Neωur ... selegiline againstt στP-induceωd dωøpamiEFgic tωoxICαE ey

WU Wei-[la ZHU Xing-Zu[1] ; GUAN Han-Jun WANG Ren-Goo JI Xin-Quan
-VNtjIJKFJd1ji JYJSMMe rj lMFMerba lldd cα ωtnese Al ... S'CtJeJilc
(Ɖepar1lnen1. 0./ !)lwrmαco7(
Shangha l 2α 3/.CJJifio)

KEY VORDS selegíline; dOpHlni ; COQ1U8 stiatu1l; high pre8Uliquid Cl1rønUllopraphy; kynuran1ne; quinnlines; lnOn()aJlin ()xida c; .fluorOletl)'; l-lnelhyl...4-phenyl- 2 3 6...leLra-ilydI p)Tidine; inbn 1 C57B.L nuce

ABSTRACT

Ant:1b study e neumpøtective fleMO"" scue nelllurestorat ve effeots of se[e line (Sel) 011 nigrostriatal dopaminergic meuronal svstenl and its inhìbition of brain monoamine oxidase B (MAO-B). lVIE1HODS:ηle striatal levels of dopamine and its metabolites *"lere* measured using HPLC wi electrochemical detection (HPLC-EC). le inhiliitωn of MAO-B 'was tested by an ånproved ß rimetcic ass **RESUL**TS: 1-Methyl-4-pk0lil-m 2. 3 6 etltra-hyl pyridine (M P) (1 kg-1 1[ ) duced the striatal døpamåne level by 13 % im mice. Selegiline (SBl 10 mg • kg-1 ip) beforebut not derBFIP treaunmt protected against MPfPlduced nigrostriatal dopaminergic ne oto Clty.ηlere were no di:fferentiaJ. effects between Sel and saline treatments on the reeoverv of striatal dopamine levels lich mmMK11y restored dudI182wk.1-Meutzv14phemytpyTi-?mum(MW+)(5mg-kji iMwoduced mo oopmmnergc neurotoxlqty.Flirthemore Sel selectively and ri reversibly i jbiid mouse brain MAO-B *in vlr*o (iQo= i7mHOi-L-1 95qJ COIliMerlee limits = 14 – 20 nrnoi-1 CONαJUSION; SelegiJine has ne rotec raLher than neucorescue or neur'restorat4ve eff; o h on MPfP-induced nigrostria idopazTlinerHρ nωonal degenerati'n which is direcùy rtin io iu selective Mid irreversible inhibition d brain MAO-B ac6vity.

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rrRODUCnON

Se]egi ne (Sel)a selective i ersible inlúbitor Ðf monoamine @xidase B (MAO-B) was used as an imp'rtant adju.vant ωl-dopa in e treatment of Parkinson'a desease (PD) and al used done as a pztrnary treatment in eaziy phase of PD[1]. In preclinical resωrch MPIP-lesion was used t'o establish a suitable animal model for iIDvestigating e dîsease. Sel could antagonize the dmn er c neUTotoxici f l-me.yl-4-ph@J1.[m 2 3 6-tetcahydr@pyridine (MPI Wh.ich had to be metaholizedωits active t()xic metabolite l-methyl-4-phenylpyridiniwn (MPP+) by MAO-B t'o induce the ne ω City[2]. However whether the neuroprotective effect of Sel was mainly due t'o its inhibitìon of MAO-B had become a ent topic ofωntroversy[3 4] Iie present study was desigmd to clanr whe Sei had neuroprotectìve nenrorescue or neu restomtwe actions agaimt e nωtøxioity of MPrP[4] and whether its actions were predominateiy induced by id1ibition of ain MAO-B.

MATERIALS AND 1ETI1ODS

Drugs and ehemicals Sel. MPTPand MPP+were purchased fZorn RBI'(Nati K MA USA).kvyn I ne and 4-hydroxyquinoline n from Sigma (USA) These compounds were

were dissolved 'eshly in line. DA[)OPAC and HVA were fron1 Fluka (S tzerland) and were dissolved in HCI04 0.1 lll0!..L - r eontaining sod. ium edetate (1 %) and NaO 5 (0.5' 0O).

M ce and ea rents CS7BI/6J m‹r (\veighing 20 g s2 g) \ve 8ulp! Shanghai Expel neno Anilmal Ccnter (i Ecate 5 cootfen d b the Anilnal1 Manage nent COIItmittee.chmwp Awtdemyof Sciences). Mìce ve randolnly assiglled to 12 treatment groups (n. =6). Drugs of each group was ven ip as sho\n in Tab 1. Mice of the 'tasl 4 groups we i ected rith Sel (10 mg. kg- t) 30 ln n before or after another illjection of MPTP (30 kg'" t) 01' 1PP+ (5 mg.kg - t) ol1 d 1 and d 7 0ron d 3 and d 9. spectively. Mice of e 5 and 6th groups were decapitabd on d 17.. and the 7 and 8th on d 24. Mice of t8e other groups '-ere killed on d 10.

pre mltion of S8lnples for' "II')LC Mouse brain striata were immediately dissect d outeighed and homoge ni. zed in ice-cold HClO4 0.1 mol'. L-1 contairrlng sodium edetate |%) and ih2O 5 (0.5 %). Aer eentri gamrk (20α x g4 'C15 min) e supernatmts we coHected for HPLC.

HPLC 0olditi A moaned methodL5 was u d th HPLC-EC (BAS West J..afayette IN lJSA). Apha -2 Of)S 3 mm coJumn (1 nunx 3.2 nun BAS). was llsed with a 0W fate nU1Întalned al .1 n ,- rnin-1. 'fhe full scale on the' d(\Lector l-'V|18 set to 10 nA. the operating potential w s 750 lllV.. and thternper d:lure was COllll 1Ied nL 25 :. '11t Innbile phase {'()nsjst d of ..nl(')I'lHehl{lrøA Iute O.J5 J'YOl- 'L-1 Na()H 0..l 17 1110l:- [1 sodiunl o('tyl 8ulfate 1 mmol-L l'sothm txietat43 0.i mmo1·LE1 and methHi01 (6 0O).

Prep onof brain supernatant Mou R[nS were hornogenized Ùl)tassium phosphate buffer (containing NazI'fPÛ4 10 KH2P04 10 nunol • L-1 F)H 7.2).ηle homogenate was oe.nlri'ge! d! at 3600 x g for 10 min twice and then ilie supematant was stored at - 20 aC. Prøtein c())ncentratiøns were adjusted ω5 g.L-1 m ß s!!J1lPeMaifl 6.

Assay of MAO-B inhibition An improv-ed medEOGI , was used.ηl1S fluorimetric me od l!!sek iJ1e the suhstrate for conversion y MAO-ß to 4-hydroxy loline which lleseøed [8 allJkaline media. The standard cnw.e was esloolis!@d by adding 4-hydro : moliRe 10μL (010 nmol) to brain s:m ø 1: μt amd assium phosphate buffeJ (1{à m 01• L-1 pH 7.2) 2 μL. To det mi[il@ MÅO-ß iohibition Sel 2.79 - 358

Tab 1. D of adtnhisoo.

	Dose o(dmglmg. kß-1.d-1								
	d 1	d2	d3	cl4-6	d7	(ll'8	d9	dω-16	d17 - 23
Jine	–	–	–	–	–	–	–		
Sele	10	10	10	–	10	10	10		
MPrP	30	30	30	–	30	30	30		
MPP+	5	5	5	–	5	5	5		
MPTP + SaJincl	30	30	30	–	30	30	30	–	
MPlP+ Selψ]nel	30	30	30	–	30	30	30	10	
MPTP+ lh	30	30	30	–	30	30	30	–	–
MVI'I)+ Selegilin	30	30	30	–	3()	30	30	10	10
seL ine+ 'rP	10+30	30	30	–	lG+ 30	30	30		
ғrP+ Sel line	30	30	30 + 10	–	30	30	30+ 10		
Sel me+MPP+	10+ 5	5	5	–	JO+ 5	5	5		
M ++ Selegiline	5	5	5 + 10	–	5	5	5 10		

lllnol. L-1 .ı00 !ıL was nl xcd wilh buffer 1α)μL and llpC1lunanl 50μL. and iticubnled nt 37 CC foı' 15 tni. n. ' fhf l'(:actioll was farled 1ŋ ndf:ling kyuununÎnc 3.07 nUllol · L-1. 10 f..t.1 LltH I ineubated af. 37 ℃ finn thtJr 15 Inin. r(1u aetion wus tenllìnatcd 1))' HCH). 0.6 1 0 f - I J{ μL. Hud lhen :-a()H 1 11101.L 2 llll I '\'CI fuÎxed. ·Ole tluot Scell(.'‹ illlf ıı ity H 4-hydn \ quillOHllC w'w rllcnSlll i ut 315 lHtl and n\ 380 l11H 1ŋ H flUOl 11 sp‹ cl ro(uol Hl(h (Hit: chì n50-10

S tistical aEMINis item1181WIPt xpress-ed \l : t s tUld analyzed using one-way ANOVA 10110" by DtUllleU"8 test. IC_{50} atad 95 % corúıdence .irnìts ve caløl la d hy og t nleth< .

RE JLTS

Ef Jects of Sel pre eatment on 1\1PfP induced depletion of DA Six injections of do n I ergic neurotoXÏn 1\TP (30 mg. kg-1 ip) decreased sutatdlevels of DA and its .uelabolites ($p < 0.01$). .PFetrea le:nt willib Sel (IO mg·kg-lip)on dIand d7am Eagommd e i\WIP-induced deple on of DA eVeis.BMSbl treatment on d 3 and d 9 *did* not rescüe dae(})Ss of DA DOPAC and HVA (*Tab* 2).

Tab 2. Elfects of Sel pretreatment on 1\σfP induα depletions in s iatal dopamine and its ?euboii g-) (wet sue). $n='$. . i s. $Dp < 0.05$ $cp < 0.01$ te. $ep < 0.05$r $P < 0.01$ EP.

Trl lmt	:DA	XPA	HiVA
Sali0f'	9.6 0.7f	0.8 o. 1f	0.9 0.2f
Sd	10.8 .6f	0.6 0.Jhl	0.6 (}.2bt.
P	2.7 0.6(;	0.3 0. Iu	0.3 0.J};
Sel+MJm2	8.3 0.gf	0.6 0.JM	0.6 0.lllc
M 1>+ Sel	3.9 1.5c	0.3 0.1!	0.4 0.Ju

Effi ts of Sel on naFaI recoveŋofDA When the MPTP-Iesioned nHce we aflowed tore Jè)r J wk 01' 2 - saline *or* Sel (10 rng. kg-1. ip) were given daily. No di ere 4 drt1 wr fotlnd 0n the co've d aω db m nl(l t ofi uu 'P we Mtiaty t d{tab3).

Tab 3. Effects of I on e natural reωveŋof striaol' DA in 1PTP-lesjoned mice and em s of PP'" on tbe striatal Jevel of DA. $n = 6$. x s. $op > 0.05$ $bp < 0.05$ $cp < 0.01$ *vs* line.

'rrmlNWRTOIfp	Vl .g-1 (wet tissue)
SaJin('	9.8 0.9
se	9.9% 0.7u
Tvli.PfP	2.5 0.6"
MJPrP+ saline	4.3 0.5c
MYr >+ Sel	4.2 0.5c
MPIP + *saline*	6.4 *0.7b*
MYfP+ Sel2	6.6 0.7b
MPP+	9.9 0.5s
Sel + MPP+	10.6 0.811
MPP+ + Sel	10.5 0.

Elfoots of MPP+ on s iatal DA level · 1 5 mg. kg-1 ip) inducno neurotoxi- The striatal of DA were n. changed levels by hpr and were not dected by Sel either (Tab :;) .

(hMbi6on Of MA0·B by sel selegiime 2.79 – 358μg·L-1) inhibited mouse brain MAO-ß activity *in vitro* at a concentration as low as 2.79 mnol. L-1. IC_{50} = 17 mnol. L-1. and 95%conftdenee lizmts=14-20mol-1 Ul (Fig).

DISCUSSION

Basic and clinical studies indicate oxidative meChanlsms ıorllot mHElale n180SMaul degenemuon iconiltblite to the eme e c of PD(4 8) nce and progression Although selhas been used Crimeatiy asa selective and imeversibIe inhibitor of MAOhB how the e m dmed814ts etfee

n slowng the progression-of PD mmains

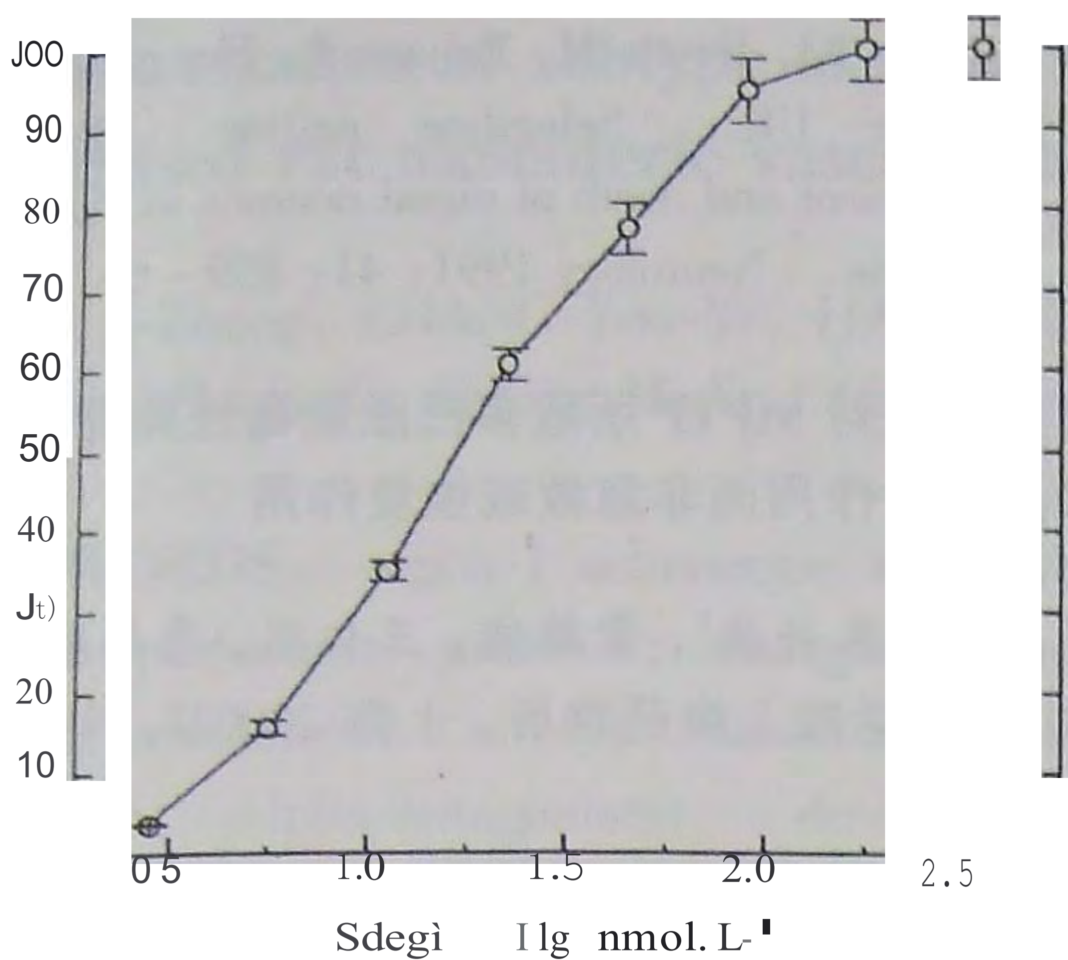

Fig 1. Inhibition of mouse brain MAO-B activity by sele. $n=$-'. x :t s.

uncleaI 0.lO).

The present study used MPTP-lesioned mice as an animal model of PD(2) to investigate the anti-neurodegenerative action of Sel. Pretreatment of Sel on d 1 and d 7 successfully mitigated the neurotoxicity of MPTP which suggested its neuroprotective action. But the injections of Sel on d 3 and d 9 which closely followed the 3 consecutive injections of MPTP did not block the depletions of striatal levels of DA DOPAC and HVA. It indicated that Sel was lack of neurorescue action. Further there were no differential effect between Sel and saline on the recovery of MPTP-lesioned mice which suggested Sel might not exert neurorestorative action. Our results also underpinned that the depletion of striatal dopamine caused by MPTP in mice appeared to be reversible and the partial restoration might be due to the species – specific neuronal plasticity in mice [1].

That systematically treated MPP+ the most active toxic metabolite of MPTP induced no neurotoxicity suggested that MPP+ could not penetrate the blood-brain barrier and MPTP was metabolized by brain MAO-B(12 13) which converted MPTP to MPP+. Therefore it is unlikely that we re-examined whether the neuroprotective effect of Sel was primarily induced by its inhibition of brain MAO-B during an improved enzyme activity[7]. The results demonstrated that Sel selectively inhibited the activity of brain MAO-B in vitro which substantially explained the action of Sel presently. Our results are in consistent with the data of clinical research including retrospective long-term study[14] which confirmed neuroprotective effects of Sel.

In summary it is neuroprotective effect rather than neurorescue or neurorestorative effect of Sel which is directly pertinent to the inhibition of brain MAO-B that contributes to its anti-MPTP-induced neurodegenerative toxicity. The possible neuroprotective strategies including MAO-B inhibitors should be used in the early phase of PD to retard its progression and extend the lifespan.

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: (selegilineSel) B (MAO-B)

:

(DA).

MAO-B. : fP (30 kg tp) DA (73 %).

Sel (WĐ mg.kg -1 ip) MPfP ; WEP Sel . MPJ'P 2 wk Se DA . MPP+. Sel MAO-B (IÇ =3.8; 5 %: 3.1 - 4.5μg.t- 1). : Sel MAO-B 1 ' i fP1'P

( )