Hacettepe University, Journal of Faculty of Pharmacy
Volume 24 / Number 1 / January 2004 / pp. 25-35

Interaction of Rizatriptan with Rat Liver Monoamine Oxidases

Received : 29.09.2004
Revised : 02.11.2004
Accepted : 04.11.2004

Gülberk Ucar *

Introduction

Migraine is a common neurological disorder characterized by paroxysmal headache, usually unilateral, and frequently accompanied by generalized sensorial hyperaesthesia and gastrointestinal disturbances such as nausea [1]. Migraine is thought to be caused by neurogenic vascular and inflamatory mechanisms and it has been suggested that serotonin (5-hydroxytryptamine; 5-HT), a neurotransmitter which has a complex role in basic processes such as sleep, memory, mood, hormone regulation, vascular and gastrointestinal smooth muscle contraction, may be involved in the genesis of migraine attacks [2,3]. Recently, successful treatment of migraine has been achieved by administration of selective 5-$HT_{1B/1D}$ agonists (triptans) such as sumatriptan, zolmitriptan, naratriptan, rizatriptan, almotriptan, and eletriptan [4,5]. Rizatriptan (MK-0462) (Figure 1) is an orally active, highly selective 5-$HT_{1B/1D}$ agonist that constricts intracranial, extracerebral blood vessels and inhibits the neuropeptides from perivascular nerves, thus causes vasoconstriction on dilated

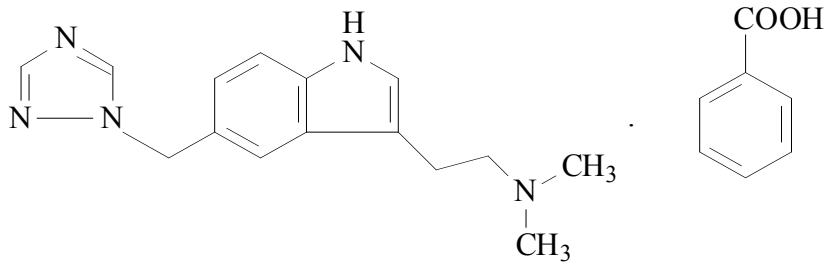


Figure 1
Rizatriptan (MK-0462)

* Department of Biochemistry, Faculty of Pharmacy, Hacettepe University, 06100 Ankara, TURKEY.

cerebral vessels [6]. Antimigraine effects of rizatriptan have been previously reported [7-9]. The *in vitro* metabolism of rizatriptan was suggested to be both species and tissue specific [10]. Although, 3-acetic acid derivative, the major metabolite of rizatriptan, was reported to be produced by oxidative deamination of the drug and rizatriptan was metabolized mainly by amine oxidases in human liver [11], the exact mechanism responsible for the oxidative deamination of rizatriptan by monoamine oxidases are still unclear.

Monoamine oxidase (MAO, EC 1.4.3.4) is a flavoenzyme which plays an essential role in the oxidative deamination of biogenic amines such as serotonin (5-HT), adrenaline (E), nor-adrenaline (NE) and dopamine both in central nervous system and in periferal tissues [12]. MAO is found in two different forms designated as MAO-A and MAO-B which are encoded by two different genes and distinguished by different substrate specificities and sensitivities to the selective inhibitors (13). MAO-A preferentially oxidizes 5-HT and NE and irreversibly inhibited by clorgyline, while MAO-B preferentially oxidizes benzylamine and phenylethylamine (PEA) and it is reversibly inactivated by pargyline. Dopamine, tyramine and tryptamine are reported as common substrates for both MAO forms[14]. However, substrate specificities of MAO isoforms differ with species and within each species with the tissues (15). MAO is capable of catalyzing the oxidative deamination of primary, secondary and tertiary amines and the oxidation is coupled to the reduction of flavin adenine dinucleotide cofactor [16].

The aim of this study is to evaluate the *in vitro* oxidative deamination of rizatriptan by MAO isoforms isolated from rat liver to assess the potential involvement of MAO isoforms in the metabolic pathway of rizatriptan and also determine the effects of known MAO inhibitors on this metabolism.

Materyal and Methods

Reagents:

All chemicals were purchased from Sigma-Aldrich,Co. The reagents were of analytical grade. The rizatriptan was kindly supplied by Merck&Co., Inc., USA.

Purification of MAO from the liver homogenates:

MAO was purified from rat liver (100-150 g) (Local Ethics Committee #: 2001/25-4) according to the method of Holt (17) with some modifications. Mitochondrial MAO was purified by isolation of mitochondria from the liver homogenates. Liver tissue (5-8 g) was homogenized 1:40 (w/v) in 0.3 M sucrose. Following centrifugation at 1,000 x g for 10 mins., the supernatant was centrifuged at 10,000 x g for 30 mins. to obtain crude mitochondrial pellet. The pellet was incubated with 3-[(3-Cholamidopro pyl)dimethylammonio]-1-propanesulfonate (CHAPS) of 1%, as detergent, at 37^{0}C for 60 mins. to isolate the enzyme from the mitochondrial membranes and centrifuged at 1,000 x g for 15 mins. The pellet was resuspended in 0.3 M sucrose and layered onto 1.2 M sucrose, centrifuged at 53,000 x g for 2 hours and resuspended in potassium phosphate buffer. It was kept at –70^{0}C until used.

Measurement of total MAO activity :

Total MAO activity was measured spectrophotometrically according to the method of Holt (17). The chromogenic solution consisted of 1 mM vanillic acid, 500 µM 4-aminoantipyrine, 4 U.ml^{-1} peroxidase in 0.2 M potassium phosphate buffer, pH 7.6. Assay mixture contained 167 µl chromogenic solution, 667 µl substrate solution (500 µM p-tyramine, 500 µM 5-HT or 20-1000 µM rizatriptan) and 133 µl potassium phosphate buffer, pH 7.6. The mixture was preincubated at 37^{0}C for 10 min. before the addition of enzyme. Reaction was initiated by addition of the homogenate (100 µl) and absorbance increase was followed at 498 nm at 37°C for 60 min. Molar absorption coefficient of 4654 M^{-1}. cm^{-1} was used to calculate the initial velocity of the reaction.

Selective measurement of MAO-A and MAO-B activities

Rat Liver homogenates were incubated with the substrates (p-tyramine at 500 µM to measure MAO-A and at 2.5 mM to measure MAO-B; 20-1000 µM rizatriptan) following the inhibition of one of MAO isoforms with selective inhibitors or at 500 µM to measure total MAO when no inhibitor had been included. Aqueous solutions of clorgyline or pargyline (50 µM), as selective MAO-A and –B inhibitor, were added to homogenates at the ratio of 1:100 (v/v) so the final inhibitor concentrations were 0.50 µM. Homogenates were incubated with inhibitors at 37^{0}C for

60 min. prior to activity measurement. After incubation of homogenates with selective inhibitors, total MAO activity was determined by the method described above.

Stock solution of rizatriptan (2 mM) was prepared in water. Working standard solutions were prepared by diluting the stock solution in the concentration range of 20-1000 µM in water daily.

The sensitivity of rizatriptan deamination with MAO isoforms was determined by using selective MAO-A inhibitors, moclobemide and clorgyline, and selective MAO-B inhibitor, pargyline, respectively. Homogenates were incubated with mentioned inhibitors at 37^{0}C for 60 min. prior to activity measurement when rizatriptan was used as substrate.

Reversibility of the inhibiton of rat liver MAO isoform activities by the inhibitor was assessed with dilution. Enzyme samples (10 times the final concentration) were incubated for 60 min. at 37^{0}C with different concentrations of moclobemide. The samples were then diluted 10 times into the assay mixture. A parallel experiment was carried out where the enzyme (10 times the final concentration) was incubated for 60 min. at 37^{0}C with an equivalent amount of water. The samples were then diluted 10 times into the assay mixture containing the same final concentrations of the inhibitor. Both sets were assayed for MAO-A and MAO-B activities using rizatriptan as substrate.

Data analysis:

The V_{max} and K_m values for deamination of substrates with MAO isoforms and kinetic data for interaction of the isoenzymes with inhibitors were determined by using the Lineweaver-Burk double reciprocal plot, by plotting 1/v vs 1/s analysed over a range of substrate concentrations. Kinetic data was analyzed using Systat (version 5.0) package program.

Protein determination:

Protein contents of the homogenates and PRP were determined according to the method of Bradford (19) with bovine serum albumin used as standard.

Results and Discussion

MAO was purified from the mitochondrial extracts of rat liver homogenates. The total specific MAO activity was found to be 43.45±6.80 nmol.mg protein^{-1} for rat liver homogenates when p-tyramine was used as substrate.

The initial rates of p-tyramine metabolism by both MAO-A and B were found to increase linearly with increasing enzyme concentration (data was not shown). K_m and V_{max} values of rat liver MAO-A and MAO-B were found to be 20.31±3.45 µM and 23.81±2.53 nmol.h^{-1}.mg protein^{-1}; 60.57±5.87 µM and 22.22±2.79 nmol.h^{-1}.mg protein^{-1}, respectively, when p-tyramine was used as substrate (Table I). Clorgyline and pargyline reduced MAO-A and B activities by 97% and 94%, respectively at 0.50 µM concentrations, indicating that the combined use of selective inhibitors with a non-selective substrate such as p-tyramine is suitable for distinguishing MAO subtypes.

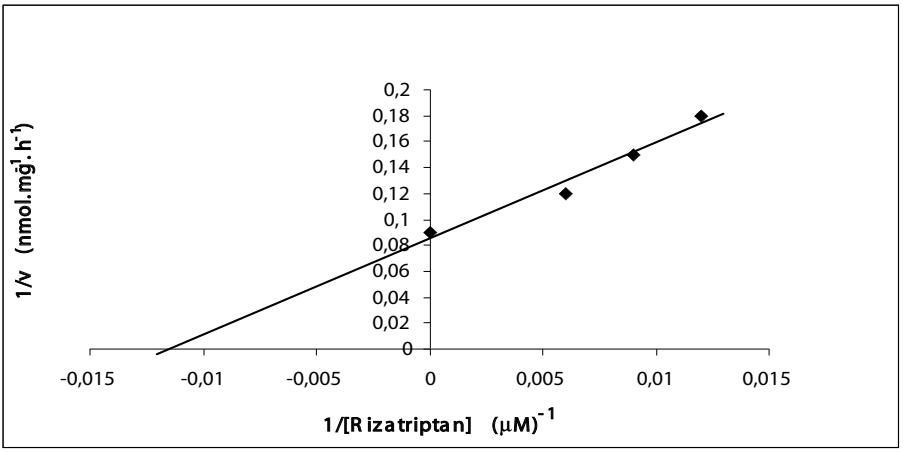
TABLE I
Apperent Michaelis-Menten Kinetic Parameters of Oxidative Deamination of Rizatriptan, p-Tyramine and 5-HT by Rat Liver Monoamine Oxidases.**

Reaction	**K_m (µM)***	**V_{max} (nmol.mg^{-1}.h^{-1})***
Oxidative deamination of rizatriptan by MAO-A MAO-B	 80.16±4.66 401.26±90.55	 11.23±3.68 25.03±5.16
Oxidative deamination of p-tyramine by MAO-A MAO-B	 20.31±3.45 60.57±5.87	 23.81±2.53 22.22±2.79
Oxidative deamination of 5-HT by MAO-A MAO-B	 68.22±6.54 -	 12.05±4.06 -

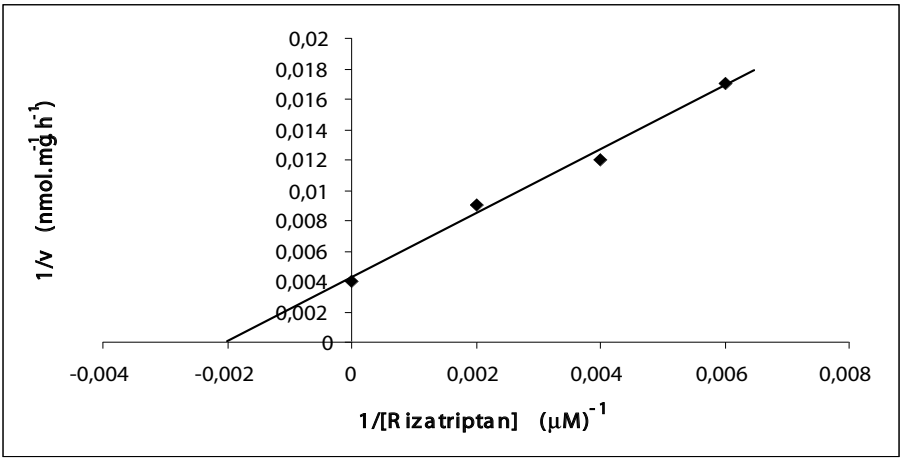
*Values represent the mean±SD of twelve measurements.

** Rat Liver homogenates were incubated with the substrates (p-tyramine at 500 µM to measure MAO-A and at 2.5 mM to measure MAO-B; 20-1000 µM rizatriptan or 5- HT) following inhibition of MAO with selective inhibitors (0.50 µM clorgyline or pargyline)at 37^{0}C for 60 min. before selective MAO activities were dete mined.

Rizatriptan was deaminated by MAO-A with K_m and V_{max} values as 80.16±4.66 µM and 11.23±3.18 nmol/h/mg protein, respectively (Table 1 and Figure 2a) while it has a little activity towards MAO-B with K_m and V_{max} values as 401.26±90.55 µM and 25.03±5.16 nmol.h^{-1}.mg $protein^{-1}$, respectively (Table 1 and Figure 2b). Since preincubation of rat liver homogenates with clorgyline and moclobemide (selective MAO-



(a)



(b)

Figure 2

Lineweaver-Burk plots of the deamination of rizatriptan by rat liver MAO-A (a) and B (b) Homogenates were preincubated either with 0.50 µM clorgyline or parglyline (selective MAOA and-B, inhibitors, respectively at 37°C for 60 min.) prior to incubation with rizatriptan, as substrate, in order to inactivate the other isoform.

A inhibitors) prior to incubation with rizatriptan caused a significant inhibition and pargyline had no inhibitory effect on MAO activity of homogenates, it was concluded that rizatriptan was mainly metabolized by rat liver MAO-A (Figure 3).

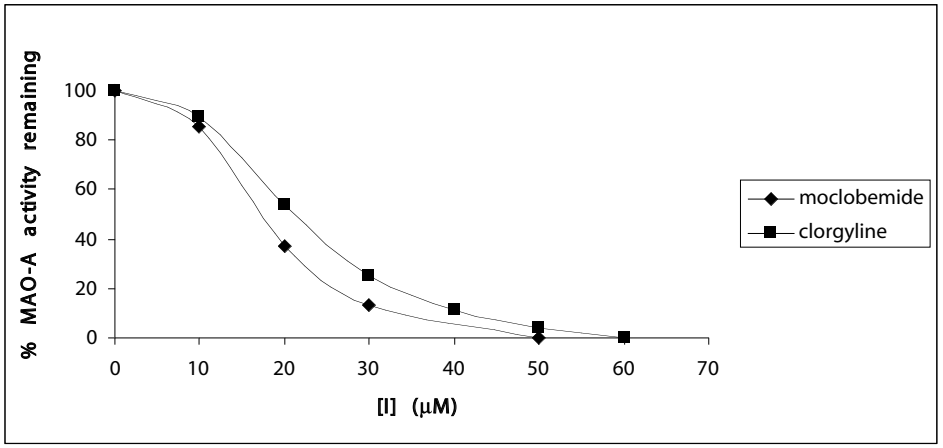


Figure 3
Inhibitory activities of clorgyline and moclobemide on the oxidative deamination of rizatriptan by MAO-A of rat liver. Liver homogenates were preincubated with various concentrations of inhibitors at 37°C for 60 min. prior to incubation with rizatriptan (100 μM). MAO-B was inhibited by incubating the homogenate with pargyline at 37°C for 60 min. prior to the inhibition experiments.

Selective and potent MAO-A inhibitor moclobemide inhibited MAO-A competitively with a K_i value of 57.10±5.20 μM in a time-dependent manner when rizatriptan was used as substrate (Figure 4). This data suggested that moclobemide might interact with the active site of the enzyme when rizatriptan was used as a selective substrate of MAO-A. Inhibition was found to be reversible.

Present study indicated that selective 5-$HT_{1B/1D}$ agonist rizatriptan was mainly metabolized via oxidative deamination through the action of MAO-A in rat liver. It was surprising to investigate that rizatriptan was metabolized by only one isoform although both isoforms present in the mitochondrial outher membrane in liver. However, it was previously reported that MAO-A and B isoforms in a tissue differ in their amino acid sequence, substrate specificity and biological function [20]. Possible

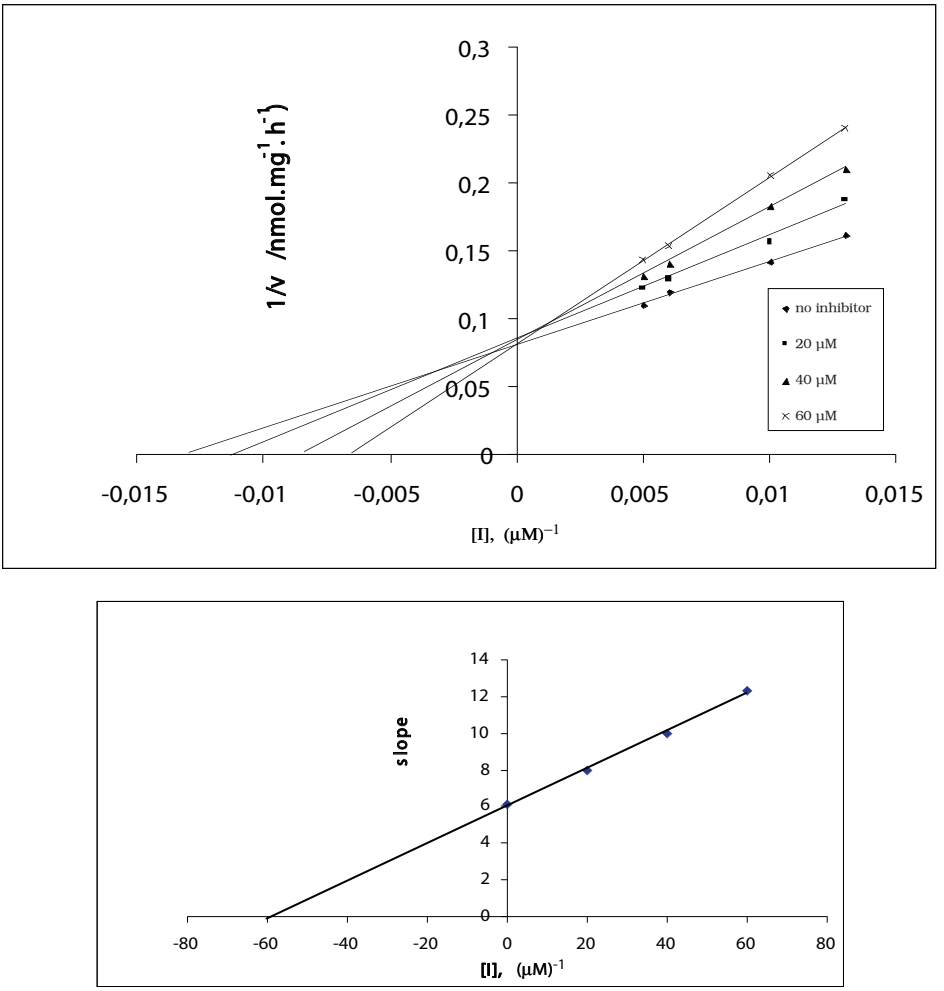


Figure 4
Lineweaver-Burk plot of inhibition of rat liver MAO-A by moclobemide (at 20 (°), 40 (5) and 60 (X) µM concentrations) when rizatriptan was used as substrate. MAO assay was performed at different concentrations of rizatriptan (20-1000 µM).
Inset shows the plot of slope of reciprocal plot, Km_{app} /V_{max} , vs 1/ [moclobemide]. K_i value was calculated as 57.10±5.20 µM.

explanation for the weak involvement of MAO-B in in vitro deamination of rizatriptan could be the presence of these isoforms in different parts of the cells in liver. It can be postulated that at least rizatriptan behaves as a preferential substrate for MAO-A in liver.

Since 5-HT, the endogen substrate of MAO-A, is oxidatively deaminated by MAO-A with a lower K_m value towards MAO-A in liver (Table 1), the possible inhibitory activity of rizatriptan on the deamination of 5-HT should be tested in further studies to clarify the mechanism of action of rizatriptan towards MAO-A in presence of endogen substrates such as 5-HT, dopamine or NE.

Briefly, present study indicated that potent MAO-A inhibitors, clorgyline and moclobemide, strongly inhibited the metabolism of rizatriptan in rat liver homogenates and MAO has to be considered as an effective enzymatic system implicated in drug metabolism in liver. Administration of MAO inhibitors together with rizatriptan is not recommended since specific MAO-A inhibitors may potentiate rizatriptan action.

Acknowledgements

The author thanks Merck&Co,Inc.,USA for the supply of pure rizatriptan.

Summary

Highly selective serotonin 5-$HT_{1B/1D}$ receptor agonist rizatriptan has recently been introduced for the treatment of acute migraine. Although it is suggested that its primary route of metabolism is via monoamine oxidases (MAO), deamination of rizatriptan with MAO isoforms is still unclear. This study was planned to investigate the *in vitro* interaction of rizatriptan with MAO-A and B isoforms of rat liver and to determine the effect of specific MAO inhibitors on this interaction. MAO was purified from rat liver mitochondrial pellets. Rizatriptan was found to be mainly deaminated by MAO-A with the K_m and V_{max} values of 20.31±3.45 µM and 11.23±3.68 $nmol.h^{-1}.mg^{-1}$, respectively. Moclobemide inhibited competetively and reversibly the activity of this form in presence of rizatriptan with a K_i value of 57.10±5.20 µM. Since the present preliminary data showed that rizatriptan was detected to be a good substrate especially for MAO-A isoform, it is suggested that tissue MAO activity may play a significant role in metabolism of rizatriptan. Administration of MAO inhibitors together with rizatriptan is not recommended since specific MAO-A inhibitors may potentiate rizatriptan action.

Keywords: Rizatriptan; Monoamine oxidase (MAO); 5-hydroxytryptamine (Serotonin); Oxidative deamination

Özet

Rizatriptan'ın Sıçan Karaciğer Monoamin Oksidazları ile Etkileşimi

Seçici serotonin 5-$HT_{1B/1D}$ reseptör agonisti olan rizatriptan, akut migren tedavisinde kullanılan yeni bir ilaçtır. Rizatriptanın başlıca monoamin oksidazlar (MAO) aracılığı ile metabolize olduğu öne sürülmekte ise de, ilacın MAO isoformları ile deaminasyonu hala açıklık kazanmamıştır. Bu çalışma, rizatriptanın sıçan karaciğer kaynaklı MAO-A ve B isoformları ile etkileşimini araştırmak ve özgül MAO inhibitörlerinin bu etkileşime olan etkisini tayin etmek üzere planlandı. MAO, sıçan karaciğer mitokondrial pelletlerinden saflaştırıldı. Rizatriptanın sırasıyla 20.31±3.45 µM and 11.23±3.68 $nmol.h^{-1}.mg^{-1}$ K_m and V_{max} değerleri ile MAO-A tarafından deamine edildiği bulundu. Moclobemide, rizatriptan varlığında bu formun aktivitesini 57.10±5.20 µM K_i değeri ile yarışmalı ve tersinir bir şekilde inhibe etti. Bu öncül veriler rizatriptanın MAO-A isoformu için iyi bir substrat olduğunu ortaya koyduğundan, doku MAO aktivitesinin rizatriptanın metabolizması açısından önemli rol oynayabileceğini düşündürdü. MAO-A inhibitörlerinin rizatriptanın etkisini potansiye edebileceği fikriyle bu inhibitörlerin rizatriptanla birlikte kullanılması önerilmedi.

Anahtar Kelimeler: Rizatriptan; Monoamin oksidaz (MAO); 5-hidroksitriptamin (Serotonin); Oksidatif deaminasyon

REFERENCES

1. Goadsyby, P.J., Hargraves, R.J.: Mechanisms of action of serotonin 5-$OH_{1B/D}$ agonists: insights into migraine pathophysiology using rizatriptan, Neurology, 55 (Suppl 2), S8 (2000).
2. Silberstein, S.D.: Rizatriptan versus usual care in long-term treatment of migraine, Headache, 35, 387 (1995).
3. Saxena, P.R., Ferrari, M.D.: 5-HT_1-like receptor agonists and the pathophysiology of migraine, Trends Pharmacol. Sci., 10, 200 (1989).
4. Jhee, S.S., Shiovitz, T.,. Crawford, A.W., Cutler, N.R.: Pharmacokinetics and pharmacodinamics of the triptan antimigraine agents, Clin. Pharmacokinet., 40(3), 189 (2001).
5. Tfelt-Hansen, P., De Vries, P., Saxena, P.R.: Triptans in migraine. A comparative review of pharmacology, pharmacokinetics and efficacy, Drugs, 60, 1259 (2000).
6. Williamson, D.J., Shepheard, S.L., Hill, R.G., Hargreaves, R.J.: The novel anti-migraine agent rizatriptan inhibits neurogenic dural vasodilation and extravasation, Eur. J. Pharmacol., 328, 61 (1997).
7. Goldstein, J., Ryan, R., Jiang, K., Getson, A., Norman, B., Block, G.A., Lines, C., The Rizatriptan Protocol 046 Study Group.: Crossover comparison rizatriptan 5 mg and 10 mg versus sumatriptan 25 mg and 50 mg in migraine, Headache, 38, 737 (1998).

8. Tfelt-Hansen, P., Teall, J., Rodriguez, F.: Oral rizatriptan versus oral sumatriptan: a direct comparative study in the acute treatment of migraine, Headache, 38, 748 (1998).
9. Block, G.A., Goldstein, J., Polis, A., Reines, S.A., Smith, M.E.: Efficacy and safety of rizatriptan versus standart care during long-term treatment for migrain. Rizatriptan Multicenter Study Groups, Headache, 38, 764 (1998).
10. Vyas, K.P., Halpin, R.A., Geer, L.A., Ellis, J.D., Liu, L., Cheng, H., Chavez-Eng, C., Matuszewski, B.K., Varga, S.L., Guiblin, A.R., Rogers, J.D.: Disposition and pharmacokinetics of the antimigraine drug, rizatriptan, in humans, Drug Metab. Dis., 28, 89 (2000).
11. Slaughter, D.E., Halpin, R.A., Davis, M.R., Maryanchik, A., Walsh, D., Vyas, K.P.: The in vitro metabolism of rizatriptan by human liver subcellular fractions, 7th North. Am. ISSX Meeting, 372 (1996).
12. Loscher, W., Lehman, H., Teschendorf, H., Traut, M., Gross, G.,: Inhibition of monoamine oxidase type A, but not type B, is an effective means of inducing anticonvulsant activity in the kindling model of epilepsy, J. Pharmacol. Exp. Ther., 288, 984 (1999).
13. Bach, A.W.C., Ian, N.C., Johnson, D.L., Abell, C.W., Bembenek, M.E., Kwan, S.W., Seeburg, A.H., Shih, J.C.: cDNA cloning of human liver MAO-A and B: molecular basis of differences in enzymatic properties, Proc. Natl. Acad. Sci., 85, 4934 (1988).
14. Yu, P.M., Tipton, T.F., Boultan, AA.: Current neurochemical and pharmacological aspects of biogenic amines, Current Prog. Brain. Res., 106, 85 (1995).
15. Ucar, G.: Substrate specificities of monoamine oxidase isoforms, FABAD J.Pharm. Sci., 27, 149 (2002).
16. Youdim, M.B.H., Harshak, N., Yoshioka, M., Araki, H., Mukai, Y., Gotto, G.: Novel substrates and products of amine oxidase catalysed reactions, Biochem. Soc. Trans., 19, 224 (1991).
17. Holt, A., Sharman, D.F., Baker, G.B., Pelcic, M.M.: Continuous spectrophotometric assay for monoamine oxidase and related enzymes in tissue homogenates, Anal. Biochem., 244, 384 (1997).
18. Segel, I.H.: Enzyme Kinetics, Wiley-Interscience Publication, New York, 125-135 (1975).
19. Bradford, M.M.: A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem., 72, 248 (1976).
20. Fowler, C.J., Tipton, K.F.: On the substrate specification of the two forms of monoamine oxidase, J. Pharm. Pharmacol. Pharm. Pharmacol., 30, 111 (1984).