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Species differences in the intracellular distribution of ciprofibroyl-CoA hydrolase. Implications for peroxisome proliferation

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Abstract Peroxisomal proliferators (HPP), such as ciprofibrate and clofibric acid, are species-specific drugs. Since HPP-coenzyme A derivatives might be involved in their action, we studied the subcellular distribution of liver ciprofibroyl-CoA hydrolase in rat and in two HPP-unresponsive species, humans and guinea pig. Total activity was similar in the three species and was not induced by clofibric acid treatment. In guinea pig, as in humans, the enzyme is localized in the mitochondrial and soluble fractions and no changes are observed after drug treatment. In the rat, the enzyme has a microsomal localization, but upon clofibric acid treatment it changes to a mitochondrial and soluble distribution, as in unresponsive species. These results raise the possibility that drug-induced hydrolases in rats might be normally expressed in humans and guinea pigs.

Key words: Peroxisome; Peroxisome proliferator; Acyl-CoA hydrolase; Ciprofibroyl-CoA; Species differences

1. Introduction

Peroxisomal proliferation in rats and mice is a well-documented phenomenon evoked by a group of diverse compounds collectively named hypolipidaemic peroxisomal proliferators (HPP; recently reviewed in [1]). HPP produce serious metabolic perturbations in the liver, and prolonged exposure generates hepatocarcinomas in experimental animals [2,3]. There are marked differences between species in the peroxisomal proliferation response: e.g. humans and the guinea pig are more resistant than rats and mice [4–6]. It is thought that HPP effects are mediated by a peroxisome proliferator-activated (PPAR) receptor [7,8]. Targeted disruption of the αPPAR isoform gene in mice results in abolishment of HPP-induced peroxisomal proliferation [9]. Isoforms of PPAR have been described in diverse species including human [7,10]. Mouse and human PPAR present a similar tissue distribution. However, the PPAR response toward HPP does not completely explain the different sensitivity of humans and mice to these xenobiotics [11]. Thus, other factors besides PPAR activation may determine the differential species response to HPP treatment. These factors could be events after PPAR activation, in the cross-talk between transcription factors inside the nucleus [12,13] or can be related to events prior to the formation of ligand-activated PPAR, for instance, the metabolic perturbation that may be induced by the formation of the CoASH thioester of HPP in liver cells [14–16]. The formation of such derivatives has been demonstrated for several HPP [14–19]; it is the first event that can be detected after drug treatment and results in the rapid depletion of the free CoASH content of the liver cell [15,16]. Since the steady-state level of HPP-CoAs might be important in determining species sensitivity to HPP, we studied the activity and subcellular distribution of HPP-CoA hydrolases in rat, human, and guinea pig liver, using the CoA derivative of ciprofibrate, a very active HPP, as substrate. The effect of clofibric acid treatment in the behavior of ciprofibroyl-CoA hydrolase was also evaluated in both rat and guinea pig liver.

2. Materials and methods

2.1. Materials

Ciprofibrate was kindly provided by Sterling-Winthrop Research Institute, NY, USA. Clofibric acid, CoASH, substrates and chemicals were purchased from Sigma Chemical Co., St. Louis, MO, USA.

2.2. Animals

Sprague-Dawley rats, weighing 180–200 g, were used. They were maintained on a standard laboratory chow diet. For ciprofibrate and clofibric acid treatment, rats were fed the same standard chow diet containing 30 mg and 3 g of the drug per kg of chow, respectively. Guinea pigs, weighing 1–1.5 kg, were used for experiments. They were maintained on a standard rabbit diet, supplemented with drug at the same concentration as the rat diet. After 14 days of treatment, animals were killed by decapitation and the liver immediately processed.

2.3. Liver biopsies

Liver biopsies were obtained from patients undergoing surgery for uncomplicated gall-stone or gastroduodenal ulcer disease. Informed consent from the patients was obtained by following the procedures approved by the Ethics Committee of the Medical School of the P. Universidad Católica de Chile.

2.4. Preparation of subcellular fractions

Tissue homogenates (20%, w/w) were prepared in 0.25 M sucrose containing 1 mM dithiothreitol and 3 mM imidazole-HCl, pH 7.4. Liver homogenates were fractionated by differential centrifugation into nuclear (N), mitochondrial (Mi), microsomal (P) and soluble (S) fractions as described [20]. The mitochondrial (Mi) fraction corresponds to the sum of the classical heavy (M) and light (L) mitochondrial fractions, that are sedimented together [20].

2.5. Ciprofibroyl-CoA hydrolase assay

Because of interference in the ciprofibroyl-CoA hydrolase spectrophotometric assay by a phosphatase capable of degrading ciprofibroyl-CoA to ciprofibroyldephospho-CoA (Urrea and Bronfman, unpublished results), the enzyme activity was estimated by directly measuring the thioester consumed and free drug formed by HPLC. Incubations were performed in the presence of phosphate, which inhibits the phosphatase activity. The reaction mixture contained 50 mM Tris-HCl (pH 8.0), 5 mM KH_2PO_4 (pH 8.0), 120 mM KCl and 25–50 μM of the drug-CoA thioester, in a final volume of 50 μl.

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Abbreviations: HPP, hypolipidaemic peroxisome proliferators; PPAR, peroxisomal-proliferator-activated receptor

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The samples were incubated at 35°C and the reaction was stopped by the addition of 200 µl of HPLC-grade methanol. After a brief centrifugation, the clear upper phase was used directly for analysis of the remaining ciprofibroyl-CoA thioester and the free acid formed. More than 95% of the free acid and the drug-CoA thioester was recovered under these conditions. HPLC separations were performed on an RP-18 (5 mm) column (LichroCart 125-4; Merck, Darmstadt, Germany), using as mobile phase 80 mM KH_2PO_4 buffer pH 5.5 containing 48% methanol. Under these conditions the retention time of ciprofibrate was 4.58 min, and that of ciprofibroyl-CoA 6.9 min.

2.6. Other assays

Established procedures described or referenced in [20] were used for the determination of protein and marker enzymes: glutamate dehydrogenase for mitochondria; NADPH-cytochrome *c* reductase for microsomal vesicles and fatty acyl-CoA oxidase for peroxisomes. Palmitoyl-CoA hydrolase was measured spectrophotometrically [20].

2.7. Statistics and calculations

For quantitative evaluation of the subcellular localization of enzymes, we used a computational method based on constrained linear-regression analysis that has been described and validated previously [20,21]. The statistical significance of the assignations was calculated by Student's *t*-test.

3. Results

3.1. Liver activity of ciprofibroyl-CoA and palmitoyl-CoA hydrolases

Table 1 shows the enzyme activities in the liver of control and drug-treated animals, and in human liver. No significant changes were observed in the liver of guinea pig treated with clofibric acid or with the more potent peroxisome proliferator ciprofibrate. Rat liver palmitoyl-CoA hydrolase was increased upon clofibric acid treatment, in agreement with published data [23–25], while fatty acyl-CoA oxidase, a positive control of peroxisomal proliferation, was induced 5-fold. As for guinea pigs, no increase in total liver ciprofibroyl-CoA hydrolase was observed in drug-treated rats. Rat liver contains 5–6-fold more palmitoyl-CoA hydrolase activity than guinea pig or human liver. However, no differences were found between rat and guinea pig for liver ciprofibroyl-CoA hydrolase activity, while human liver contains about half of the rat liver activity.

3.2. Subcellular distribution of hydrolases

The subcellular distribution of ciprofibroyl-CoA and palmitoyl-CoA hydrolase and that of marker enzymes in rat and guinea pig liver from control and drug-treated animals and in human liver are shown in Fig. 1. For comparative purposes, the subcellular distributions of ciprofibroyl-CoA and palmitoyl-CoA hydrolase presented in Fig. 1 were quantified using assignation to marker enzymes by constrained linear regression [20,21] and are presented in Table 2.

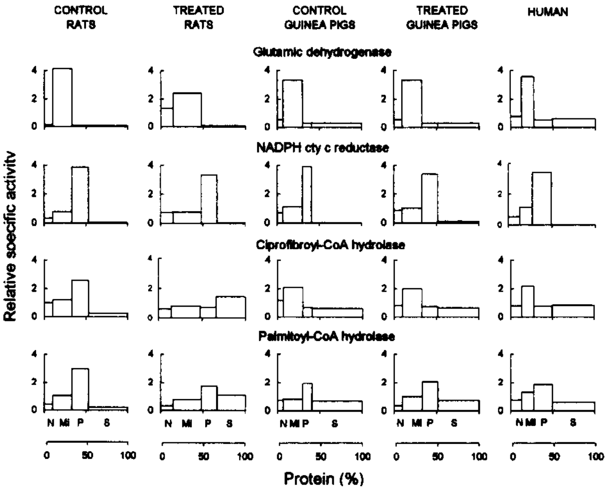

Fig. 1. Subcellular fractionation of rat and guinea pig liver from control and drug-treated animals, and of human liver. Distribution of ciprofibroyl-CoA and palmitoyl-CoA hydrolase. For each distribution pattern, the abscissa represents the protein content of the fraction as a percentage of the total protein of the liver. The ordinate represents the percentage, in the fraction, of the liver content of the enzyme divided by the percentage of liver protein in that fraction. Recoveries ranged from 89 to 119%. Standardized and averaged results [20] from three experiments are presented, except for human liver (one experiment). Maximal variation from the mean was ±18%. Animals were treated with clofibric acid, as described in Section 2.

In control rats ciprofibroyl-CoA hydrolase was present mainly in the microsomal fraction, while in drug-treated rats most of the activity was present in the cytosol. Quantification of these changes (Table 2) show that while the percentage of the total enzyme present in the mitochondrial fraction did not change upon treatment, the microsomal contribution decreases from 52.1% to 21.4%, and the soluble contribution increased from 12.6% to 48.9%. Thus, although in rats the absolute value of ciprofibroyl-CoA hydrolase activity, on a wet weight liver basis, is not modified by drug treatment, its subcellular distribution changes as a consequence of increased soluble activity. In an earlier report on the subcellular distri-

Table 1
Absolute values of fatty acyl-CoA oxidase and acyl-CoA hydrolase activities

	No. of experiments	Fatty acyl-CoA oxidase	Palmitoyl-CoA hydrolase	Ciprofibroyl-CoA hydrolase
Rats				
Control	4	0.950 ± 0.270	4.410 ± 0.460	0.401 ± 0.062
Clofibric acid treated	4	4.964 ± 1.714[a]	6.940 ± 1.950[a]	0.467 ± 0.141
Guinea pigs				
Control	5	0.698 ± 0.321	0.820 ± 0.340	0.487 ± 0.140
Clofibric acid treated	3	0.864 ± 0.258	0.780 ± 0.270	0.631 ± 0.169
Ciprofibrate treated	3	1.088 ± 0.135	0.980 ± 0.210	0.633 ± 0.132
Human	2	0.169 ± 0.023	0.360 ± 0.110	0.155 ± 0.042

Values of activity refers to 1 g wet wt of liver, and are given in units as means ± S.D. The number of independent assays is indicated.
[a]Statistically different from control value.

Table 2
Assignation by constrained linear regression of ciprofibroyl-CoA and palmitoyl-CoA hydrolases

			Mitochondrial	Microsomal	Soluble
Rat	Ciprofibroyl-CoA hydrolase	control	26.61 ± 8.21	52.11 ± 9.57	12.61 ± 7.33
		treated	28.94 ± 4.11	21.35 ± 4.49[a]	48,91 ± 2.81[a]
	Palmitoyl-CoA hydrolase	control	12.03 ± 5.01	77.04 ± 7.01	7.60 ± 5.50
		treated	14.83 ± 5.06	50.77 ± 6.01[a]	36.40 ± 3.75[a]
Guinea pig	Ciprofibroyl-CoA hydrolase	control	58.86 ± 4.54	13.40 ± 4.73	22.98 ± 3.18
		treated	51.78 ± 5.15	18.06 ± 6.76	29.14 ± 3.86
	Palmitoyl-CoA hydrolase	control	7.38 ± 4.60	49.63 ± 5.03	38.78 ± 3.46
		treated	10.26 ± 5.42	59.40 ± 6.49	32.37 ± 5.64
Human	Ciprofibroyl-CoA hydrolase		58.20 ± 7.79	13.62 ± 5.24	25.37 ± 4.28
	Palmitoyl-CoA hydrolase		21.45 ± 9.51	51.23 ± 6.41	23.92 ± 5.23

Glutamate dehydrogenase and NADPH-cytochrome *c* reductase were used as marker enzymes of mitochondria and microsomes, respectively. A hypothetical enzyme with 100% of its activity in the soluble compartment was used as marker of the cytosol for the calculations. Results are given in percentages (± S.D.) and correspond to analysis of the data shown in Fig. 1.
[a]Statistically different from control value.

bution of clofibroyl-CoA hydrolase in rat liver, more than 75% of the enzyme was found to be present in the soluble compartment in both control and clofibrate-treated rats [22]. In contrast with our results, no activity was observed by the latter authors in the microsomal fraction. Differences between our HPLC-based assay and the spectrophotometric assay used by these authors, or between the HPP-CoA used, might be responsible for these differences. In our hands, the activity of the microsomal hydrolase was similar when using ciprofibroyl-CoA, nafenopin-CoA or tibryl-CoA as substrate (not shown).

No changes in the subcellular distribution of ciprofibroyl-CoA hydrolase were observed in the liver of treated guinea pigs when compared to that of control animals. A minor amount of ciprofibroyl-CoA hydrolase was found in the microsomal fraction while most of the activity was in the mitochondrial (58.8%) and soluble fractions (29.1%). In fact, in the unresponsive species, the intracellular distribution of ciprofibroyl-CoA hydrolase resembles better the enzyme localization pattern observed in clofibric acid-treated rats rather than that observed in normal rat liver. On the other hand, ciprofibroyl-CoA and palmitoyl-CoA hydrolases presented a very different distribution in HPP-unresponsive species, while in control rats both enzymes present a similar microsomal distribution.

In agreement with previous data [22,23], soluble palmitoyl-CoA hydrolase was increased in drug-treated rats. No changes were observed in the distribution of the enzyme in the liver of clofibric acid-treated guinea pigs. As for ciprofibroyl-CoA hydrolase, it is noteworthy that the intracellular distribution of palmitoyl-CoA hydrolase in guinea pig and human liver is different from that of control rats but similar to the distribution of the enzyme in the liver of clofibric acid-treated rats.

From the data in Tables 1 and 2, the absolute value of the enzyme activities in each subcellular fraction can be estimated and compared (Table 3). In the rat, palmitoyl-CoA hydrolase is increased in the mitochondrial and soluble fractions, confirming earlier observations [23–25], while ciprofibroyl-CoA hydrolase is increased mainly in the soluble fraction. It is noteworthy that in the guinea pig absolute ciprofibroyl-CoA hydrolase activity in the soluble and mitochondrial fractions is 2-fold higher than the corresponding fractions in the control rat, while the reverse is true for the microsomal activity. Thus, in untreated animals, the subcellular capacity for destroying ciprofibroyl-CoA is totally different in the guinea pig or in the rat according to the subcellular compartment involved.

4. Discussion

We have previously proposed that the coenzyme A thioesters of HPP might be the common pharmacologically active

Table 3
Absolute values of ciprofibroyl-CoA and palmitoyl-CoA hydrolase activities in subcellular compartments

		Mitochondria	Microsomes	Soluble
Rats				
Ciprofibroyl-CoA hydrolase	control	0.107 ± 0.016	0.209 ± 0.032	0.051 ± 0.008
	treated	0.135 ± 0.041	0.100 ± 0.03	0.228 ± 0.069
Palmitoyl-CoA hydrolase	control	0.531 ± 0.055	3.397 ± 0.354	0.335 ± 0.035
	treated	1.029 ± 0.289	3.523 ± 0.990	2.526 ± 0.710
Guinea pig				
Ciprofibroyl-CoA hydrolase		0.287 ± 0.092	0.065 ± 0.019	0.112 ± 0.032
Palmitoyl-CoA hydrolase		0.061 ± 0.025	0.407 ± 0.169	0.318 ± 0.132

Values are given in units per g wet wt of liver, as mean ± S.D., and were calculated from the total activity in the liver (Table 1) multiplied by the percent of activity in each compartment (Table 2). In the case of the guinea pig, only control values are presented, since there was no effect of the treatment upon total enzyme activity.

metabolite that unifies the structural diversity of these compounds [14–16]. Most HPP present a carboxylic acid functional group, or a group that can be readily oxidized to carboxylic acid and activated to a CoASH thioester. Even in HPP, lacking a carboxylic function as azole compounds, carboxylic groups can arise from metabolic degradation [26]. HPP-CoA has been shown to activate protein kinase C [27,28], to bind specifically to rat liver cytosolic proteins [29], to inhibit acetyl-CoA carboxylase [30] and to modify the phosphorylation status of proteins [31]. In this report, we investigated the possible existence of differences in the degradation of ciprofibroyl-CoA in rat liver and in the liver of two HPP non-responsive species, humans and the guinea pig.

Striking differences were found between the rat and the HPP-non responsive species in the subcellular distribution of ciprofibroyl-CoA and palmitoyl-CoA hydrolase and in the response to clofibric acid treatment. In the rat the activity of palmitoyl-CoA hydrolase is 5–6-fold higher than in the unresponsive species. In agreement with published data [23–25] it is increased in clofibric acid treated animals, while no effect is observed in the guinea pig. In contrast, similar liver absolute activities were observed for ciprofibroyl-CoA hydrolase in the three species. In the guinea pig, the enzyme is not sensitive to clofibric acid or ciprofibrate treatment. However, its distribution pattern, as the human enzyme, shows a relatively high activity in the mitochondrial and soluble fractions, when compared with the rat. This intracellular distribution follows the pattern of ciprofibroyl-CoA hydrolase in drug-treated rats, which show increased soluble and mitochondrial activities when compared to controls. Since, in the rat, HPP treatment has been shown to induce cytosolic and mitochondrial long chain acyl-CoA hydrolases which are only weakly expressed, if at all, under normal conditions [25], it is tempting to speculate that the clofibric acid-inducible ciprofibroyl-CoA hydrolase from rat liver is being normally expressed in the liver of the guinea pig and other HPP non-responsive species, resulting in a more effective degradation of HPP-CoAs.

Whether or not the greater mitochondrial and cytosolic capacity of ciprofibroyl-CoA degradation of the liver guinea pig can be extended to other HPP-CoAs, or be related with the lack of HPP-induced peroxisomal proliferation of this species remains to be established. On the other hand, the true subcellular localization of the enzyme in the mitochondrial fraction also remains to be established, since our fractionation method does not discriminate between peroxisomal and mitochondrial contributions to enzyme activity in that fraction.

If HPP-CoAs are indeed involved in HPP-induced pleiotropic effects, other factors such as the liver activity of HPP-CoA synthetase may be also involved. Human liver forms ciprofibroyl-CoA at a rate comparable to that of the rat [15], while the guinea pig liver is as active as the rat organ in the activation of ciprofibrate (Urrea and Bronfman, unpublished results). In the rat, long chain mitochondrial and microsomal acyl-CoA synthetases have been shown to activate HPP [14,15]. In addition to these acyl-CoA synthetases, separate microsomal xenobiotic-CoA synthetases exist [32], and also a peroxisomal nafenopin-CoA synthetase has been demonstrated [33]. The problem is further complicated by the fact that acyl-CoA synthetases present different affinities for HPP which results in very different intracellular concentrations of HPP-CoAs [15], and by the recent report of liver HPP-CoA binding proteins with different affinities for HPP-CoAs [29], which may also be involved in their effects. Further research on species-comparative evaluation of the intracellular concentration and distribution of HPP-CoAs in vivo, and on the liver activity and subcellular distribution of HPP-CoA synthetases and hydrolases might help us to establish whether or not metabolic differences are responsible for the existence of HPP responsive and unresponsive species.

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References

[1] Lake, B. (1995) Annu. Rev. Pharmacol. Toxicol. 35, 483–507.
[2] Reddy, J.K., Rao, M.S., Azarnoff, D.L. and Sell, S. (1979) Cancer Res. 39, 152–161.
[3] Reddy, J.K., Azarnoff, D.L. and Hignite,C.E. (1980) Nature 208, 856–859.
[4] Reddy, J.K. and Lalwani, N,D. (1983) CRC Crit. Rev. Toxicol. 12, 1–58.
[5] Hawkins, J.M., Jones, W.E., Bonner, F.W. and Gibson, G.G. (1987) Drug. Metab. Rev. 18, 441–515.
[6] Watanabe, T., Horie, S., Yamada, J., Isaji, M., Nishigaki, T., Naito, J. and Suga, T. (1989) Biochem. Pharmacol. 38, 367–371.
[7] Issemann, I. and Green, S. (1990) Nature 347, 645–650.
[8] Issemann, I., Prince, R.A. and Green, S. (1993) J. Mol. Endocrinol. 11, 37–47.
[9] Lee, S.S.-T., Pineau, T., Drago, J., Lee, E., Owens, J.W., Kroetz, D.L., Fernandez-Salguero, P.M., Westphal, H. and Gonzalez, F. (1995) Mol. Cell Biol. 15, 3012–3022.
[10] Sher, T., Yi, H.-F., Mcbride, O.W. and Gonzalez, F. (1993) Biochem. J. 32, 5598–5604.
[11] Mukherjee, R., Jow, L., Noonan, D. and McDonnell, D.P. (1994) J. Steroid Biochem. Mol. Biol. 51, 157–166.
[12] Jow, L. and Mukherjee, R. (1995) J. Biol. Chem. 270, 3836–3840.
[13] Juge-Aubry, C., Gorla-Bajszczak, A., Permin, A., Lemberger, T., Wahli, W., Burger, A. and Meier, C.A. (1995) J. Biol. Chem. 270, 18117–18122.
[14] Bronfman, M., Amigo, L. and Morales, M.N. (1986) Biochem. J. 239, 781–784.
[15] Bronfman, M., Morales, M.N., Amigo, L., Orellana, A., Nuñez, L., Cárdenas, L. and Hidalgo, P. (1992) Biochem. J. 284, 289–295.
[16] Bronfman, M. (1993) in: Peroxisomes. Biology and Importance in Toxicology and Medicine (Gibson, G. and Lake, B. eds.) pp. 217–245, Taylor and Francis, London.
[17] Hertz, R. and Bar-Tana, J. (1988) Biochem. J. 254, 39–44.
[18] Aarsland, A., Berge, R.K., Bremer, J. and Aarsaether, N. (1990) Biochim. Biophys. Acta 1033, 176–183.
[19] Nishimaki-Mogami, T., Takahashi, A. and Hayashi, Y. (1993) Biochem. J. 296, 265–270.
[20] Bronfman, M. and Leighton, F. (1984) Biochem. J. 224, 721–730.
[21] Bronfman, M., Leighton, F. and Feytmans, E. (1982) Ann. N.Y. Acad. Sci. 386, 408–410.
[22] Berge, R., Stensland, E., Aarsland, A., Tsegai, G., Osmundsen, H., Aarsaether, N. and Gjellesvik, D.R. (1987) Biochim. Biophys. Acta 918, 60–66.
[23] Berge, R., Skrede,S. and Farstad, M. (1981) FEBS Lett. 124, 43–47.
[24] Miyazawa, S., Furuta, S. and Hashimoto, T. (1981) Eur. J. Biochem. 117, 425–430.
[25] Svensson, L.T., Wilcke, M. and Alexson, S.E.H. (1995) Eur. J. Biochem. 230, 813–820.
[26] Nakaoka, M. and Hakusui, H. (1987) Xenobiotica 17, 1329–1339.
[27] Bronfman, M., Orellana, A., Morales, M.N., Bieri, F., Waechter,

F., Stäubli, W. and Bentley, P. (1989) Biochem. Biophys. Res. Commun. 159, 1026–1031.
[28] Orellana, A., Hidalgo, P., Morales, M.N., Mezzano, D. and Bronfman, M. (1990) Eur. J. Biochem. 190, 57–61.
[29] Morgan, C. and Bronfman, M. (1995) Xenobiotica 25, 1293–1300.
[30] Tomaszewski, K. and Melnick, R. (1994) Biochim. Biophys. Acta 1120, 118–124.
[31] Orellana, A., Holuigue, L., Hidalgo, P.C., Faúndez, V., González, A. and Bronfman, M. (1993) Eur. J. Biochem. 215, 903–906.
[32] Knights, K.M. and Roberts, B.J. (1994) Chem-Biol. Interact. 90, 215–223.
[33] Roberts, B.J., Macleod, J.K., Singh, I. and Knights, K.M. (1995) Biochem. Pharmacol. 49, 1335–1339.