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SOME BIOCHEMICAL CHANGES ASSOCIATED WITH FENARIMOL-INDUCED LIVER GROWTH IN THE RAT

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Liver enlargement was observed in adult rats treated with single and repeated doses of fenarimol (250 and 125 mg/kg body weight per day). The initial response of the liver appeared to be primary hyperplasia followed by cellular hypertrophy.

Fenarimol (alpha-/2-chlorophenyl/-alpha-/4-chlorophenyl/-5-pirimidine-methanol) is widely used in agriculture as a synthetic fungicide. No published data on the mutagenic properties of fenarimol are available in the open literature, but its chemical structure, similar to DDT and some other organochlorine insecticides, appear to support a lack of mutagenic potential of this compound. However, our previous experiments have shown that repeated oral doses of 250 mg of fenarimol/kg body weight per day produce regenerative liver hyperplasia, resulting from hepatocyte injury in juvenile rats [13]. It should be emphasized that elevated rate of cell proliferation, induced by chronic cytotoxicity of non-genotoxic compounds, have been suggested by many authors to increase the tumorigenicity at the initiation [3, 11, 21] and/or promotion stage [5, 9, 18].

The present studies involved the measurement of DNA synthesis, total hepatic DNA and protein content in the liver of adult rats receiving single and repeated doses of 250 and 125 mg fenarimol/kg body weight per day, respectively. The effect of fenarimol on liver weight was also recorded.

MATERIALS AND METHODS

Fenarimol from the *Elanco Company* (98.8%); 6-/^{3}H/ thymidine, specific activity 1020 MBq/mmol, produced by the Institute for Research, Production and Use of Radioisotopes, Praque, Czechoslovakia.

Male *Wistar* rats of own breed were used. Prior to use, the rats were housed (5 animals per cage) at controlled temperature (22°C$\pm$1°C), relative humidity 50$\pm$10% and 12-h light cycle. When rat body weight approached 200–210 g, they were divided into 3 groups, and fenarimol was administered orally in oil suspension. In the first group rats were given at 1-days intervals, a single dose of 250 mg fenarimol/kg body weight per day on 1st, 3rd and 5th day of experiment. In the second group the dose of 125 mg/kg body weight per day was applied analogically. In the control groups, rats were adminitered an equal volume of olive oil. Each fenarimol and olive oil administration was performed between 8–9 a.m. After 1, 3 and 5 days from the first

fenarimol administration, animals of the all three groups were once interperitoneal injected /^{3}H/thymidine (1,2 MBq per rat) for estimation of DNA synthesis. After 1 h from thymidine administration (i.e. after 1, 3, and 5 days of experiment), animals of all three groups were sacrified. Livers were immediately removed, liberated from blood and weighted. Samples of liver tissue were used immediately for biochemical determination.

A sample of liver tissue (1g) was taken from right lobe, suspended in cold 0.25 M sucrose solution and homogenized. An aloquot of the homogenate was diluted with water and the concentration of protein was determined by the *Lowry* at al. method [8] using bovine serum albumin as reference standard. The isolation of nuclear DNA and radioactivity measurements were performed as previously described [13]. DNA was determined by *Burton's* method [2].

RESULTS

The relative liver weight – RLW – (liver weight/body weight × 100) did not increase significantly after the single dose of 250 and 125 mg fenarimol/kg body per day. RLW reached a maximum after three doses of 125 mg fenarimol/kg body weight per day (a 1.3-fold increase in RLW), and after five doses there was no further rise of RLW (a 1.3-fold increase). In rats receiving three and five doses of 250 mg/kg body weight per day the increase in RLW was 1.4-fold ($P<0,01$) and 1.8-fold ($P<0,01$), respectively, as compared with control (Fig. 1A and 1 B).

DNA synthesis was measured by means of /^{3}H/thymidine incorporation into nuclear DNA which accounts for 99% of hepatic DNA. It was found that rate of utilization of /^{3}H/thymidine for DNA synthesis in rat liver increased significantly at 24 h after oral application of fenarimol in one single dose of 250 or 125 mg/kg body weight per day, respectively (Fig. 2A and 2 B). The increase in thymidine incorporation, as compared to control, was 4.1-fold ($P<0,01$) and 8.0-fold ($P<0,01$) respectively. Subsequently, after three and five doses of fenarimol, respectively, DNA synthesis decreased. Nevertheless, the difference between the group receiving 250 mg of fenarimol/kg for 3 and 5 days and control animals, was still significant. In the case of the dose of 125 mg/kg body weight per day, the above difference returned to control level after 5 days.

The concentration of nuclear DNA, expressed as mg DNA/g of liver decreased owing to considerable enlargement of the organ after repeated doses of fenarimol. For this reason the content of DNA was calculated and expressed as total hepatic DNA (mg DNA/ total liver weight).

One single dose of 250 or 125 mg of fenarimol/kg body weight did not result after 24 h from administration in a significant increase in total hepatic DNA content. A 1.4-fold ($P<0,01$) and 1.2-fold ($P<0,05$) increase in total hepatic DNA was observed after five single doses of 250 and 125 mg fenarimol/kg body weight per day, respectively (Fig. 2A and 2B).

It was found that repeated administration of fenarimol resulted in a significant increase in protein content expressed as grams per total liver weight (Table 1).

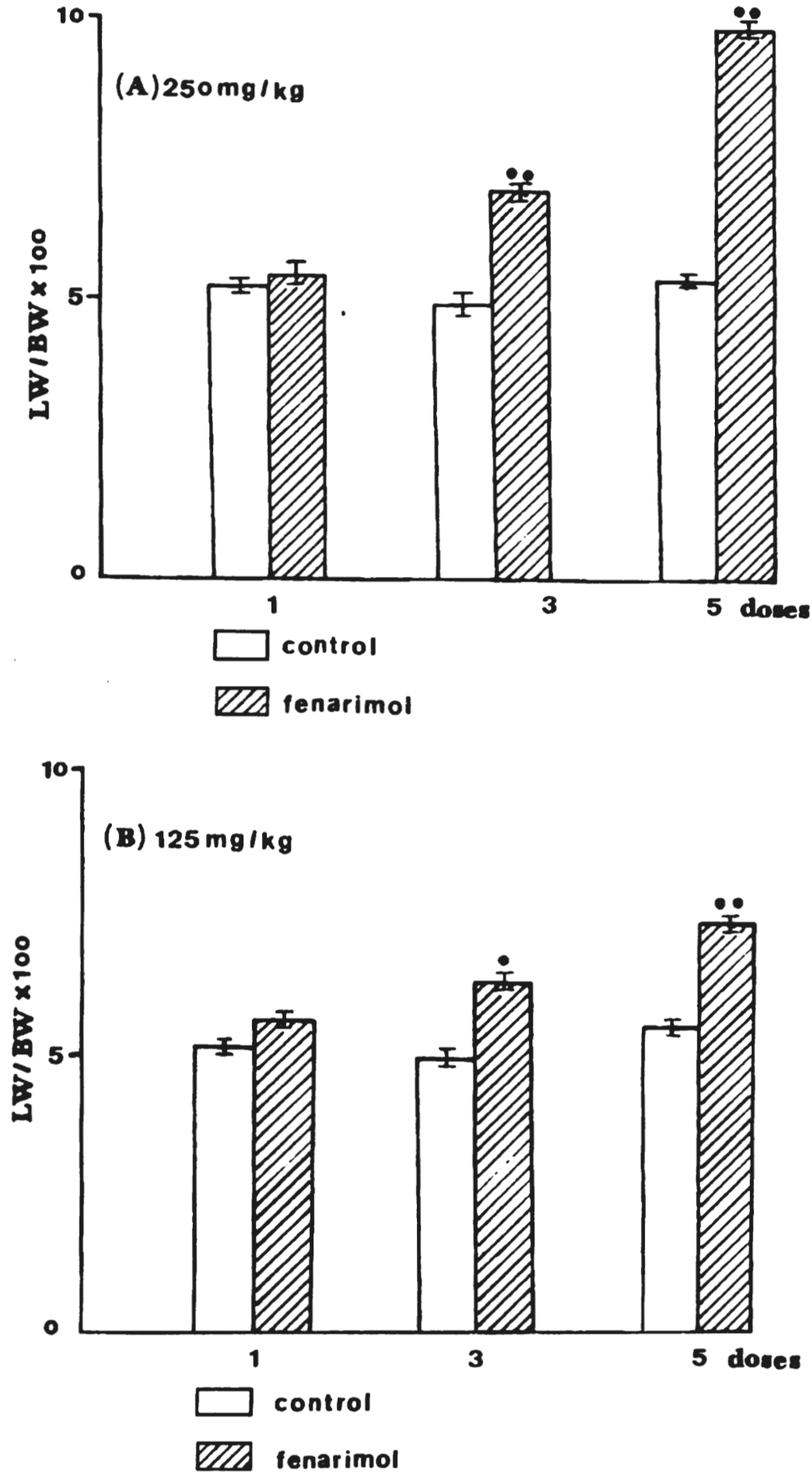

Fig. 1. Relative liver weight (RLW) in rats, following single or repeated administration of fenarimol. (A) 250 mg/kg per day; (B) 125 mg/kg per day. Means for five animals ±SEM
. – $P < 0.05$, .. – $P < 0.01$

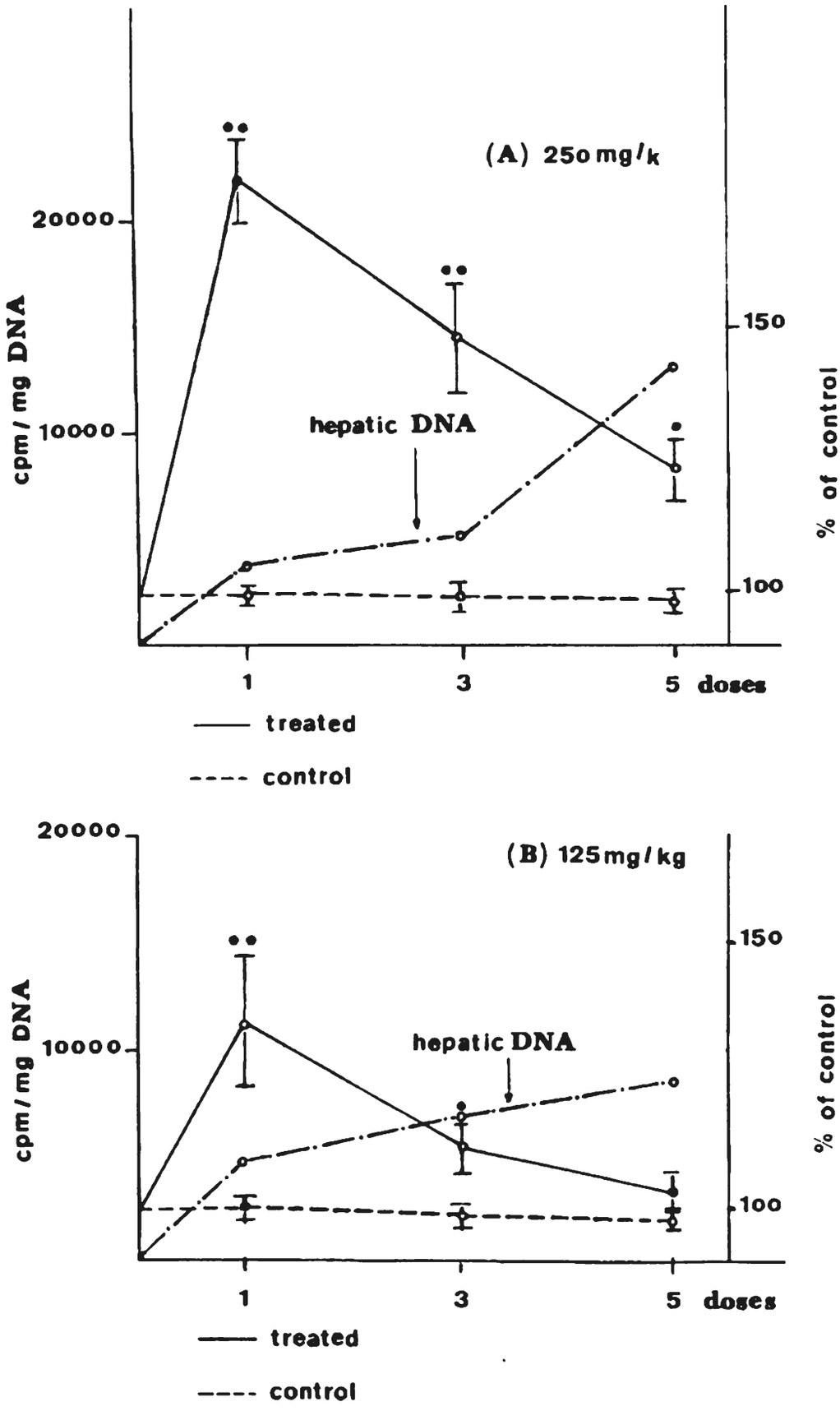

Fig. 2. The synthesis of DNA and content of total hepatic DNA in rat liver, following single or repeated administration of fenarimol (A) 250 mg/kg per day; (B) 125 mg/kg per day. Means for five animals ± SEM. Total hepatic DNA content, calculated against control taken as 100%, amounted to 26,02 ± 1,46; 17,83 ± o,43 and 18,85 ± 1,42 mg/total liver weight, after 1, 3 and 5 doeses, repectively. . – $P < 0.05$, .. – $P < 0.01$.

Tabela I. Protein content in rat liver, following repeated administration of 250 and 125 mg fenarimol/kg body weight per day, respectively

Experimental condition	Protein content g/total liver weight [1]	Percent of control
250 mg/kg 3[2]	2.09±0.03[3]	140
125 mg/kg 3	1.97±0.04[3]	132
250 mg/kg 5	2.59±0.02[3]	174
125 mg/kg 5	1.89±0.10[4]	127
Control	1.49±0.09	

1) Means for five animals ±SEM
2) Number of consecutive doses
3) $P<0.01$
4) $P<0.05$

DISCUSSION

The hepatic response to the administration of fenarimol is of particular interest, since this fungicide is a structural analoque of some phenobarbital-type of smooth endoplasmatic reticulum (SER) proliferators, which are regarded as non-genotoxic hepatocarcinogens with putative tumor-promoting activity [4, 20]. Conventionally non-genotoxic hepatocercinogens have been divided into three major classes of compounds, namely those which cause proliferation of SER and those which cause proliferation of peroximes and a minor class, namely compounds which induce hepatocyte necrosis and tissue regenaration [4]. There is, however, some doubt as to whether this classification reflects real differences in the mechanisms of carcinogenicity. Nevertheless, the liver enlargement by primary or regenerative hyperplasia and hypertrophy is a common feature associated with the action of these non-genotoxic carcinogens [4].

Fenarimol at 250 mg/kg per day has been found to lead to hepatomegaly, chronic hepatotoxicity and elevated rate of regenerative cell hyperplasia (increase in DNA synthesis and mitotic activity) in juvenile rats [13].

The present results indicate that under experimantal conditions repeated oral dose of 250 and 125 mg fenarimol/kg body weight per day produced liver enlargement which was asocciated with a wave of /^{3}H/thymidine uptake, at 24 h after one single dose. Subsequently, even though fenarimol treatment was continued, the DNA synthesis was supressed as soon as a new steady liver DNA level was reached. The peak of DNA synthesis in fenarimol-treated rats was indicator of hyperplastic response. There was significant increase in /^{3}H/thymidine incorporation into nuclear DNA and no significant increase in RLW. This early hyperplastic episode was evidently primarily responsible for the liver growth, that appeared within the first 3 days of administration of the compound. This later stage of hepatomegaly was mainly due to cellular hypertrophy involving an increase in RLW accompanied by a decrease

in DNA concentration (mg DNA/g of liver) and an increase in protein content (g protein/liver weight). This initial response of the liver to the administration of fenarimol appeared to be primary hyperplasia followed by cellular hypertrophy.

Althought numerous compounds have been shown to cause an increase in liver weight, it is importatnt to note that besides of some chlorinated hydrocarbons [19], none produced as great an increase in liver mass as we demonstrated with five doses of 250 mg fenarimol/kg body weight per day; apparently the maximal response was not yet reached at this time.

Primary hyperplasia (or cell proliferation without any initial cell death) has been observed upon treatment with phenobarbital [14], butylated hydroxytoluene [1, 16], cyproterone acetate [17], ethinyl-estradiol [12], peroxime proliferating agents such as clofibrate, fenofibrate, nafenopin, Wy-14,643 [7, 10, 15, 16] and other non-genotoxic hepatocarcinogens studies so far. The role of primary hyperplasia in hepatocarcinogenesis has not yet been clearly defined, although as *in vivo/in vitro* evidence suggests that non-genotoxic agents may enhance tumorigenesis by a direct proliferative action on initiated cells [6, 18]. Although hepatomegaly and mitogenesis are less defined indicators of carcinogenicity, but they occur in a sufficient number of liver-specific carcinogens [4] that their role as early indicators is worth of confirmed studies.

SUMMARY

Fenarimol administred in one single or repeated oral doses of 250 or 125 mg/kg body weight per day, respectively, stimulated rat liver enlargement which was associated with a wave of DNA synthesis on the first day after start of treatment. Subsequently, even though fenarimol treatment was continued, the DNA synthesis was supressed as soon as a new steady liver DNA level was reached. The early hyperplastic episode was evidently primary responsible for the liver growth that occured within the first 3 days of administration of compound. Liver enlergement achevied maximum growth by 3 or 5 days from first administration of 125 and 250 mg fenarimol/kg body weight per day, respectively.

This later stage of hepatomegaly was mainly due to cellular hypertrophy involving an increase in RLW accompanied by an increase in liver protein content. Hepatomegaly and DNA synthesis appeared to be threshold related.

D. Palut

BIOCHEMICZNE ZMIANY TOWARZYSZĄCE POWIĘKSZANIU WĄTROBY SZCZURÓW POD WPŁYWEM FENARIMOLU

Streszczenie

Fenarimol podawany szczurom jednorazowo oraz wielokrotnie w odstępach dobowych w dawkach wynoszących odpowiednio 250 i 125 mg/kg/dzień wywoływał powiększenie wątroby. Przerost wątroby poprzedzał wzrost syntezy DNA z maksimum przypadającym w 24 godz. po jednorazowym podaniu badanego związku. Podczas dalszej ekspozycji szczurów na fenarimol obserwowano stopniowy spadek syntezy DNA do wartości obserwowanych w grupach kontrolnych, natomiast zawartość całkowitego DNA w wątrobie wzrastała osiągając najwyższy poziom po podaniu ostatniej, piątej dawki fenarimolu wynoszącej 250 lub 125 mg/kg/dzień. Przejściowa hyperplazja związana była z powięk-

szeniem wątroby występującym po 3 dniach podawania związku. Masa wątroby osiągała maksymalną wartość po 3 lub 5 dniach podawania fenarimolu w ilościach wynoszących odpowiednio 125 lub 250 mg/kg/dzień. Powiększenie wątroby dokonywało się głównie na zasadzie komórkowej hypertrofii, ponieważ masa badanego narządu wzrastała proporcjonalnie do wzrostu zawartości całkowitego białka. Zarówno powiększenie wątroby jak i wzrost syntezy DNA wykazywały zależność dawka-odpowiedź.

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