MUTAGENIC POTENTIAL OF THE HYPOLIPIDEMIC DRUG CLOFIBRATE

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ABSTRACT

The mutagenic potential of clofibrate, a hypolipidemic drug, was assessed in human lymphocytes *in vitro* and in rat bone marrow cells *in vivo*. Chromosomal aberrations and sister chromatid exchanges (SCE) were analysed in human lymphocyte exposed to three different molar concentrations of the drug *in vitro*. Significant chromosomal aberrations were induced only at 10^{-4} M concentration. A slight increase in SCE was observed at all concentrations.

Analysis of bone marrow cells of rats exposed to clofibrate *in vivo*, failed to show any significant increase in the number of aberrant cells. Moreover, these changes were reversible on withdrawal of the drug, suggesting a possible promoter role for clofibrate in hepatocarcinogenesis.

INTRODUCTION

SEVERAL aryloxyisobutyrate derivatives such as clofibrate, developed as hypolipidemic drugs, are known to induce hepatocellular carcinomas in rats[1]. In contrast to conventional carcinogens, these compounds failed to induce mutations in the Salmonella microsome assay or produce DNA damage in the lymphocyte (^{3}H)-thymidine incorporation assay[2]. Furthermore, these agents do not interact covalently with DNA of target tissue[3,4]. However, very little is known about the induction of chromosomal aberrations or SCEs by clofibrate. Hence, we employed both these cytological end points to test the genotoxic potential of the drug *in vitro*. In addition, the *in vivo* effect was also assessed by analysing metaphase chromosomes from the bone marrow of drug-treated rats.

MATERIALS AND METHODS

In vitro studies

Heparinized blood samples from two normal subjects were cultured using the modified method of Hungerford[5]. The method for the differential staining of sister chromatids was adapted from Kulkarni *et al*[6]. Clofibrate was tested[7] at three different subtoxic molar concentrations (10^{-5}, 10^{-4} and 10^{-3}). The anticancer drug cis-Platin, was used as the positive control at a concentration of 1μg/ml (3×10^{-6} M), a concentration reported to induce the maximum number of aberrations and SCEs[8]. Dimethylsulphoxide (DMSO), at a final concentration of 0.2%, was used as the solvent. DMSO alone served as the negative control. Cultures were exposed to the drug for the entire culture period from the time of initiation.

In vivo studies

Bone marrow cells from 4–month–old male Wistar rats fed 0.5% clofibrate for 3 weeks, were processed[9] for chromosome analysis. Animals in the control group received basal diet without the drug.

Aberrations both *in vitro* and *in vivo*, were categorized[10] as chromosomal and chromatid type. Aberrations were classified into different groups: chromatid gap (G'), isochromatid gap (G''), chromatid break (B'), isochromatid break (B''), fragment (F), minute (M), double minute (DM), exchange figure (EF), pulverization (Pul) and multiple aberration (MA).

Standard statistical procedures were employed for the comparison of sample proportions and means[11].

RESULTS

Table 1 shows the induction of chromosomal aberrations by various concentrations of clofibrate added to human lymphocyte cultures *in vitro*. Data are presented with and without the inclusion of gaps. When gaps were included, clofibrate exhibited significant clastogenic activity at 10^{-4} M concentration. On exclusion of gaps, the clastogenic effect was more pronounced at 10^{-5} and 10^{-3} M concentrations. Aberrations induced by clofibrate were mostly gaps and breaks.

Clofibrate produced slight but significant increase in the means of SCE at all concentrations of the drug tested (table 2). However, there was no clear dose-related increase in the frequency of SCEs.

Table 1 *Chromosomal aberrations induced by clofibrate in human lymphocytes in vitro*

Treatment	No. of aberrant cells	% of aberrant cells		Types of aberrations							
		Including gaps	Excluding gaps	G'	G"	B'	B"	F	M	EF	MA	Pul
Solvent control	8	4.0	0	6	2	–	–	–	–	–	–	–
Positive control (3×10^{-6} M cis-Platin)	42	21.0**	15	12	–	1	6	8	–	13	1	1
Clofibrate												
10^{-5} M	10	5.0	2	5	1	4	–	–	–	–	–	–
10^{-4} M	21	10.5*	1	14	5	2	–	–	–	–	–	–
10^{-3} M	12	6.0*	3	4	2	3	–	2	1	–	–	–

Data represent analysis from 200 metaphase plates from duplicates of two donors; *Statistically significant difference from solvent controls $P < 0.05$; **Statistically significant difference from solvent controls $P < 0.001$; (Z test).

Table 2 *Frequency of SCE induced by clofibrate in human lymphocytes in vitro*

Treatment	No. of cells analysed	Total No. of exchanges	Mean SCE/Cell
Solvent control	80	508	6.3 ± 3.0
Positive control (3×10^{-6} M cis-Platin)	41	2615	63 ± 16.0**
Clofibrate			
10^{-5} M	64	593	9.4 ± 3.8*
10^{-4} M	50	444	8.8 ± 2.8*
10^{-3} M	50	520	10.4 ± 3.5*

Values are mean + SD from duplicates of two donors; * Statistically significant difference from solvent controls $P < 0.05$; ** Statistically significant difference from solvent controls $P < 0.001$; (Student's t test).

Table 3 *Chromosomal damages induced by clofibrate in rat bone marrow*

Treatment	No. of aberrant cells	% of aberrant cells		Types of aberrations				
		Including gaps	Excluding gaps	G'	G"	B'	B"	F
Control	7	1.75	0.00	7	–	–	–	–
Clofibrate	11	2.75	2.00	3	–	7	–	1
Drug withdrawn	10	2.50	0.75	7	–	2	–	1

Data represent analysis from 400 well-spread metaphases from each group. Two groups of 4 animals each received 0.5% clofibrate in the diet for 3 weeks. The drug was withdrawn from the diet 1 week prior to sacrifice in one group. A group of 4 animals receiving only basal diet was maintained as control.

Table 3 shows chromosomal aberrations in bone marrow of rats fed clofibrate. Aberrations induced by clofibrate were not significant when gaps were included. The percentage of aberrant cells excluding gaps, was however, higher in the clofibrate-fed group than in the controls. Similar to *in vitro* observations, aberrations induced *in vivo* were also mostly gaps and breaks.

To see whether clofibrate induced changes in bonemarrow were reversible, the drug was withdrawn from the diet for a week prior to sacrifice. The percentage of aberrant cells including gaps was

not significantly different from the drug-fed group. However, when gaps were excluded, the percentage of aberrant cells tended to return to control levels.

DISCUSSION

Clofibrate failed to show a dose-related increase in chromosomal aberrations in human lymphocyte cultures *in vitro*. Only weakly positive clastogenic activity was detected even in bone marrow cells of treated animals. Furthermore, these changes appeared to be reversible. Aberrations induced by clofibrate both *in vitro* and *in vivo*, were predominantly chromatid type, indicating its effect on G_2 phase of cell cycle.

Our data on SCE agree with the results of Linnainmaa[7], who reported a dose-independent but significant increase in SCE frequency in Chinese hamster ovary cells *in vitro*. The increase in SCE frequency was statistically significant, nonetheless clofibrate cannot qualify as a potent inducer of SCE, because it failed to induce a 2-fold increase over base line[12].

These studies suggest that clofibrate can at best be considered only as a weak mutagen, substantiating the observations of other workers[2-4] that hypolipidemic drugs do not damage DNA directly, unlike the majority of chemical carcinogens. The exact mechanism of hypolipidemic drug-induced hepatocarcinogenesis is still not clearly understood. These agents are known to cause sustained peroxisome proliferation resulting in excessive generation of H_2O_2 and other reactive oxygen species[13]. Production of reactive oxygen species is consistent with the tumor-promoting ability of these agents[14,15]. The present data also lend support to our biochemical findings that clofibrate acts more as a promoter than as an initiator in hepatocarcinogenesis[16].

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