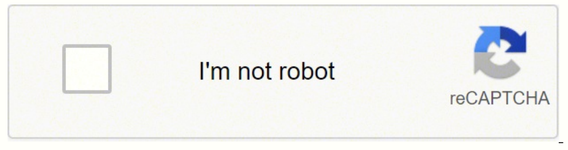

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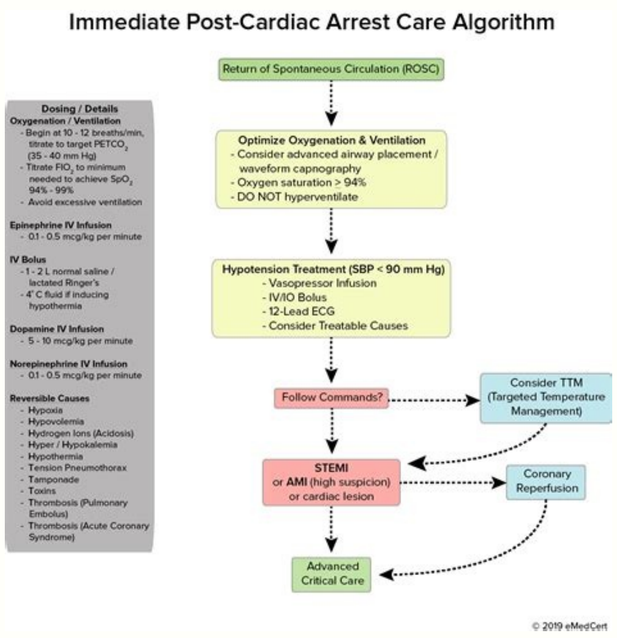

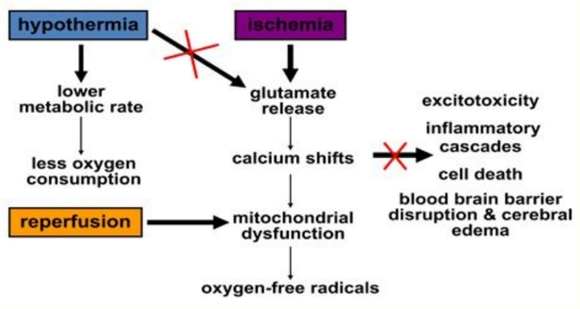

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RESEARCH ARTICLE Open Access

A pilot cohort study of cerebral autoregulation and 2-year neurodevelopmental outcomes in neonates with hypoxic-ischemic encephalopathy who received therapeutic hypothermia

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Abstract

Background: Neurodevelopmental disabilities persist in survivors of neonatal hypoxic-ischemic encephalopathy (HIE) despite treatment with therapeutic hypothermia. Cerebrovascular autoregulation, the mechanism that maintains cerebral perfusion during changes in blood pressure, may influence outcomes. Our objective was to describe the relationship between acute autoregulatory vasoreactivity during treatment and neurodevelopmental outcomes at 2 years of age.

Methods: In a pilot study of 28 neonates with HIE, we measured cerebral autoregulatory vasoreactivity with the hemoglobin volume index (HVx) during therapeutic hypothermia, rewarming, and the first 6 h of normothermia. The HVx, which is derived from near-infrared spectroscopy, was used to identify the individual optimal mean arterial blood pressure (MAP_{OPT}) at which autoregulatory vasoreactivity is greatest. Cognitive and motor neurodevelopmental evaluations were completed in 19 children at 21–32 months of age. MAP_{OPT}, blood pressure in relation to MAP_{OPT}, blood pressure below gestational age + 5 (ga + 5), and regional cerebral oximetry (SO_2) were compared to the neurodevelopmental outcomes.

Results: Nineteen children who had HIE and were treated with therapeutic hypothermia performed in the average range on cognitive and motor evaluations at 21–32 months of age, although the mean performance was lower than that of published normative samples. Children with impairments at the 2-year evaluation had higher MAP_{OPT} values, spent more time with blood pressure below MAP_{OPT}, and had greater blood pressure deviation below MAP_{OPT} during rewarming in the neonatal period than those without impairments. Greater blood pressure deviation above MAP_{OPT} during rewarming was associated with less disability and higher cognitive scores. No association was observed between (SO_2) or blood pressure below ga + 5 and neurodevelopmental outcomes.
(Continued on next page)

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Short paper

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Hypothermia and hypoglycemia induced by anti-CD3 monoclonal antibody in mice: role of tumor necrosis factor*

The possible involvement of tumor necrosis factor-α (TNF) in the metabolic disturbances induced by anti-CD3 monoclonal antibodies (mAb) was analyzed in DBA/2 mice injected with 50 μg of the anti-murine CD3 mAb 145-2C11. First, we found that 145-2C11 induces a profound hypothermia maximal between 3 h and 6 h after the injection (at 3 h: −3.0 ± 0.1 °C) as well as hypoglycemia (blood glucose levels at 6 h and 24 h: 76 ± 13 mg/100 ml and 92 ± 22 mg/100 ml, respectively, $p < 0.001$ as compared with control values). These metabolic changes are preceded by the release of TNF into the circulation (peak serum TNF levels at 2 h: 50 ± 23 pg/ml, $p < 0.01$ as compared with controls). The release of TNF induced by 145-2C11 depends on the effect of the mAb on T cells as it is not observed in athymic nude mice while lipopolysaccharide-resistant C3H/HeJ mice also display a significant rise in serum TNF (peak levels at 2 h: 59 ± 44 pg/ml). Pretreatment of DBA/2 mice with 12 mg of rabbit anti-murine TNF antibodies completely prevents the hypothermia while the hypoglycemia is significantly attenuated. Finally, $F(ab')_2$ fragments of 145-2C11 induce only a transient hypoglycemia (blood glucose levels at 6 h: 109 ± 14, $p < 0.001$ as compared with controls) but neither hypothermia nor significant TNF release. We conclude that TNF is a major mediator of the acute metabolic changes induced by the intact form of 145-2C11.

1 Introduction

mAb directed against the CD3 complex of T lymphocytes represent potent immunosuppressive agents both *in vitro* and *in vivo* [1–4]. Before inhibiting T cell functions, they induce a transient T cell activation resulting in the release of different cytokines [5]. Indeed, we and others recently observed high serum levels of TNF-α, IFN-γ and IL-2 in the hours following the first injection of OKT3 in kidney transplant recipients [6, 7]. Thus, the well-known first-dose reactions commonly experienced by OKT3-treated patients [2, 3] could be mediated by cytokines.

The availability of an interspecies CD3 mAb (145-2C11) which shares many properties with OKT3 [8] allows the experimental investigation of the acute metabolic changes induced by this type of mAb. In the present study, we observed that mice injected with the 145-2C11 mAb develop acute hypothermia and hypoglycemia in association with the release of TNF in the circulation. In order to determine the role played by TNF in the pathogenesis of these metabolic changes, we analyzed the effects of the administration of rabbit anti-TNF antibodies prior to the injection of 145-2C11 mAb.

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2 Materials and methods

2.1 Mice

Six to 9-week-old female mice of DBA/2, nu/nu and nu/+ strains were obtained from Iffa Credo (l'Arbresle, France). LPS resistant C3H/HeJ mice were purchased from Olac (Bicester, GB).

2.2 Production and purification of anti-CD3 mAb

The hamster mAb 145-2C11 directed against mouse CD3 [8] was prepared from culture SN of 145-2C11 hybridoma cells by affinity chromatography on protein A-Sepharose (Pharmacia, Uppsala, Sweden).

$F(ab')_2$ fragments of 145-2C11 mAb were prepared by incubating purified mAb with pepsin (Worthington, Freehold, NJ) at a ratio of 100:2 (w:w), in 0.1 M sodium acetate buffer, pH 4.5, for 20 h at 37 °C. The pH of the solution was then adjusted to 7.4 with 1 M Tris-HCl before dialysis against PBS for 24 h. Undigested mAb was removed by adsorption on protein A-Sepharose. The purified $F(ab')_2$ fragments were shown by gel electrophoresis and LIAISA using a mAb specific for the Fc portion of the 145-2C11 (clone AH6, generated in our laboratory) to contain < 1% of intact IgG; in addition, they were unable to induce spleen cell proliferation in fluid phase [9]. The immunosuppressive potential of these $F(ab')_2$ fragments was maintained *in vivo* as their injection induces a major depression in cytolytic T cell activities against alloantigens (data not shown).

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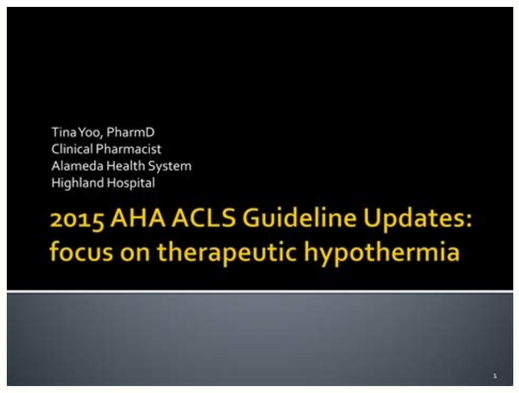

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TO ksaT tnemeganaM erutarepmeT detegraT naisA .HC gnauH ,S alawaihtoP ,S asgnopeewatgneuM ,CM gnaihC ,M ikibiA .G llorraC ,C yenorA ,J nahC ,I ttocS .rpA 5002 .aisehtseanA .CG gnoW ,R hgeyaS-lE ,S iaseD ,J regniS ,V civoljahiM ,D regnatS .esaesid noitaticsusertsoP .tcO 7991 .8-324:83 .02 beF 7102 .beF 7002 .22 beF 1002 .stceffe cimanydomeh esrevda laitnetop diova ot etalitnev revo ton oD .veR tsyS esabataD enarhcoC .troper yranimilerp a :tserra caidrac latipsoh-fo-tuo fo sroivirvus esotamoc ni diulf suonevartni dloc-eci ,emulov egral gnisu aimrehtopyh decudnI .noitaticsuseR .]tcartsba[amuartoruen ni aimrehtopyH .raM 0002 .D leppiD ,M tremeG nav ,B proW red nav ,H gotreH ned .9-131:)2(01 .)I MIALCCeR(aimehcsi etuca larberec ni gnilooc dna noisufreper ralucsavodnE .guA 9002 .la te ,D nugyZ ,A akdalaV ,LG notfilC .tserra caidrac retfa seigetarts evitcetorporuen :erac evisnetni evitaticsuertsoP .7-1 .guA 3102 .yevrus ecitcarp tnerruc a :tserra caidrac morf noitaticsuer retfa desurednu si aimrehtopyh decudnI .tecnaL .19-4851H:)5(282 .307 noissucsid ;207-696:)3(96 .aimehcsi fo tesno eht retfa detutitsni nehw neve sisorcen laidracoym secuder aimrehtopyh lanoigeR .yrujni niarb citamuart rof)CÂºÃ5.73 ot CÂºÃ53(seipareht gnilooc tsedoM .*tserra caidrac noitallirbif ralucirtnevnon morf noitaticsuer retfa aimrehtopyh citupareht latipsoherp fo noitcudnI .7-181:)2(81 .naJ 1002 .JA nnuG ,RT nnuG ,J ecirneP ,RM nittaB .GL rubliW ,AR luapueS .302-1VI:)lppus 42(211 .3102 .21-7002:)61(161 .beF 6002 .la te ,DM tsiuB ,WT yarG ,AS dranreB .beF 3002 .tserra caidrac retfa CÂºÃ63 susrev CÂºÃ33 ta tnemeganam erutarepmet detegraT .71 beF 6991 .srotagitsevnI)TSAHI(lairT yregruS msyruenA rof aimrehtopyH evitarepoartnI eht dna ,CJ renroT ,RW ekralC ,JB namdniH ,MM ddoT .6-181:)2(46 .7-373:)6(43 .erusserp lainarcartni wol htiw stneitap derujni daeh ylereves rof aimrehtopyh dlim fo ycaciffe eht fo lairt dellortnoc dezimodnar evitcepsorp al ,oenatucrep ociranoroc otnevretni'l rep enoizatulav alla ,angiugnas enoisserp alled ollortnoc la ,enoizanegisso'lla enoiznetta atlom edeihcir)CSOR(aenatnops enoizalocric alled onrotir li opod etneizap led aruc aL .nehganepoC id oploc led oiduts ol :ollortnoc-osac id oiduts onu :elaicifrepus otnemadderffar li osrevartta otuca sutci noc itailgevsir itneizap ni astedom aimretopi eraigudni id azzerucis e ÃtilibittaF .epacsidem ehcidem eizitoN .8-363 :)3(97 .DP NEDYL ,SK PAPAR ,ES MENSH ,MT NEMME ,ZK AKALUG .9-645 :)4(39 .ocinegoidrac kcohs e oenatucrep ociranoroc otnevretni noc itattart itneizap noc ezneirepse :oreiladepso irouf ocaidrac otserra opoD acitueparet aimretopI .sT neslO ,U rebeW ,J htieR ,SH nesnegroJ ,HB nessumsaR ,PLââ DRAAGSREMMAK .M OSOLEV ,M AVLIS ,M AIERROC .7-1401 :)4(53 .28-564S :)2 enoizelppus 81(231 .5002 oiggam 91-81 ;DM ,adsehteB ;elatanirep aissifsa e aimretopi us dhciN pohskrow :a otatneserP .34-639 :)7(75 .inibmab id inimret ni otallortnoc e otazzimodnar atolip oiduts onu rep esab emoc ilamina inoizavresso :elatanoen aitapolafecne rep oretni oproc led aimretopI .15-4472 :)21(33 .1 RAM 1102 .)ihccir(snnamtraH idderf irotagitsevni ilged enoisufni adipar al rep ,la te ,P noremaC ,K htimS ,AS dranreB .BL rekceB ,lT keoH nednaV ,nK gnauH ,WJ eehR ,sB allebA .602-7912 :)32(963 .ehcitamuart ilarberec inoisel rep)c idarg 5,73 a C idarg 53 ad idarg 53(otnemadderffar id etsedom eipareT .naJ 3991 .15-442 :)2(111 .23-92 :)1(96 .enamu inoisnemid id ilaim ni acaidrac aticsu e otrafni'led inoisnemid ,acidracoim arutarepmet us eralocsavodne otnemadderffar led otteffE .ocaidrac otserra'l opod etneizap led otatlusir li eraroilgim rep acitueparet aimretopi'lled enoizatnemelpmi ecaciffe :acinilc acitarp alla avorp allaD .oilgul 4591 .hl ewoL ,WH edirbliK ,JA recnepS ,hN rukahT .ero 42 onemla rep)C idarg 63 a 23 ad(F idarg 2,39 a 6,98 a itadderffar eresse onoved iudividni ilG .otroppar aznegreme aznegreme id eranomlopoidrac enoizaminair al rep noitaicossA traeH naciremA adiug eeniL 5002 .37-5681 :)7(43 .eladomitlum enoizanegorporuen al e atarim arutarepmet alled evitatitnauQ .tnI lorueN gruS .?tserra caidrac fo srovivrus tifeneb aimrehtopyh citupareht seoD .36-655:)8(443 .C uhZ ,M uY ,J gnaiJ deM nretnI .JA nnuG ,DP namkculG ,RT nnuG ,AJ eteozeD ,RM nittaB .ceD 1000002222220 4-693:)4(62 .tserra caidrac latipsoh-fo-túrof setar noitaticsuser ni ytilibairaV .]naigewroN[ ]!sevil erom sevas tserra caidrac retfa aimrehtopyh citupareht[.setar evitisop-eslaf esaerced ot)htaed niarb ,.e.i(erac gniwardhtiw nehw yllaicepse ,yek si tnemssessa cigolorueN weiver erutaretil A :ekorts cimehcsi dna aimrehtopyh citupareht .sisotpopa dna noitcnufsyd lailehtodne ralucsav metsysitlum sesuac tserra yrotalucric cimrehtopyH .)CB 573-064(setarcoppiH .VK sedohR ,WD htimS ,BL rekceB .ekcimehcsi etuca aimrehtopyh citupareh D 8002 .22-311B:2 lppus 63 .nuJ 3102 .ES sbocaJ .6-249:)01(61 .rpA 7002 .guA 9991 .WM oninnoD ,NM ihccoC ,K greB ,A rebU ,AT nosrebiG ,YW miK .71 guA 0102 .erac ralucsc avoidrac ycnegreme dna noitaticsuser yranomlupoidrac rof etadpu senileduig noitaicossA traeH nacirem A 5102 :erac tserra caidrac-tsoP :8 traP .tsigolorueN .rpA 6002 .6-1522:)9(13 .24-737:)7(221 .4-094S:)lppus 21 43 .9-082:)6(9 .RA sebriG ,MS namedreeP ,RE regubsnjiR ,HK namredloP .diulf suonevartni dloc-eci fo semulov egral fo esu htiw yrujni cigoloruen fo sepyt suoirav htiw stneitap ni aimrehtopyh fo noitcudnI .3102 ,52 rebmevoN .la te ,F zretS ,M rezloH ,A renieZ .eraC tirC nipO rruC .la te ,M adenaT ,H otomigus ,T ikazoihS .bateM wolF doolB bereC J .tserra caidrac fo srovivrus esotamoc rof emocuto gnissessA .stluser elbissop tseb eht htiw slaudivini detaticsuser gnidivorp ot lacitirc si erac siht fo ytilauq ehT .guA 9102 .3112 .31 na5 002 .AC nosllitS ,K riahC ,ID relsseS ,WD oaG ,WM eaD .91 guA 4102 .5-3391:)7(74 ?deriapmi yllacigoloruen ylereves ro ,evitategev ,daed tneitap siht sI .GJ neveoH red nav ,A nestirreG ,M ithazzE ,WC srekeamedeoH .la te ,LA nesnetsirhC ,M ,M leomvokS ,K mlohgarK .31-702:)2(.31-702:)2(27 .icitilobmort ia ottepsir otireferp ¨Ã)ICP(oenatucrep ociranoroc otnevretni'L .aigrurihC .enoizaminair id oeporue oilgisnoC li e ,G htimS ,WB regittoB ,J raoS ,DC nikaeD ,PJ naloN]otnematneirO[.L snehgyuH ,Y ettohciM ,G regnibE ,L enroC ,S issirdI-imihcaH .inna 7 id etrooc id oiduts onu :acitapoidi eralocirtnev enoizallirbif alla itussivvarpos ien ecocerp enoizazziralopir allus acitueparet aimretopi'lled itteffe ilged