SAFETY OF TRAMADOL - CHANGES IN THE LEVELS OF MICROSOMAL CYP450 IN RAT BRAIN WITHOUT NOCICEPTION

P. Sahitya Chetan[1,2]*, P.Saritha[2], L.A.R. Sangeetha[3], G.Visweswari[2], P. Murali Mohan[4], and W. Rajendra[2]

1. **Department of Biological Sciences, Arkansas State University, Arkansas, USA.**
2. **Division of Molecular Biology, Dept. of Zoology, Sri Venkateswara University, Tirupati, A.P. India**
3. **Department of Pharmaceutical Sciences, S.P. Mahila University, Tirupati, A.P. India.**
4. **Department of Sericulture, S.P. Mahila University, Tirupati, A.P. India.**

Summary

In the present investigation the activity levels of the enzyme cytochrome-P450 in different areas of rat brain were examined during administration of the synthetic opioid analgesic drug tramadol without induction of pain. Male adult Wistar rats were used in the study. Tramadol was injected subcutaneously, and the changes in CyP450 activity in cortex, cerebellum, pons-medulla, hippocampus and hypothalamus of the brain were studied at 3, 6, 12 and 24 hours following administration of the drug (31 mg/kg body wt). The present study showed an increase in CyP450 activity in all areas of the brain by 6 hours following the injection of tramadol, and by 24 hours it returned more or less to the respective control levels. Maximal increase of enzyme activity levels was recorded in hippocampus followed by cortex, pons-medulla, cerebellum and hypothalamus. The results indicate that the levels of CyP450 get increased as a measure of metabolizing the drug for one-time administration of tramadol. However, the activity got reverted to control levels after the time limit of change, indicating that tramadol would not have any adverse affects on the brain and can be administrated safely during total absence of pain or non-induction of pain.

Key Words: Cytochrome P450, Tramadol, Rat brain areas, Microsomes, Non-induction of pain

*Dr. P. S. Chetan
Department of Biology
Arkansas State University
Arkansas,USA.
Cell: 870-882-1648
Email: drchetan@aol.in

Introduction

Pain-perception is modulated by a variety of opioids through changes in neurotransmitter levels including those of nor-epinephrine and serotonin [1]. Opioids are widely used for the treatment of moderate to severe pain [2,3]. Studies have demonstrated that many experimental pain models are sensitive analgesic assays [4]. Some studies suggested that opiates are ineffective in treating neuropathic pain [5], while it is said in other reports that opiates are merely less potent in treating neuropathic pain than nociceptive pain [6,7].

Opioids are broad-spectrum analgesics with potent pain-relieving qualities but also with potential adverse effects related to both short-term and long-term therapy. Researchers have attempted to alter existing opioid analgesics, utilize different routes/ formulations, or combine opioid analgesics with other compounds in efforts to improve analgesia while minimizing adverse effects. Opioid effects are mediated by central and peripheral opioid receptors [8].

Tramadol is a centrally acting analgesic in widespread use throughout the world [9]. It is endowed with opioid, noradrenergic and serotonergic properties. Various data suggest that, in addition to its analgesic effect, tramadol may have antidepressant and anxiolytic-like effects [10].

Acting in a synergistic manner and being more efficacious, tramadol is used world wide for the treatment of moderate to severe acute or chronic pain. Abuse and dependence of tramadol as well as tramadol-related deaths have been reported, either ingested alone or taken in combination with other potentially interacting drugs. The possible toxic effect of tramadol was reviewed from aspects of its analgesic mechanisms, adverse effects, dependence, and abuse [11].

Tramadol is a racemic mixture of 2 enantiomers that have comparable pharmacokinetic profile and this lack of difference is also observed with their main active metabolite, O-demethyl tramadol (M1). The serum concentrations of this metabolite depend largely on the activity of the enzyme Cytochrome P450 (CyP450). Nevertheless, the inter-individual variability observed in the pharmacokinetics of tramadol and consequently in the pharmacodynamic profile is mainly due to the genetic polymorphism of CyP450 [12].

In the oxidation-reduction process, two microsomal enzymes, viz. NADPH-CYP450 reductase and CyP450 play a very important role. The presence of CyP450 in rat brain was reported [13]**).** Much work has been carried out on the P450 gene variants (polymorphisms) that cause individual variability in drug response [14,15]. CyP450 activity was examined with reference to opioid analgesia. It was suggested that the opioid activity of tramadol may well be consequent to metabolism of the parent drug to the active demethylated metabolite by the polymorphic CyP450 enzyme, debrisoquine 4-hydroxylase [16,17].

Although the behavior of tramadol-induced changes in CyP450 levels is not different from what it actually has to be as reported by several scientific observations in the presence of pain, in order to assess the possible interaction under presumably non-nociceptive conditions, the present investigation was carried out to see if there would be any changes during non-induction of pain. Despite the logical assumption that the actions of an analgesic drug would be same in the presence or absence of pain, the biochemical events in different areas of the brain during experimental induction of pain remain relatively unexplored.

The work was intended to examine the change in the levels of a primary drug-metabolizing enzyme CyP450 without nociception.

Material and Methods

Tramadol (Ultram), was obtained as a commercial grade chemical from Apollo Pharmacy (Hyderabad, INDIA). Male adult Wistar rats of 3 months age group, weighing 150 ± 20 grams were used as the experimental animals in the present investigation. The rats were purchased from the Indian Institute of Science, Bangalore, maintained in the animal house of the department in polypropylene cages under laboratory conditions of temperature (28 ± 2ºC), photoperiod (LD 12:12) and relative humidity (75%). The rats were fed with standard pellet diet and given free access to water. The rats were maintained according to the animal ethical guidelines for animal protection and welfare bearing the CPCSEA 438/01/a/cpcsea/dt 17.07.2001 in its resolution No: 9/IAEC/SVU/2001/dt 04.03.2002.

Dosage for Administration

After the rats were acclimated to the laboratory conditions, they were divided into groups depending on the dosage and time for sacrificing the animals. Five groups, with six rats in each group, were housed in separate cages. All doses were given in the morning between 9-10 AM, keeping in view the altered activity of rats during the nights compared to the daytime. Controls were maintained individually for each group. The dosage was administered subcutaneously (*sc*) according to (ED50) value obtained in rats at 31 mg/kg in hot plate test conducted as per [18].

Isolation of Tissues

The present study was carried out on different areas of the brain, viz. cerebral cortex (CC), cerebellum (CB), pons-medulla (PM), hippocampus (HI) and hypothalamus (HY). The animals were sacrificed at the chosen time periods of 3h, 6h, 12h and 24h following tramadol administration. The brain was isolated immediately and placed on a chilled glass plate. The brain areas were separated and frozen in liquid nitrogen (-180ºC) and stored at -70ºC until further use. At the time of analyses the tissues were thawed and used. Isolation of microsomes was done using the established methods of Cotman and Matthews, Dodd *et al.* and Kodavanti *et al.* [19,20,21].

Assay of Cytochrome P450 Levels

The microsomal CyP450 levels were estimated by the method of Omura and Sato [22,23]. Microsomes were solubilized prior to the assay. Solubilization was achieved by adding detergents such as Triton X-100, to obtain a final concentration of 0.5%. To the sample and reference cuvettes containing solubilized microsomes in buffer solution (10 mM phosphate buffer of pH 7.4, and 0.1 M EDTA), solid sodium dithionate was added to reduce the CyP450**.** The sample cuvette was read before carbon monoxide bubbling and again after bubbling with carbon monoxide for 1 min and absorbance was read, and the difference in spectra was recorded. The CyP450 activity was calculated using the extinction coefficient of 91 mM/cm for the absorbance of 450 nm minus the absorbance at 490 nm. The CyP450 activity was expressed as nmoles of CyP450/mg protein.

Results

The results are presented in Table-1. In the brain of control rats, highest levels of CyP450 were recorded in cortex (CC) and hypothalamus (HY), and the lowest activity was registered in cerebellum (CB).

Consequent on the injection of single dose of tramadol, the CyP450 activity showed an increase in all areas of the brain. Maximal increase was recorded in hippocampus (HI) followed by pons-medulla (PM), cortex (CC), Hypothalamus (HY) and cerebellum (CB) at 6 h after tramadol administration (Table-1).

Table-1: Changes in the Cytochrome P450 (CyP450) levels in different brain regions of rats at different time periods after administration of single effective dose of tramadol. Values are expressed in n moles of CyP450 /mg protein/h.

Brain Area	Indices	Control	3 Hours	6 Hours	12 Hours	24 Hours
Cortex	**Mean**	0.78	1.07	1.22	1.03	0.92
	SD (±)	0.12	0.11	0.15	0.13	0.09
	% Change		+37.18*	+56.41*	+32.05*	+17.95*
Cerebellum	**Mean**	0.13	0.17	0.19	0.15	0.12
	SD (±)	0.06	0.07	0.08	0.06	0.05
	% Change		+30.77*	+46.15*	+15.38*	-7.69
Pons-Medulla	**Mean**	0.44	0.62	0.69	0.56	0.48
	SD (±)	0.09	0.13	0.14	0.09	0.06
	% Change		+40.91*	+56.82*	+27.27*	+9.09
Hippocampus	**Mean**	0.19	0.27	0.30	0.22	0.18
	SD (±)	0.05	0.07	0.09	0.06	0.06
	% Change		+42.10*	+57.89*	+15.79*	-5.26
Hypothalamus	**Mean**	0.74	1.03	1.1	0.96	0.82
	SD (±)	0.05	0.06	0.06	0.08	0.09
	% Change		+39.19*	+48.65*	+29.73*	+10.81

Each value is the mean ± standard deviation (S.D.) of observations from six separate experiments. Values are significant at least at P < 0.05 in SNK test. * Significant.

After recording the highest increase in cortex and hypothalamus at 6 h following drug administration, the CyP450 levels showed a return towards the control levels during the subsequent periods. By 24 h following tramadol administration, CB and HI recorded non-significant decrease from the respective controls: CC, HY and PM recorded slight positive deviations at 24 h following tramadol injection (Table-1).

Discussion

The CyP450 system is responsible for the metabolism of a multitude of diverse pharmacological agents [24]. CYP enzymes in the mammalian brain have been shown to be highly localized in discrete areas and may thus alter the local action or concentration of neuroactive drugs [25]. Brain regions differ tremendously in their cellular composition, cell density and function, and the expression pattern of brain CYPs is also extremely varied [26].

Drugs or their metabolites can affect CyP450 enzymatic catalytic activity. The opioid activity of tramadol may well be consequent to metabolism of the parent drug to the active demethylated metabolite by the polymorphic CyP450 enzyme, debrisoquine 4-hydroxylase [17, 18]. Drug inhibition of a CyP450 enzyme occurs with the first dose of an inhibiting drug and reaches maximum inhibition when the drug reaches a steady state (4-7 half-life) level [27,28,29]. In the present study, the enzyme activity showed an increase in all areas of the brain following drug administration, which is an indication of the measure of metabolizing the drug. Tramadol is primarily metabolized into O-demethyl tramadol (M1) by CYP2D6. However, CYP2D6 polymorphism on the variability in pharmacokinetics, metabolism and pharmacodynamics of tramadol remains to be established [30]. With prodrugs like codeine and tramadol, people who metabolize them poorly exhibit little analgesic activity with the intake of these drugs because they do not form the necessary active metabolites, while people who metabolize them readily form the active metabolites exhibit significant analgesia [31].

An evaluation of the mechanism for the metabolic clearance of 315 different drugs revealed that 56% of them were primarily cleared through the action of the CyP450 enzymes [32]. Drug metabolizing enzymes represent a further major target of ongoing research in order to identify associations between an individual's genetic profile and drug response (pharmacogenetics). Polymorphisms of the CyP450 enzymes influence analgesic efficacy of codeine, tramadol and tricyclic antidepressants (CYP2D6). Blood levels of some NSAIDs are dependent on CYP2C9 activity, whereas opioid-receptor polymorphisms are discussed for differences in opioid mediated analgesia and side-effects [33]. The present study was intended only to examine the change in activity of the enzyme representing the CyP450 super-family in different areas of brain under different time periods, notwithstanding its polymorphism. Although it is unlikely that brain CYPs contribute to overall clearance of xenobiotics, they are able to metabolize a variety of compounds, including many drugs that cross the blood–brain barrier to produce their pharmacological effects within the brain [34].

Maihöfner et al. [35] suggested that there is a difference in the brain areas modulated by analgesia and antihyperalgesia. The differential activity of CyP450 recorded from different areas of the brain in controls as well as in tramadol-administered rats in the present study point towards the concept of metabolic compartmentation in the brain explained several years ago [36] and the possible implications of such compartmentation [37].

Most evidence to date suggests that metabolic characteristics of brain CYPs are similar to their hepatic forms. However, it is unlikely that brain CYPs contribute to the overall metabolism and clearance of xenobiotics. Rather, their importance lies in their localization in specific brain regions and brain cells, where they are most likely involved in the *in situ* metabolism of xenobiotic drugs and toxins and endogenous neurotransmitters and neurosteroids. Metabolism in the brain by CyP450 enzyme may have a profound influence on the on- and off-set of action and therapeutic efficiency of some of these drugs.

The changes were significantly observed in the cortex and hypothalamus which are in good correlation with the pain sensing centers (cortex and thalamus). Changes in the other areas were also found, although lesser when compared to those in cortex and hypothalamus.

It was reported that chronic administration caused a slight but significant elevation in the concentration of D-serine in the cortex, striatum, and hippocampus [38]. Tramadol (20 mg/kg, i.p.) administered acutely (single dose) in male wistar rats, induced a significant decrease in the α_2-adrenergic receptors at 24 h after dosing in all brain regions studied. The most pronounced effects were observed in all sub-regions of the olfactory system, nucleus accumbens and septum, thalamus, hypothalamus, amygdala, and cerebral cortex [39].

The enzyme CyP450 showed highest levels in activity at 6h time period, and then the activity returned to normal levels as in controls. The increase in levels of this enzyme following the administration of tramadol is clearly indicative of the enzyme getting quickly activated to metabolize the drug. It was apparent that as the drug got accentuated by 3 or 6 h after its administration, the CyP450 activity also shows a concurrent increase. Following this, as the effect of the drug started to gradually vane, the CyP450 activity also began to decline concurrently, and reverted to control levels.

Chronic use of morphine and/or tramadol in increasing doses causes neuron degeneration in the rat brain, which could contribute to cerebral dysfunction [40]. However the current work where only a single dose of tramadol is given indicated that even during non-induction or absence of pain in the rat, tramadol causes changes in the levels of the CYP450 enzyme activity that are not lethal.

Conclusion

The observed changes in CyP450 activity levels, without reference to the polymorphic forms of CyP450, presumably do not cause any physiological lesion since they got reverted to control levels after the time-limit of change under tramadol influence. This observation indicates that tramadol can be administered safely within reasonable limits without any adverse limits.

People in the field generally carry out investigations as related to nociceptive situations only. Our interest is to look at drug-effects leading to changes, if any, in biochemical parameters which may or may not be associated with pain conditions. It is not necessary to induce pain, since we do not expect that the drug effects on biochemical parameters will be any different under conditions of pain or no-pain, barring quantitative variations. In the process, however, if there are any qualitative differences as well in the biochemical parameters under the influence of the drug, they will be revealed.

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