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Oral Allopurinol for Preventing Mortality and Morbidity in Neonates with Moderate Hypoxic Ischemic Encephalopathy

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Abstract

Background: *Despite progress in neonatal care, the mortality and the incidence of neuromotor disabilities after perinatal asphyxia have failed to show substantial improvement. Delayed neuronal death following a perinatal hypoxic insult is partially due to xanthine oxidase-mediated production of cytotoxic free radicals. Evidence exists that allopurinol, a xanthine oxidase inhibitor,reduces delayed cell death in experimental models of perinatal asphyxia.*

Objectives: *To determine the effect of oral allopurinol on mortality and mobidity in newborn infants with hyoxic ischemic encephalopathy.*

Methods/Design: *Term newborns(gestional age≥ 36 weeks and birth weight ≥1800g) with precocious metabolic, clinical and electroencephalographic signs of moderate hypoxic ischemic encephalopathy will be recruited from the neonatal intensive care unit of menoufyia university hospital and will be randomised to receive allopurinol added to standard treatment of asphyxia. Allopurinol will be administered orally within the first 4 hours after birth through a nasogastric tube in total daily dose of 40 mg/kg of birth weight and continued for three days after birth. To evaluate the efficacy of allopurinol, the neurologic outcome of enrolled cases will be evaluated by serial neurologic and neuroradiologic examinations at 1 week after birth, at 3 months and at 12 months of age.*

Results: *Our results showed a significant decrease in morbidities in neonates who received allopurinol as an adjunctive to ordinary therapy ,as the incidence of seizures beyond the neonatal period, continued nasogastric tube feeding, epilepsy and its control with drugs and also the incidence of epilepsy were all less in those who received allopurinol. Also the results of neuroimaging were in favour of those neonates.*

Discussion: *This study will explore the possible theraputic role of allopurinol. Any favourable results of this research might open new perspectives about the reduction of cerebral insults following hyoxic ischemic encephalopathy.*

Conclusion: *Allopurinol is a simple and inexpensive intervention that serves as a beneficial adjunctive treatment for newborns suffering from hypoxic ischemic encephalopathy*

Keywords: *Hypoxic ischemic encephalopathy, allopurinol, xanthine oxidase inhibitor, morbidity, mortality.*

Background

Neonatal hypoxic-ischemic encephalopathy: disease incidence and pathogenesis

Neonatal encephalopathy is a common clinical condition affecting approximately 2 in 1000 neonates [1], and accounts for a substantial proportion of admissions to neonatal intensive care; 10%–15% of cases will die in the neonatal unit, 10%–15% will develop cerebral palsy and up to 40% will have other significant disabilities including blindness, deafness, autism, epilepsy, global developmental delay, as well as problems with cognition, memory, fine motor skills and behavior [2–6]. These problems are observed throughout development.

The principal pathogenic mechanism underlying neurological damage resulting from hypoxic ischemia (HI) is the deprivation of the glucose and oxygen supply, which causes a primary energy failure and initiates a cascade of biochemical events leading to cell dysfunction and ultimately to cell death [7-10]. Brain damage following a perinatal HI is an evolving process, which is comprised of two phases [8]. A first phase consists of an early energetic failure, where the oxidative energy metabolism of cells decreases and it promotes necrotic death. This is followed by a second phase of cell death, a late energetic failure, which occurs during reperfusion and reoxygenation several hours after the initial event and lasts for days [7,11,12]. The pathophysiology of this late energetic failure initiates a cascade of biochemical events , which involve nitric oxide synthetases activation,the production of cytotoxic free radicals, inflammation, membrane dysfunction and apoptosis[13].

During the late energetic failure, a consequent reperfusion injury often deteriorates the brain metabolism by increasing the oxidative stress damage. Particular roles for increase in extracellular glutamate, excessive activation of glutamate receptors (excitotoxicity), increase in cytosolic calcium (Ca2+) and generation of free radicals are emphasized [14,15–17]. Loss of mitochondrial membrane potential, combined with high concentrations of glutamate, opens calcium-permeable N methyl D aspartate (NMDA) glutamate channels and voltage-gated calcium channels allowing calcium to move into neurons [18]. This fact triggers enhanced production of free radicals and activation of lipases, proteases, and endonucleases.

As a consequence of lipases and proteases activation, the release of free fatty acids, especially arachidonic acid, will activate cyclooxygenase and will catalyze the formation of prostaglandins, which will liberate super-oxide free radicals. In addition, the formation of oxygen free radicals is also enhanced via hypoxanthine metabolization. Hypoxanthine is formed during the HI and metabolized to uric acid. Collectively, these processes will lead to a surge of the superoxide free radicals, which play a central role in further production of free radicals and other toxic compounds [14,15–17].

Description of the intervention

In part, the production of cytotoxic free radicals is dependent on xanthine oxidase-mediated

metabolism of hypoxanthine [19]. Studies using experimental animal models have found that the xanthine oxidase inhibitor, allopurinol and its metabolic product, oxypurinol, reduce free-radical formation and limit the degree of post-asphyxial brain damage [20-22].At high concentrations, allopurinol scavenges free radicals such as hydroxyl, chelates free iron, and inhibits lipid peroxidation and heat shock factor expression [23].

The most commonly reported adverse effects of allopurinol are skin rashes and hypersensitivity reactions [24].A very rare but severe hypersensitivity syndrome consisting of skin reactions (erythema multiforme, toxic epidermal necrolysis), fever, eosinophilia, and multiorgan failure has been described in people with concomitant renal impairment or thiazide diuretic use[25-26].

Methods

Type of study:

Controlled trials using randomised patient allocation from the neonatal intensive care unit (NICU) of Menoufyia university hospital.

Inclusion criteria:

The treatment with allopurinol will be reserved to newborns with gestational age≥36 weeks and birth weight≥1,800 g with moderate degree of asphyxia who will fulfill the following criteria:

1. Metabolic criteria: Apgar score ≤5 at 10 min, or persisting need for resuscitation, including endotracheal intubation or mask ventilation for more than 10 min after birth, or acidosis (pH≤7.0 and/or base deficit≥−16 mmol/L in umbilical cord blood or arterial, venous, or capillary blood) within 60 min from birth.
2. Neurological criteria (modified from Sarnat and Sarnat [27]) of moderate encephalopathy consisting of altered state of consciousness (irritability) and ≥1 of the following signs: hypotonia, or abnormal reflexes including oculomotor or pupil abnormalities, or absent or weak suctioning, or clinical seizures.
3. EEG criteria: moderately abnormal indicates a background tracing with upper margin >10μV and lower margin≤5μV; severely abnormal pattern refers to a tracing with upper margin <10μV and lower margin <5μV; often this is accompanied by bursts of high voltage activity (burst suppression).

Seizures are identified as periods of sudden increase in voltage, accompanied by narrowing of the band of EEG activity followed by a brief period of suppression or by a buildup of rhythmic activity of increasing amplitude and decreasing frequency [28].

At least one of the parents of the newborns meeting the inclusion criteria will be approached by the study investigator/nurse and informed of the study. A signed parental informed consent must be obtained.

Exclusion criteria

1. Newborns with gestational age less than 36 weeks, with birth weights less than

1800 g, or admitted at the NICU after 6 hours of life.

2. Newborns with major congenital abnormalities or other syndromes that include brain malformations, congenital viral infections or evidence of encephalopathy other than hypoxic ischemic encephalopathy (HIE).
3. Informed Consent refused.

Study population setting:

The included newborns were further subdivided into two groups:

Group I (study group): this group included 20 neonate with moderate HIE who received allopurinol plus the conventional treatment.

Group II (control group): this group included another 20 neonate who received only the conventional treatment.

Type of interventions

Allopurinol versus no drug administered orally through a nasogastric tube after dissolving 300 mg tablets in 5 cm of distelled water and given within four hours of delivery in a total daily dose of 40 mg/kg of birth weight and continued for three days after birth. Blood sampling will be performed to check renal, liver and metabolic balance. Allopurinol could have been given in conjunction with another intervention provided both treatment and control groups received the intervention.

Neurological follow-up

The duration of follow-up necessary for possible neuro-motor disabilities will be 12 months.

Every newborn will be evaluated at the end of the first week, at 3 and at 12 months of life by undergoing detailed neurological examination, Standard electroencephalographic (EEG) evaluation and a magnetic resonance imaging (MRI). The neurologic examination in the neonatal period went through the eliction of any abnormalities in autonomic system, motor system, state system and responsiveness while later on, the classic neurological examination was done with emphasize on motor, fine motor and social developmental abnormalities.

Types of outcome measures

Primary outcome

The neurological and neuroimaging outcome of these two groups of infants will be compared to determine whether adjunctive treatment with allopurinol improves the outcome. The primary outcome will be the combined frequency of mortality and severe neurodevelopment disability in survivors at 12 months of age. Severe disability is defined as any one of the components of severe sensorimotor disability (e.g. non ambulant cerebral palsy, severe developmental delay assessed using validated tools, auditory and visual impairments).

Secondary outcomes

The following secondary outcomes will also be assessed

1. Seziures in the neonatal period, either apparent clinically or by electroencephalo-graphic recordings.
2. Time to achieve full or all feeding independent of enteral tube feeding (days after birth), and/or incidence of continued enteral tube feeding at four weeks after birth.
3. Epilepsy (recurrent seizures beyond the neonatal period requiring anticonvulsant treatment).
4. Magnetic resonance imaging abnormal-lities.
5. Potential adverse effects of allopurinol (skin rashes, hypersensitivity reactions) that necessitates discontinuation of therapy.

Results

Our results showed that, there was non significant difference between the studied groups regarding the occurrence of seizures in the 1st 48h of life(p value=0.41)while the persistence of seizures beyond the neonatal period was significantly higher in group II (80%) than in group I (45%).Neonates who received allopurinol as an adjuctive treatment reached full oral feeding in a period shorter(X±SD=5.41±2.12)than those who didn't receive it(X±SD=11.54±5.98).In group I only 10 % of the studied neonates continued enteral feeding by nasogastric tube while a larger number of neonates of group II (40%) used these tubes in their feeding. In our study a significant difference in the studied groups in incidence of cerebral palsy was found(P=0.026) being higher in neonates who didn't receive allopurinol (65%)than in those who received it(30%).Also the occurrence of epilepsy was higher in group II(80%) than in group I (45%) and its control with monotherapy was higher in group I(30%) when compared with group II which needed two or more drugs to control .There were non significant difference between the two groups regarding the occurrence of auditory and visual impairments or even in death(P=0.51,0.72 and 0.61).All studied neonates were subjected to electroencephalographic (EEG) imaging ,which showed non significant difference between both groups in the percentage of normal and abnormal findings regarding the background at the end of the 1st week(P=0.14). while at 3rd and12th months of age there were significant differences ,with more abnormalities in neonates who didn't receive allopurinol (P=0.06).At the end of 1st week ,65% of neonates of group I had normal EEG background while only 40% of group II has normal findings. The abnormalities found at the end of 1st week were isoelectric EEG background in 5% of group I and 25% of II and burst suppression in 30% of neonates of group I and 35% in group II.

At the end of 3rd and 12th month, 75% of neonates of group I had normal EEG background while only 40% of group II had normal findings. The abnormalities found were, diffuse slowing in EEG background in 25% of group I and 55% of II and low voltage records in 0% of neonates of group I and 5% in group II. Regarding the Epileptogenic activity, although there were non significant difference between the studied groups at the end of 3rd and 12th months after birth, we can notice

that the percentage of generalized activities were higher in group II than I. At the end of 3rd month, focal activities were found in 15% of neonates of group I and 10% of group II while focal activities with secondary generalization were found in 0% in neonates of group I and 15% in group II and generalized activities in 20% of group I and 40% of group II. At the end of 12Th month, focal activities were found in 0% of neonates of group I and II while focal activities with secondary generalization were found in 0% of neonates of group I and 15% of group II and generalized activities in 20% of group I and 40% of group II. Magnetic resonance imaging(MRI) done at the end of 1st week, end of 3rd and 12th month showed significant difference between the studied groups as regards the abnormal findings which were higher in group II than in group I(P =0.03,0.06 and 0.6 respectively). At the end of 1st week,25% of neonates of group I had brain oedema while 45% of group II had it and non of the neonates of group I suffered from intra cranial hemorrhage while 15% of group II suffered from it.MRI done at the end of 3rd and 12th month showed that 20% of neonates of group I had brain atrophy while 60% of II had it and also showed that temporal and occipital encephalomalecia was found in 15% of group I and 5% of II.

Table 1 Clinical data among the studied groups

	The studied group				Test	P value
	Group I N = 20		GroupII N = 20			
	No	%	No	%		
Seizure in 1st 48 h	15	75	18	90	1.56#	0.41
Seizure beyond neonatal period	9	45	16	80	X^2=5.23	0.02
Period till achieving full oral feeding X ± SD Range	5.41±2.12 3 – 10		11.54±5.98 2 – 20		U 2.72	0.006
	No	%	No	%		
Continued nasogastric tube feeding after neonatal period	2	10	8	40	X^2=4.80	0.03
Cerebral palsy	6	30	13	65	X^2=4.91	0.026
Epilepsy	9	45	16	80	X^2=5.23	0.02
Control of epilepsy with mono therapy	6	30	3	15	1.29#	0.45
Control of epilepsy with two or more drugs	3	15	13	65	X^2=10.4	0.001
Auditory impairment	8	40	6	30	X^2=0.44	0.51
Visual impairment	5	25	6	30	X^2=0.13	0.72
Death in the 1st year	1	5	3	15	X^2=1.11	0.61

= Fisher's Exact test, X^2= Chi Square test, U = Mann Whitney U test

Table 2 Electroencephalographic (EEG) changes among the studied groups

	The studied group				Test	P value
	Group I N = 20		Group II N = 20			
	No	%	No	%		
Back ground						
EEG results at the end of 1st week						
Normal	13	65	8	40	3.93	0.14
Isoelectric	1	5	5	25		
BS	6	30	7	35		
EEG results at the end of 3 months						
Normal	15	75	8	40	5.38	0.06
Diffuse slowing	5	25	11	55		
Low voltage records	0	0	1	5		
EEG results at the end of 12 months						
Normal	15	75	8	40	5.38	0.06
Diffuse slowing	5	25	11	55		
Low voltage records	0	0	1	5		
Epileptogenic activity						
EEG results at the end of 3 months						
Focal	3	42.9	2	18.2	1.73	0.42
Focal with secondary generalization	0	0.0	1	9.1		
Generalized	4	57.1	8	72.7		
EEG results at the end of 12 months					FE	
Focal	0	0	0	0.0	1.36	0.52
Focal with secondary generalization	0	0	3	27.3		
Generalized	4	100	8	72.7		

FE= Fisher's Exact test

Table 3 Magnetic resonance imaging (MRI) changes among the studied groups

	The studied group				Test	P value
	GroupI N = 20		Group II N = 20			
	No	%	No	%		
MRI results at the end of 1st week						
Normal	15	75	8	40	5.01	0.03
Abnormal	5	25	12	60		
MRI results at the end of 3 months						
Normal	13	65	7	35	3.60	0.06
Abnormal	7	35	13	65		
MRI results at the end of 12 months						
Normal	13	65	7	35	3.60	0.06
Abnormal	7	35	13	65		
MRI results at the end of 1st week						
Normal	15	75	8	40	6.27	0.04
Brain oedema	5	25	9	45		
Intra cranial hemorrage	0	0	3	15		
MRI results at the end of 3 months						
Normal	13	65	7	35		
Brain atrophy	4	20	12	60	6.80	0.03
Temporal and occipital encephalomalecia	3	15	1	5		
MRI results at the end of 12 months						
Normal	13	65	7	35		
Brain atrophy	4	20	12	60	6.80	0.03
Temporal and occipital encephalomalecia	3	15	1	5		

Discussion

Hypoxic ischemic encephalopathy (HIE) currently constitutes one of the non-excluding causes of child cerebral palsy (CP) and, together with prematurity, is potentially preventable. For this reason there is an increasing interest in prevention policies as well as in research on neuro protection therapies that minimize cerebral lesion and concomitant disabilities. The xanthine-oxidase inhibitor, allopurinol reduces free radical formation, thereby limiting the amount of brain damage. Our study had demonstrated a beneficial effect of oral allopurinol administration on morbidities affecting the asphyxiated newborns as seen in reducing the risk of having seizures beyond the neonatal period, allowing better achievements of full oral feedings, reducing the incidence of epilepsy and its better control with mono therapy instead of two or more drugs and also reducing the occurrence of cerebral palsy. This goes with (Peeters-Scholte et al, 2003) who stated that treatment with allopurinol reduces cerebral energy failure and cytotoxic oedema due to reduction of free radical production following ischaemia and it also reduces tissue injury in in vitro [(29)]. A more recent paper by Gunes et al, 2007 reported an improved neurological outcome after postnatal allopurinol administration (40 mg/kg/day, 3 days, within 2 hours after birth) compared to a placebo in term asphyxiated neonates [(30)].

Our results also showed less EEG abnormal findings in neonates who received allopurinol when compared with those who didn't receive it.

Van Bel et al, 1998 showed an improvement of electrocortical brain activity after neonatal allopurinol administration[(31)].MRI studies that were done to our neonates showed significant difference between the studied groups at all periods of study, with less abnormal findings in neonates who received allopurinol ,this ensures the beneficial effects of allopurinol.

Conclusion

Our data shows that the use of oral allopurinol as an adjunctive therapy in treatment of neonatal asphyxia is beneficial and can reduce the consequent morbidities. The results of this study could be confirmed with additional studies that include larger numbers of newborns with asphyxia.

Competing Interests

The authors declare that they have no competing interests.

Author's Contributions

Dina Midan:conceived the study, she is the chief investigator, designed the study, collected data and drafted the manuscript.

Sameh Abd el nabi: participated in the design of the study, collection of data and is the performer of the EEG and the interpreter of its results.

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References

1. Pierrat, V.;Haouari, N.; Liska, A.; Thomas, D.; Subtil, D.; Truffert, P.; Groupe d'Etudes en Epidemiologie Perinatale. Prevalence, causes, and outcome at 2 years of age of newborn encephalopathy: Population based study. Arch. Dis. Child. Fetal Neonatal Ed.2005,90, F257–F261.
2. Marlow, N.; Budge, H. Prevalence, causes, and outcome at 2 years of age of newborn encephalopathy. Arch. Dis. Child. Fetal Neonatal Ed.2005, 90, F193–F194.
3. Cowan, F. Outcome after intrapartum asphyxia in term infants. Semin. Neonatol.2000, 5, 127–140.
4. Al-Macki, N.; Miller, S.P.; Hall, N.; Shevell, M.The spectrum of abnormal neurologic outcomes subsequent to term intrapartum asphyxia. Pediatr. Neurol. 2009, 41, 399–405.
5. Barnett, A.; Mercuri, E.; Rutherford, M.; Haataja, L.; Frisone,M.F.; Henderson, S.; Cowan, F.; Dubowitz, L. Neurological and perceptual-motor outcome at 5–6 years of age in children with neonatal encephalo-pathy: Relationship with neonatal brain MRI.Neuropediatrics2002,33, 242–248.
6. Glass, H.C.; Ferriero, D.M. Treatment of hypoxic-ischemic encephalopathy in newborns. Curr. Treat. Options Neurol. 2007, 9,414–423.
7. Du Plessis, A.J.; Volpe, J.J. Perinatal brain injury in the preterm and term newborn. Curr. Opin. Neurol.2002, 15, 151–157.
8. Ferriero, D. Neonatal brain injury. N. Engl. J. Med.2004, 351,1985–1995.
9. Perlman, J.M. Summary proceedings from the neurology group on hypoxic-ischemic encephalopathy. Pedia.2006, 117, 28–33.
10. Volpe, J.J. Neurology of the Newborn, 5th ed.; Elsevier Health Sciences: New York, NY, USA, 2008.
11. Wyatt, J.S.; Edwards, A.D.; Azzopardi, D.; Reynolds, E.O. Magnetic resonance and near infrared spectroscopy for investigation of perinatal hypoxic-ischaemic brain injury. Arch. Dis. Child.1989, 64, 953–963.
12. Yager, J.Y.; Brucklacher, R.M.; Vannucci, R.C. Cerebral energy metabolism during hypoxia-ischemia and early recovery in immature rats. Am. J. Physiol.1992, 262, 672–677.
13. Inder, T.E.; Huppi, P.S. In vivo studies of brain development by magnetic resonance techniques. Ment. Retard. Dev. Disabil. Res. Rev2000, 6, 59–67.
14. Fan, X.; Kavelaars, A.; Heijnen, C.J.; Groenendaal, F.; van Bel,F. Pharmacological neuroprotection after perinatal hypoxic-ischemic brain injury. Curr. Neuropharmacol. 2010, 8, 324–334.
15. Girard, S.; Kadhim, H.; Larouche, A.; Roy, M.; Gobeil, F.;Sébire, G. Pro-inflammatory disequilibrium of the IL-1 beta/IL-1ra ratio in an experimental model of perinatal brain damages induced by lipopolysaccharide and hypoxia-ischemia. Cytokine2008, 43, 54–62.

16. Hilton, G.D.; Nunez, J.L.; Bambrick, L.; Thompson, S.M.; McCarthy, M.M. Glutamate-mediated excitotoxicity in neonatal hippocampal neurons is mediated by mGluR-induced release of Ca2+ from intracellular stores and is prevented by estradiol. Eur. J.Neurosci.2006, 24, 3008–3016.
17. Kumar, A.; Mittal, R.; Khanna, H.D.; Basu, S.Free radical injury and blood-brain barrier permeability in hypoxic-ischemic encephalopathy. Pediatrics2008, 122, 722–727.
18. Cross, J.L.; Meloni, B.P.; Bakker, A.J.; Lee, S.; Knuckey, N.W. Modes of Neuronal Calcium Entry and Homeostasis following Cerebral Ischemia. Stroke Res. Treat.2010, 2010, doi:10.4061/2010/316862.
19. Warner DS, Sheng H, Batinic-Haberle I. Oxidants, antioxidants and the ischemic brain. Journal of Experimental Biology 2004; 207 :3221–312.
20. Palmer C, Vannucci RC, Towfighi J. Reduction of perinatal hypoxic-ischemic brain damage with allopurinol. Pediatric Research 1990; 27 :332–6.
21. Palmer C, Smith MB, Williams GD. Allopurinol preserves cerebral energy metabolism during perinatal hypoxic-ischemic injury and reduces brain damage in a dose dependent manner. Journal of Cerebral Blood Flow and Metabolism 1991; 11 :S144–9.
22. Palmer C, Towfighi J, Roberts RL, Heitjan DF. Allopurinol administered after inducing hypoxia ischemia reduces brain injury in 7-day-old rats. Pediatric Research 1993; 33 :405–11
23. Pacher P, Nivorozhkin A, Szabo C. Therapeutic effects of xanthine oxidase inhibitors: renaissance half a century after the discovery of allopurinol. Pharma-cological Reviews 2006; 58 :87–114.
24. Vazquez-Mellado J, Morales EM, Pacheco-Tena C, Burgos- Vargas R. Relation between adverse events associated with allopurinol and renal function in patients with gout. Annals of the Rheumatic Diseases 2001; 60 :981–3.
25. Arellano F, Sacristán JA. Allopurinol hypersensitivity syndrome: a review. Annals of Pharmacotherapy 1993;27:337-43.
26. Kumar A, Edward N, White MI, Johnston PW, Catto GR. Allopurinol, erythema multiforme, and renal insufficiency. BMJ 1996; 312 :173–4.
27. Sarnat HB, Sarnat MS: Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. Arch Neurol 1976, 33: 696 –705.
28. Al Naqeeb N, Edwards AD, Cowan FM, Azzopardi D: Assessment of neonatal encephalopathy by amplitude-integrated electroencephalography. Pediatrics 1999, 103: 1263 –1271.
29. Peeters-Scholte C, Braun K, Koster J, Kops N, Blomgren K, Buonocore G, et al.

F: Effects of allopurinol and deferoxamine on reperfusion injury of the brain in newborn piglets after neonatal hypoxia-ischemia. Pediatr Res 2003; 54:516-522.

30. Gunes T, Ozturk MA, Koklu E, Kose K, Gunes I. Effect of allopurinol supplementation on nitric oxide levels in asphyxiated newborns. Pediatr Neurol 2007; 36:17-24.
31. Van Bel F, Shadid M, Moison RM, Dorrepaal CA, Fontijn J, Monteiro L, et al. Effect of allopurinol on postasphyxial free radical formation, cerebral hemodynamics, and electrical brain activity. *Pediatrics.* 1998, 101:185-193.