Biochemical Pharmacology, Vol. 33, No. 14, pp. 2179–2184, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

THE PREVENTION BY (+)-CYANIDANOL-3 OF HEPATITIS-INDUCED CHANGES IN THE DISPOSITION OF IMIPRAMINE IN THE RAT

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(*Received* 18 *October* 1983; *accepted* 17 *January* 1984)

Abstract—The administration of (+)-Cyanidanol-3 ((+)-catechin) to the rat using a subchronic dosing regime based on that currently used in the therapy of acute viral hepatitis in man, largely prevented the changes in the disposition of a single dose of [^{14}C]imipramine hydrochloride induced by the hepatotoxin, D-(+)-galactosamine hydrochloride in rats. Complete return to normal pharmacokinetics was not attained due to interaction between (+)-Cyanidanol-3 and imipramine. Biliary excretion of imipramine metabolites was 79.3% of the dose in control rats. This was reduced to 69.3 and 39.8% by the separate administration of (+)-catechin and galactosamine respectively. Concurrent administration of (+)-Cyanidanol-3 and galactosamine resulted in 64.8% of the imipramine dose appearing in bile. These results were reflected in changes in faecal and renal excretion of imipramine metabolites in surgically unmodified rats in which galactosamine injection caused an elevation of urinary excretion from 31.0 to 69.8% of the imipramine dose. Concurrent Cyanidanol administration reduced the effect of galactosamine so that only 46.9% was excreted in urine. These changes were due to decreased biliary excretion and increased renal excretion of the glucuronide conjugates of 2-hydroxyimipramine, 2-hydroxydesmethylimipramine and 10-hydroxyimipramine. None of the treatments used impaired the overall ability of the rat to metabolize imipramine, although the plasma clearance of imipramine was reduced by 42% as a result of galactosamine administration and by 21% during treatment with (+)-catechin alone or combined catechin and galactosamine treatment.

(+)-Cyanidanol-3 ((+)-catechin) is currently used in the therapy of acute viral hepatitis in man [1]. It has also been shown to exert hepatoprotective effects in animal systems, for example, against injury by paracetamol [2], carbon tetrachloride [3] and lipid peroxidation [4, 5] whether induced chemically or by depletion of hepatic glutathione content [6]. In such systems it is likely that the protective effects are based on the ability of (+)-catechin to function as a free radical scavenger [7, 8].

Cyanidanol-3 has also been reported to protect the rat liver against damage by the hepatotoxin, D-galactosamine [3, 9]. Galactosamine-hepatitis is held to show analogous changes to those observed in human AVH [10] and the rat galactosamine model has been used to explore the possible effects of AVH on the disposition of drugs including (+)-Cyanidanol-3 [11] ((+)-catechin) 3-O-methyl-(+)-catechin [12] and imipramine [13]. In this study we have investigated the effect of administration of the liver-protective agent (+)-Cyanidanol-3, on the development of the abnormal patterns of imipramine metabolism excretion and pharmacokinetics observed following the induction of an experimental hapatitis.

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MATERIALS AND METHODS

D-(+)-Galactosamine hydrochloride (2-amino, 2-deoxy-D-(+)-galactose-HCl) and imipramine hydrochloride were purchased from the Sigma Chemical Co., Poole, United Kingdom. (+)-Cyanidanol-3 was a gift from Zyma S.A. Nyon, Switzerland.

[^{14}C]Imipramine hydrochloride (N-(dimethylaminopropyl)imino[dimethylene-^{14}C] benzyl HCl) was purchased from Amersham International PLC, Amersham, U.K. and found to be 98% radiochemically pure. Unlabelled desipramine was obtained from the dispensary at the Queen Elizabeth Hospital, Birmingham and authentic samples of six other unconjugated imipramine metabolites (see Table 3) were gifts from Ciba Geigy, Basel, Switzerland.

Animals and animal experiments. Male rats (225–275 g body weight) of the Birmingham albino Wistar strain were used. They were fed on the diet described by Griffiths [14] and allowed free access to drinking water.

Groups of rats were subjected to one of four 48 hr dosing schedules designed to test the effects of galactosamine, Cyanidanol-3 and a combination of these on the disposition of [^{14}C]imipramine hydrochloride. The individual treatments are detailed in Table 1.

Non-surgically modified rats were housed for 96 hr following imipramine administration in all glass metabolism cages allowing separate collection of urine and faeces under solid CO_2 [15]. All oral doses

were administered by gavage. In the case of imipramine such doses were of 5 mg (2 μCi) of imipramine [^{14}C]-HCl. Intravenous doses of imipramine were administered at the level: 15 mg/kg rat body weight (0.5 μCi).

Bile duct cannulations were carried out where appropriate according to the method of Barrow and Griffiths [16]. Bile duct cannulated rats were housed in specially designed restraining cages and bile collected in successive 2 hr fractions for 36 hr after imipramine administration.

Analyses of samples. Total radioactivity in urine, bile, plasma and faeces was measured by liquid scintillation counting and combustion techniques [15]. Imipramine concentrations in plasma were measured by the extraction and high performance liquid chromatographic method of Proelss *et al.* [17].

Other techniques used in the analysis of samples of urine, bile and faeces including incubation with β-glucuronidase preparations, thin layer chromatography of metabolites and quantification of individual metabolites by radioscanning and high performance liquid chromatography of metabolites have been previously described [13].

RESULTS

Influence of various regimes upon the total excretion of ^{14}C following ^{14}C-imipramine administration. The excretion of ^{14}C in the urine and faeces of surgically unmodified rats following oral administration of [^{14}C]imipramine HCl is shown in Table 2. Statistical analysis of the measurements of urinary excretion of ^{14}C by Student's *t*-test showed that the excretion of ^{14}C in rats predosed with galactosamine (69.8% of the dose) was significantly increased over the control value (31.0%, $P < 0.001$). Concurrent treatment of galactosamine treated rats with Cyanidanol (treatment 4, Table 1) resulted in a urinary excretion of 46.9% of the dose. Statistically this was significantly different from both control rats and rats treated with galactosamine only ($P < 0.001$ for both observations). Interestingly, the administration of Cyanidanol alone (treatment 3, Table 1) also significantly increased urinary ^{14}C excretion to 40.7% of the dose (cf. 31.0% in controls, $P < 0.05$), but the urinary ^{14}C excretion in Cyanidanol/galactosamine rats (treatment 3) was still just significantly elevated above this value ($P < 0.05$) showing that the reversal of the galactosamine effect by Cyanidanol was not complete, even when the interaction between Cyanidanol and imipramine was taken into account.

If the excretion of ^{14}C in the urine of rats treated with Cyanidanol only is taken as the maximum extent of any protective effect, then the subchronic Cyanidanol administration gave an approx. 70% reversal of the effect of galactosamine on the urinary excretion of imipramine metabolites.

The observed changes in urinary excretion of ^{14}C were complemented by changes in faecal excretion; although statistical analysis of the faecal data was less informative due to the larger inter-individual variation within the four treatments (Table 2). None

Table 1. Dose regimes for rats

Treatment	Time after start of treatment (hr) 0	12	12	24	30	36	48
Control	Water	Water	Saline	Water	Imipramine ^{14}C	Water	Water
Galactosamine	Water	Water	D-Galactosamine-HCl	Water	Imipramine ^{14}C	Water	Water
Cyanidanol	Cyanidanol-3	Cyanidanol-3	Saline	Cyanidanol-3	Imipramine ^{14}C	Cyanidanol-3	Cyanidanol-3
Cyanidanol + galactosamine	Cyanidanol-3	Cyanidanol-3	D-Galactosamine-HCl	Cyanidanol-3	Imipramine ^{14}C	Cyanidanol-3	Cyanidanol-3

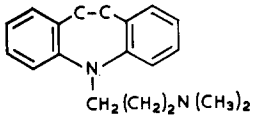
(+)-Cyanidanol-3 (10 mg in 3 ml H_2O) was administered by gavage; rats not receiving Cyanidanol were given 3 ml of water only.
D-Galactosamine HCl was injected intraperitoneally (1 g/kg body weight in 1.5 ml of sterile 0.9% saline); 1.5 ml of 0.9% saline was injected into non-galactosamine treated rats.
Imipramine ^{14}C was administered orally (5 mg) dissolved in water or by i.v. injection (15 mg/kg body weight) in 0.9% saline.
In appropriate experiments bile duct cannulations were carried out 27 hr after T_0.

Table 2. The excretion of ^{14}C in the urine and faeces of rats following a single dose of imipramine ^{14}C-HCl (5 mg; oral) ± S.D.

	Control	Galactosamine only	Cyanidanol only	Galactosamine + Cyanidanol
Excretion in urine				
0–6 hr	7.1	15.1	8.0	9.1
6–24 hr	10.0	20.1	18.3	13.6
24–48 hr	9.5	19.1	9.2	17.1
48–72 hr	3.5	11.9	3.2	5.4
72–96 hr	0.9	3.6	2.0	1.7
Total ± S.D.	31.0 ± 4.2	69.8 ± 1.3	40.7 ± 2.8	46.9 ± 0.4
Excretion in faeces				
0–24 hr	24.6	15.1	27.3	5.6
24–48 hr	16.1	9.9	28.4	27.6
48–72 hr	20.2	2.9	3.6	14.6
72–96 hr	7.4	1.3	0.4	0.0
Total ± S.D.	68.3 ± 5.1	29.2 ± 3.7	59.7 ± 3.9	47.8 ± 8.3
Total Recovery ± S.D.	99.3 ± 6.3	99.0 ± 4.9	100.4 ± 5.9	94.7 ± 8.5

of the treatments affected the complete excretion of ^{14}C within the 96 hr collection period.

Measurements of biliary excretion of ^{14}C in bile duct cannulated rats showed that administration of galactosamine alone decreased biliary ^{14}C excretion from 79.3 to 39.8% of the [^{14}C]imipramine dose ($P < 0.001$) (Fig. 2). Administration of Cyanidanol concurrently with galactosamine (treatment 4, Table 1) partly prevented this reduction, the biliary excretion being 64.8% in 36 hr after dosing. This value was significantly different from the values seen in both control and galactosamine treated rats. Again an interaction between Cyanidanol and imipramine was observed, treatment with Cyanidanol alone decreasing biliary excretion from 79.3% to 69.3% of the dose ($P < 0.05$). The biliary excretion of ^{14}C in rats treated with Cyanidanol and galactosamine was not significantly different from that observed where Cyanidanol dosing alone was carried out, indicating a highly efficient reversal by Cyanidanol of the galactosamine induced changes. This apparent inconsistency with conclusions drawn from renal excretions in surgically unmodified rats (which indicate a less efficient protective effect) may be due to the occurrence of enterohepatic circulation in the surgically unmodified rats resulting in magnification of small changes in the bile:urine partitioning of imipramine metabolites by successive circulations.



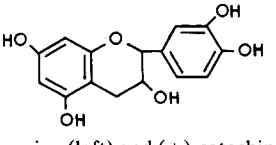


Fig. 1. The structures of imipramine (left) and (+)-catechin (right).

If the biliary excretion of ^{14}C in rats treated with Cyanidanol alone is taken as the baseline, then the effect of Cyanidanol was approx. 85% effective in counteracting the effect of galactosamine or the biliary excretion of imipramine metabolites.

The metabolites of imipramine excreted in bile, urine and faeces. Incubation of bile samples with β-glucuronidase followed by chromatography showed that bile from rats undergoing all four treatments contained four compounds. These were the glucuronide conjugates of 2-hydroxyimipramine, 10-

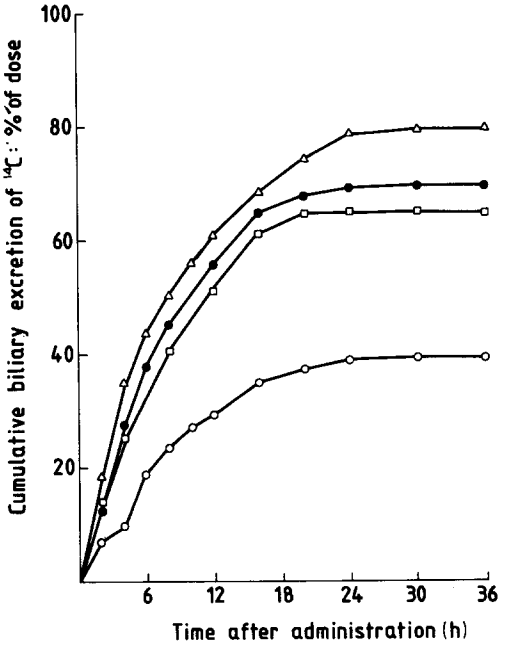


Fig. 2. The cumulative excretion of ^{14}C by bile-duct cannulated rats following oral administration of [^{14}C]imipramine (5 mg, 2 μCi orally). Means of 4 experiments. △——△ Control rats, ●——● Cyanidanol treated rats, ○——○ galactosamine treated rats, □——□ galactosamine treated rats receiving concurrent Cyanidanol dosing.

Table 3. The excretion of imipramine and its metabolites in urine and bile following oral administration of imipramine [^{14}C]HCl (% of dose) ± S.D.

	Control		Galactosamine		Cyanidanol		Galactosamine + Cyanidanol	
	Urine	Bile	Urine	Bile	Urine	Bile	Urine	Bile
2-Hydroxyimipramine glucuronide	2.9 ± 2.1	30.4 ± 3.9	12.7 ± 3.1	11.7 ± 2.7	6.1 ± 1.3	25.1 ± 4.1	10.2 ± 1.7	22.7 ± 3.1
2-Hydroxydesmethylimipramine glucuronide	13.1 ± 1.9	26.4 ±3.0	30.2 ± 4.6	12.7 ± 1.9	16.9 ± 2.7	24.3 ± 3.1	18.3 ± 2.2	27.8 ± 5.1
10-Hydroxyimipramine glucuronide	1.4 ± 0.7	12.8 ± 3.7	2.8 ± 1.5	8.2 ± 0.3	2.2 ± 0.8	9.1 ± 1.2	3.1 ± 1.0	5.3 ± 1.1
Imipramine	0.8	ND	0.9	ND	0.7	ND	ND	ND
Desipramine	2.7	ND	1.2	ND	1.1	ND	0.7	ND
Didesmethylimipramine	1.8	ND	1.6	ND	1.0	ND	1.5	ND
2-Hydroxyimipramine	1.2	ND	2.2	ND	0.7	ND	2.6	ND
2-Hydroxydesmethylimipramine	2.2	ND	4.5	ND	1.9	ND	3.1	ND
10-Hydroxyimipramine	1.0	ND	1.8	ND	0.7	ND	0.9	ND
Imipramine N-oxide	1.5	ND	2.1	ND	0.8	ND	1.7	ND
Iminodibenzyl	0.7	9.0	0.3	4.6	0.9	2.9	1.1	4.1

hydroxyimipramine and 2-hydroxydesmethylimipramine (Table 3). Some iminodibenzyl was also detected but this is considered to be an artefact formed spontaneously from the aglycone of one of the three conjugates under the conditions of β-glucuronidase incubation [13]. The relative amounts of these compounds in bile in the present study was similar: none being selectively affected by any of the treatments.

Chromatography (TLC, HPLC) of the urine of surgically unmodified rats dosed with imipramine [^{14}C] showed the presence of imipramine and 10 other metabolites (Table 3). Amounts of imipramine were small and unaffected by treatment with galactosamine, Cyanidanol or both. Similarly seven other phase I metabolites of imipramine were detected in small amounts only and like imipramine the extent of their excretion was not influenced by any treatment.

Urine was also found to contain the three conjugated metabolites noted in bile and the changes in excretion of these accounted for the changes in total ^{14}C excretion discussed in the previous section.

It is important to note that none of the treatments used impaired the overall ability of the rat to metabolize imipramine and there was no increase in the excretion of those metabolites like desipramine and imipramine N-oxide which are known to be pharmacologically active.

Effects on plasma clearance of imipramine and its metabolites. The concentration of imipramine in the plasma of groups of rats undergoing the four described treatments (Table 1) was measured by an HPLC technique [17] over a period of 8 hr following i.v. injection of 15 mg/kg [^{14}C]imipramine. The results of these studies are shown in Fig. 3. The areas under these curves were measured by microcomputer (CBM Commodore PET) and clearance values calculated from the equation

$$Cl = \frac{Dose}{AUC}.$$

In the control group of rats the mean clearance was 12.4 ml/min. Mean clearance was decreased by galactosamine treatment to 7.2 ml/min (a reduction

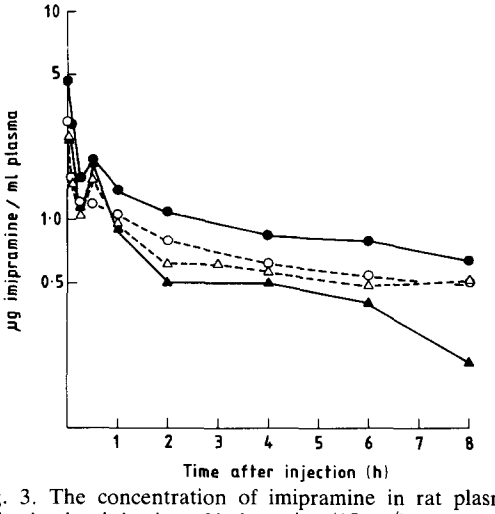


Fig. 3. The concentration of imipramine in rat plasma following i.v. injection of imipramine (15 mg/kg, means of 3 experiments for each point). ▲——▲ Control (treatment 1), ●——● galactosamine (treatment 2), ○——○ Cyanidanol only (treatment 3), △——△ Cyanidanol + galactosamine (treatment 4).

of 42%). Interaction between Cyanidanol alone and imipramine was apparent in the clearance of imipramine. Mean plasma clearance of imipramine in rats treated with Cyanidanol alone was 9.9 ml/min which is a 21% reduction from the control value. The mean plasma clearance of imipramine in rats treated with both galactosamine and Cyanidanol was 9.8 ml/min indicating that the effect of the galactosamine on plasma clearance is totally prevented by Cyanidanol.

Total ^{14}C in plasma was also measured by liquid scintillation counting (Fig. 4) Concentrations of ^{14}C were for each treatment higher than could be accounted for by the presence of imipramine alone indicating the presence of metabolites. Although the concentrations of ^{14}C in plasma were apparently increased by galactosamine treatment (Fig. 4) the effect of Cyanidanol alone and during concurrent administration with galactosamine was not distinct from the control curve. Calculations of total metabolite clearances were made by subtracting mean imi-

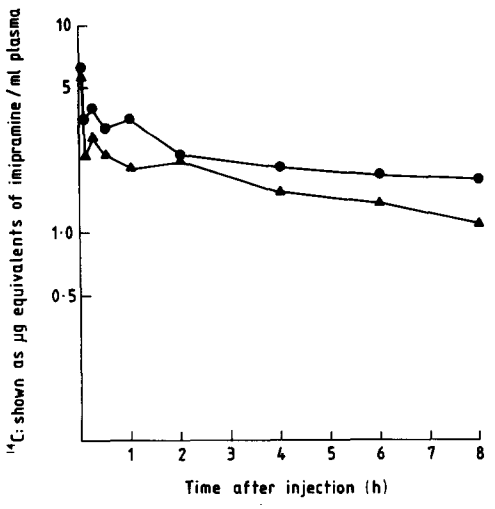


Fig. 4. The concentration of ^{14}C in rat plasma following i.v. injection of ^{14}C-imipramine-HCl (15 mg/kg, means of 3 experiments for each point). ▲——▲ Control (treatment 1), ●——● galactosamine (treatment 2).

pramine concentrations from total ^{14}C concentration for each time point and computing the area under the resulting curve. All four treatments gave clearance values for total metabolites between 5 and 7 ml/min and none was significantly different from the others. This means that the effect of galactosamine was under these conditions dependent entirely on the primary clearance of imipramine and not upon that of its metabolites.

DISCUSSION

In previous studies it has been demonstrated that (+)-Cyanidanol-3 exerts a protective effect against galactosamine-induced liver damage in the rat by the use of the classical plasma tests of liver function and damage such as BSP clearance, bilirubin concentration and SGOT activity [3, 9]. In the present study we have demonstrated that the changes in the disposition of imipramine caused by galactosamine administration to the rat (decreased biliary-faecal excretion, decreased plasma clearance and increased urinary excretion of metabolites) can also be largely prevented by a concurrent sub-chronic administration of (+)-Cyanidanol-3 similar to that in current clinical use in the treatment of acute viral hepatitis in man [1]. The present study does not show whether the effect of Cyanidanol is purely preventative or whether it would be effective if administered only after the onset of liver damage but one of the clinical implications of our study is that Cyanidanol therapy in man may have the added benefit of allowing the concurrent use of other drugs, like imipramine, normally contraindicated in liver disease.

In none of our experiments where galactosamine and Cyanidanol were co-administered did excretion and clearance values return fully to control values. This was apparently due to an interaction between Cyanidanol and imipramine which was observed in the urinary, and biliary excretion of imipramine metabolites and the plasma clearance of imipramine itself. Interference by Cyanidanol in the disposition of co-administered drugs must therefore be considered by clinicians prescribing Cyanidanol. The effect of other drugs on Cyanidanol disposition has yet to be investigated.

The present studies *in vivo* give little insight into the specific subcellular changes involved in the observed galactosamine induced changes in imipramine disposition or into the effect of (+)-Cyanidanol in preventing these changes. Decker *et al.* [18] have attributed the hepatic toxicity of galactosamine to its metabolism in liver to UDP-hexosamines and UDP-N-acetylhexosamines and decreased levels of UTP, UDP glucose and UDP galactose. Reutter *et al.* [9] have termed this the "primary biochemical response". The resulting inhibition of RNA synthesis and disturbance of the biosynthesis of proteins and glycoproteins leading to deterioration of cell organelles and membranes and other hepatitis-like changes has been termed the "secondary biochemical response". It is possible that changes in the metabolism and excretion of drugs may be mediated by either the primary or the secondary response. The primary response involves a dramatic, short term decrease in liver UDPGA concentration [19, 20] which Gregus *et al.* [21] have used to demonstrate the dependence of a group of compounds on the glucuronidation pathway as a prerequisite for biliary excretion. The primary biochemical response would be expected to play little direct part in the galactosamine induced changes in imipramine disposition or in the effect of Cyanidanol on these changes, since the decrease in UDP-hexose levels is transient [19] and Cyanidanol is known not to influence the concentration of [^{14}C]galactosamine and its metabolites in rat liver [9].

The mechanisms of the effects of galactosamine and Cyanidanol are probably both associated with the secondary biochemical response leading to alterations in the activity of drug metabolizing enzymes. The metabolism of imipramine is largely dependent on cytochrome P450 [22] the activity of which is decreased in rat liver by galactosamine treatment [23]. Although Cyanidanol is not metabolized by cytochrome P-450, it binds to P-450 in rat liver microsomes and inhibits several P-450 and P-448 mediated oxidations *in vitro* [24, 25]. Subchronic administration of Cyanidanol may also lead to prolonged hexobarbital sleeping time and reduced N-demethylation of aminopyrine *in vivo* [24, 25]. Beyeler *et al.* [25] also report that Cyanidanol can *in vitro* stabilize cytochrome P-450, protecting it from lipoperoxidative destruction and have shown the binding of Cyanidanol is based on its ligand properties towards the Fe of the haeme. This type of binding is consistent with non-competitive inhibition of aminopyrine demethylation and biphenyl-4-hydroxylation *in vitro*. In the context of the present study these observations are consistent with the protective effect of Cyanidanol and with the observed interaction between Cyanidanol and imipramine. However the phase I metabolites of imipramine and Cyanidanol and its major metabolite, 3'-O-methyl-(+)-catechin all undergo glucuronidation and biliary excretion offering further scope for interaction.

It is also interesting to note that the 40% reduction in plasma clearance of imipramine caused by galac-

tosamine injection is associated with a 40% decrease in biliary excretion of imipramine metabolites, whereas the 21% reduction in plasma clearance of imipramine due to Cyanidanol alone is accompanied by only a 10% decrease in biliary excretion, suggesting that galactosamine also impairs the ability of the liver to conjugate and/or excrete imipramine phase I metabolites.

Acknowledgements—The authors wish to thank Zyma S.A., Nyon, Switzerland for financial support; the Medical Research Council for provision of a Research studentship (to ICS) and Miss Rosemary Dring for capable technical assistance.

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