rats (50—60 *g*, both sexes) received fractions or ltiples of LD_{50} or ED_{50} as daily doses. The effective and lethal doses were determined the Pharmacology Section of this Institute. In every experiment a control group of about ual number of animals received only the vehicle. All compounds were given

orally except phenaquinn (an analeptic) and buciridin (a local anaesthetic) which were given *ip* and *sc* respectively. Animals received the compounds once a day for 90 days unless otherwise stated (Table I). All animals were maintained under uniform conditions throughout the experiment. They were observed for behaviour, appetite, activity and body weight. The animals were killed by ether inhalation and after gross observation the liver was weighed and its pieces were fixed in Bouin's fluid with buffered 10% formaldehyde solution. Histological sections of liver were stained with standard haematoxylin eosin stain.

TABLE I: Changes in liver weight and cellular constituents after prolonged administration of different compounds in rats.

Compound (1)	*Dose-multiple of E.D. 50 or L.D. 50 (No. of animals in each sex group)* (2)	*Liver weight as percent of the body weight ± S.E.(Sex)* (3)	*Liver cells/ H.P.F. ± S.E.* (4)	*Endothelial cells/ H.P.F. ± S.E.* (5)
1. Naphthofuron (antifertility agent)	X 2.5 (8)	4.0 ± 0.13 (M)** 4.1 ± 0.02 (F)	37.1 ± 1.68	7.3 ± 0.53 **
ED_{50}=10 *mg/kg*	Control, gum acacia (6)	2.9 ± 0.20 (M) 3.9 ± 0.15 (F)	40.1 ± 1.77	11.3 ± 0.63
2. Diphenyl sulphone-4, : 4-Di-iso-thic-cyanate	X 2.5 (9)	5.8 ± 0.23 (M)** 4.9 ± 0.09 (F)*	39.9 ± 1.16**	6.4 ± 0.59
(anthelminthics agent)	X 5 (9)	6.1 ± 0.23 (M)** 4.9 ± 0.14 (F)*	32.0 ± 1.64	6.6 ± 0.36
ED_{50}=100 *mg/kg*	Control, gum acacia (9)	4.3 ± 0.10 (M) 4.1 ± 0.24 (F)	32.3 ± 1.68	6.4 ± 0.58
3. 3-p-Chlorophenoxy l-n-(3,4 di =methoxy phenyl piperazinopropanol -2)	X 2.5 (2)	4.30 ± 0.12 (M)** 4.50 ± 0.13 (F)*	30.4 ± 1.00	7.0 ± 0.35
(anticonvulsant agent) ED_{50}=40 *mg/kg*	Control, gum acacia (6)	2.9 ± 0.20 (M) 3.9 ± 0.15 (F)	31.4 ± 1.93	6.4 ± 0.49
4. l(m-trifluoromethyl)-4-(3-nitro-4 pyridyl) piperazine	X 2.5 (9)	4.6 ± 0.09 (M)** 4.4 ± 0.18 (F)**	38.8 ± 1.07**	5.8 ± 0.18*
	X 5 (9)	4.1 ± 0.13 (M)** 4.9 ± 0.16 (F)**	28.5 ± 1.20	8.1 ± 0.31
(anticonvulsant agent) ED_{50}=12.6 *mg/kg*	Control, gum acacia (10)	3.1 ± 0.11 (M) 3.6 ± 0.10 (F)	28.3 ± 1.29	7.3 ± 0.53
5. Phenaquinn hydrochloride (new analeptic agent, under trial)	X 2.5 (8)	5.1 ± 0.18 (M)* 3.7 ± 0.16 (F)**	32.3 ± 1.62	7.0 ± 0.54

1	2	3	4	5
(administered for 15 days)]	X 5 (8)	6.2±0.13 (M)** 4.9±0.15 (F)**	30.0±1.26	7.3±0.62
	X 20(8)	6.5±0.24 (M)** 6.4±0.26 (F)*	32.5±1.61	7.5±0.64
ED_{50}=10 *mg/kg*	Control, normal saline (8)	4.5±0.12 (M) 5.6±0.16 (F)	32.4±1.66	6.3±0.83
4′ [2-hydroxy-3(-1-(4-phenyl piperazine) propoxy] propiophenone (antidepressant agent)	X 2.5 (5)	3.5±0.26 (M) 3.9±0.22 (F)	27.9±1.53	6.5±0.61
	X 5 (6)	4.2±0.15 (M)*	29.9±1.88	6.0±0.22
ED_{50}=20 *mg/kg*	X 15(6)	3.4±0.08 (M) 4.2±0.27 (F)*	29.9±1.87	6.7±0.52
	Control, gum acacia (8)	3.3±0.47 (M) 3.4±0.10 (F)	32.2±2.13	6.5±0.53
Paspalum scrobiculatum dried ethanol extract (tranquillizer)	2.5 (6)	4.1±0.19 (M)* 4.1±0.17 (F)*	26.2±1.48	7.4±0.49
	5 (4)	4.3±0.27 (M)** 4.1±0.16 (F)**	28.2±1.63	7.6±0.32*
ED_{50}=41.6 *mg/kg*	Control, gum acacia (5)	3.4±0.18 (M) 3.3±0.13 (F)	29.1±1.61	6.7±0.21
Phenacetin U.S.S.R.P.	X 2.5 (4)	4.1±0.23 (M) 4.8±0.32 (F)**	24.8±1.07*	6.4±0.89
Phenacetin B.P.	X 2.5 (4)	4.2±0.38 (M) 4.3±0.22 (F)*	25.6±0.98*	7.1±0.52
ED_{50}—24 *mg/kg*	Control, distilled water (4)	3.6±0.12 (M) 3.6±0.21 (F)	29.7±1.26	6.4±0.41
Buciridin (new local anaesthetic under trial) (administered for 30 days)	X 0.1 (9)	2.9±0.12 (M)** 3.4±0.11 (F)	30.7±1.28	7.1±0.57**
	X 0.01 (10)	3.3±0.07 (M)** 4.1±0.10 (F)	32.4±1.68	6.8±0.31**
LD_{50}=45 *mg/kg*	Control, distilled water (10)	4.8±0.33 (M)	31.2±1.28	11.3±0.87
3 ethyl-8, methyl-1, 3,8-triazali-cyclo (4,n,o,)-decan-2-one	X 2.5 (9)	3.1±0.14 (M) 3.2±0.18 (F)	30.9±1.15	11.3±0.83 **
	X 5 (9)	2.9±0.11 (M) 3.5±0.12 (F)	28.6±1.64	7.4±0.61*
ED_{50}=1 *mg/kg*	Control, distilled water (16)	3.3±0.47 (M)	32.2±1.67	5.8±0.31

H.P.F.=high power field under the microscope, M=male, F=female, S.E.=standard error.

**=P<0.01
*=P<0.05

One liver slide of each animal was studied. The parenchymatous and endothelial cells in 25 high power (x430) fields were counted. Average cell counts per field of the individual animals (both sexes together) of every group were calculated (7).

RESULTS

The data are are shown in Table I. The first 8 of the 10 compounds induced statistically significant hepatomegaly as judged by the liver weights. The percent increase in liver weights varied from 3 to 50 ($P = <0.05$ to <0.01 by "t"—test) as compared to the control weights. Compound numbers 5 and 9 significantly reduced liver weights in some groups. Apparently the dose of the drugs did not influence the degree of hepatomegaly. Compound numbers 2 and 4 significantly increased and 8 decreased the number of liver cells. Compound numbers 1, 4 and 9 significantly decreased while 7 and 10 increased the endothelial cells.

DISCUSSION

This work demonstrates that in the chronic feeding study of some compounds (numbers 3, 5 and 6 in Table I), liver enlargement can occur as a common denominator without gross morphological change or hyperplasia; with other drugs it is often accompanied by alteration of hepatic and endothelial cell counts. In such cases it is likely that the liver enlargement is a compensatory mechanism. The ratio of parenchymal to endothelial cells indicates the state of vascularity of the parenchyma. The capillary space increases by division of the endothelial cells (7). When thei ncreases (compound numbers 1, 2, 4 and 9 in Table I) it indicates poorer blood supply to the parenchyma which is undesirable. Increase in the number of liver cells per in field drug-treated animals indicates slight decrease in the size of the individual liver cells. It cannot be categorically stated that drug-induced hepatomegaly is harmless in every case; one way to investigate the issue would be to see how animals with larger livers of normal histological appearance react to the stress of shock, infection, surgery and drugs. Further work on this line is in progress.

ACKNOWLEDGEMENT

Dr. S.T. Gogte, Department of Pharmacology, All-India Institute of Medical Sciences, New Delhi-16, kindly helped in the preparation of the manuscript.

REFERENCES

1. Arcos, J.C., A.H. Conney and N.P. Buu-Hoi. Induction of microsomal enzyme synthesis by polycyclic aromatic hydrocarbons of different molecular size. **J. *Biol. Chem.*, 236** : 1291-1296, 1961.

2. Conney, A.H. and J.J. Burns. Advances in Pharmacology, *Academic Press Inc.*, New York & London, 1962, Vol. 1, p. 31-58.

Fouts, J.R. and L.A. Rogers. Morphological changes in the liver accompanying stimulation of microsomal drug metabolizing enzyme activity by phenobarbital, chlordane, benzpyrene and methylcholanthrene in rats. *J. Pharmac. Exp. Ther.*, **147** : 112-119, 1965.

Hart, L.G. and J.R. Fouts. Studies of the possible mechanism by which chlordane stimulates hepatic microsomal drug metabolism in the rat. *Biochem. Pharmac.*, **14** : 263-272, 1965.

Hess, R., W. Staubli and W. Riess. Nature of the hepatomegalic effect produced by ethyl-chlorophenoxy-isobutyrate in the rat. *Nature (Lond.)*, **208** : 856-858, 1965.

Kato, R., G. Frontino and P. Vassanelli. Phenobarbital markedly increases liver weight in walker carcinosarcoma bearing rats. *Experientia*, **19** : 30-31, 1963.

Kunz H.W., G. Schande, H. Schimassek, W. Schmid and M. Siess. Stimulation of liver growth by drugs. (i) Morphological analysis. (ii) Biochemical analysis. *Proceedings of the European Society for the Study of Drug Toxicity*, **7** : 113-153, 1966.