UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE FARMÁCIA

Bianca de Moraes Fracasso

Caracterização de produtos finais de glicação avançada (AGEs) em modelo animal de infarto agudo do miocárdio

PORTO ALEGRE

2013

Bianca de Moraes Fracasso

Caracterização de produtos finais de glicação avançada (AGEs) em modelo animal de infarto agudo do miocárdio

Trabalho de Conclusão do Curso de Farmácia da Universidade Federal do Rio Grande do Sul – UFRGS.

Orientador: Drª. Gabriela Corrêa Souza

Co-Orientador: Dr. Michael Everton Andrades

Porto Alegre

Sumário

O presente trabalho foi realizado no formato de artigo de acordo com normas de publicação da revista: Journal of Molecular and Cellular Cardiology

Caracterização de produtos finais de glicação avançada (AGEs) em modelo animal de infarto agudo do miocárdio

Bianca de M. Fracasso[1*], Amanda Phaelante Pinto[1,2], Fernando Schwengber[1], Virgílio da Rocha Olsen[1], Michael Andrades PhD[1,2] and Gabriela Corrêa Souza PhD[2,3].

[1]Laboratório de Pesquisa Cardiovascular, Centro de Pesquisa Experimental, Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcelos 2350, Sala 12201, Porto Alegre, RS, 90035-003, Brasil; [2]Programa de Pós-Graduação em Ciências da Saúde: Cardiologia e Ciências Cardiovasculares, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil; [3]Professora de Nutrição, Faculdade de Medicina, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil.

***Autor correspondente**

Bianca de M. Fracasso, Laboratório de Pesquisa Cardiovascular, Centro de Pesquisa Experimental, Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcelos 2350, Sala 12201, Porto Alegre, RS, 90035-003, Brasil. Tel./Fax: +55 51 33598844. E-mail: biancafracasso@gmail.com

Resumo

Produtos finais de glicação avançada (AGEs) são continuamente gerados pelo organismo e sua produção pode ser estimulada em algumas enfermidades tendo impacto no processo inflamatório. A formação dos AGEs esta aumentada na insuficiência cardíaca e no infarto agudo do miocárdio (IAM) mas ainda faltam estudos de caracterização em modelos animais representativos dessas condições A partir disso, o objetivo deste trabalho é caracterizar a formação de AGEs em ratos submetidos ao IAM e acompanhados por 28 dias. Ratos Wistar machos foram randomizados para receber cirurgia *sham* (n=8) ou de indução de IAM (n=9). Coletou-se sangue nas primeiras 12 e 48 horas e 7, 14 e 28 dias após a cirurgia. Análises foram realizadas pelas técnicas de formação de *browning*, fluorescência e imunodetecção por dot blot. Os resultados encontrados não mostraram diferença significativa nos níveis de AGEs entre o grupo *sham* e o grupo IAM quando comparados dentro de cada tempo. Entretanto, o grupo IAM apresentou aumento progressivo na flourescência plasmática ao longo do tempo, diferente do grupo *sham* que apresentou retorno ao nível de AGEs no baseline ($P < 0,05$). Usando imudodetecção, encontramos aumento de AGEs no grupo IAM tanto 12 horas (99,6 ± 4,1 vs. 126,9 ± 9,2, *sham* e IAM, respectivamente; $P < 0,05$) quanto 48 horas (92,7 ± 5,0 vs. 135,5 ± 10,1, *sham* e IAM, respectivamente; $P < 0,01$). Os nossos resultados sugerem que o modelo da indução de IAM por ligadura da artéria coronária descendente anterior promove um aumento transitório precocemente de AGEs que não é sustentado ao final dos 28 dias de seguimento.

Palavras-chave: AGEs, glicação, infarto agudo do miocárdio, insuficiência cardíaca

1 INTRODUÇÃO

Produtos finais de glicação avançada (AGE - do Inglês *Advanced Glycation End-Products*) são continuamente gerados pelos organismos sadios, principalmente no metabolismo energético. Além das fontes endógenas, os AGEs podem ser adquiridos na dieta, principalmente em alimentos assados ou fritos [1]. Sua produção também é estimulada em condições pró-inflamatórias ou hiperglicêmicas [2, 3].

A glicação inicia com a formação de uma base de Shiff entre uma amina primária (presente nas proteínas) com grupamentos carbonil, encontrados na glicose ou em pequenos aldeídos reativos, derivados do estresse oxidativo (ex. metilglioxal) [4]. Após rearranjos moleculares, é formado o Produto de Amadori, estrutura estável, que poderá sofrer alterações pela presença de compostos oxidantes formando os AGEs. A sigla AGE não determina uma molécula específica e sim um grupo heterogêneo de moléculas. Dentre essas, algumas com importante função biológica já foram descritas, como a carboximetil lisina [5].

A formação de AGEs no organismo pode ter impacto funcional importante, gerando ligações cruzadas intra- e entre moléculas e alterando a meia-vida e função de proteínas [6, 7]. Além disso, os AGEs têm propriedades sinalizadora de cascatas pró-inflamatórias [8]. Já foi visto que pacientes com *Diabetes Mellitus* (DM) ou Insuficiência Renal Crônica (IRC) apresentam níveis elevados de AGEs em tecido e essa concentração tem correlação com a piora do prognóstico da doença [9, 10]. A partir disso, vem sendo estudada a presença desses compostos em outras enfermidades e eventos patológicos.

A insuficiência cardíaca (IC) é uma condição clínica caracterizada pela incapacidade do coração de suprir o organismo com quantidades de sangue suficientes

para as atividades normais. A sua principal causa é o infarto agudo do miocárdio (IAM), onde uma área de necrose ocorre após a interrupção do fluxo sanguíneo das artérias coronárias [11]. Já foi demonstrado que os AGEs estão aumentados após o IAM e que sua concentração plasmática é preditora independente no desenvolvimento da IC [12]. Sabe-se que os níveis plasmáticos aumentados de dois tipos importantes de AGEs, a carboximetil lisina (CML) e a pentosidina, estão associados à maior chance de hospitalização em pacientes com IC, sendo que a CML também apresenta associação com um maior risco de mortalidade nesse grupo de indivíduos [13]. Esses resultados abrem a possibilidade de testes com drogas anti-AGEs [14-17], porém, até o presente momento não existe a caracterização dos AGEs em um modelo pré-clínico de IC. A partir disso, o objetivo deste trabalho é caracterizar a formação de AGEs em ratos submetidos ao IAM e acompanhados por 28 dias.

2 MATERIAIS E MÉTODOS

Ratos Wistar machos (60 dias) foram randomizados para receber cirurgia *sham* (n=8) ou de indução do IAM (n=9). A indução do IAM foi realizada pela técnica de ligadura da coronária descendente anterior esquerda [18]. Para a cirurgia *sham* foram realizados todos os procedimentos da cirurgia para indução do IAM, mas sem a ligadura da coronária. Os animais foram mantidos com comida e bebida à vontade, em caixas com 5 animais em ambiente com temperatura controlada (21 ± 1 °C).

Após as cirurgias, os animais foram avaliados por ecocardiografia (Philips Systems – HD7, Andover, MA, EUA, transdutor 3-12 MHz e profundidade de 2 centímetros) por operador treinado e cegado nos dias 2 e 28 para confirmação do IAM. O sangue para as análises foi coletado (1 mL) por punção do plexo retro-orbital com capilar heparinizado nos seguintes momentos após a cirurgia: 12 e 48 horas, 7, 14 e 28 dias, centrifugado (1000 xg por 10 minutos) e o plasma foi armazenado em freezer -80 °C.

2.1 Identificação de AGEs

O plasma foi utilizado para dosar a concentração de AGEs por três técnicas: fluorescência [19], formação de *browning* [20] e método de dot blot (imunodetecção).

As amostras (20 µL) foram pipetadas em triplicata em placa opaca de 96 poços e diluídas com 180 µL de água destilada. Após, as amostras foram excitadas (340 nm) e a emissão (440 nm) foi registrada, de acordo com Munch et al. (1997). Paralelamente, as amostras foram pipetadas em placa transparente Greiner® e a absorbância em 340 nm foi registrada como um indicativo de *browning*, conforme descrito por Valencia et al. (2004).

A metodologia anterior foi alterada para verificar a quantidade de AGEs das amostras de acordo com o peso molecular da proteína formada. As amostras foram preparadas com ácido tricloroacético (TCA) 0,15 M e clorofórmio [21]. Em seguida, foram centrifugadas e o sobrenadante foi pipetado em triplicata tanto na placa opaca como na transparente. As placas foram analisadas conforme as técnicas e os comprimentos de ondas utilizados anteriormente.

Para o dot blot, os plasmas foram diluídos com Tris 0,1M, pH 6,8, e as proteínas foram desnaturadas com o Tampão de Laemmli 4x (Tris 250 mM, SDS 8%, Glicerol 40%, azul de bromofenol 0,008%, β-mercaptoetanol 20%, pH 6,8) obtendo uma alíquota de 200 µL numa concentração de 2 µg/µL de proteína [22]. As amostras foram aquecidas em banho-maria por 15 min em temperatura de 70 °C e pipetadas (3 µL) em membrana de PVDF previamente ativada com metanol, água e tampão (Tris 48 mM, Glicina 39 mM, metanol 20%, pH 9,1). Após secagem, a membrana foi incubada com o anticorpo monoclonal específico para CML (6D12, cedido por Ryoji Nagai, Tokai University; 1:10.000) em TTBS (Tris 20 mM, NaCl 150 mM, Tween20 0,1%, pH 7,6) suplementado com BSA 1% por 2 horas, sob agitação, à temperatura ambiente. Em seguida, foram realizadas 4 lavagens de 10 min com TTBS e preparada a solução com o anticorpo secundário conjugado com peroxidase (Sigma-Aldrich®, código A4416, 1:10.000) com TTBS por 1 hora, sob agitação, à temperatura ambiente. Após lavagens (4 x 10 min) com TTBS, a membrana foi incubada com Reagente de Revelação (Bio-Rad, código WBKLSO500) por 3 minutos e as bandas foram observadas em fotodocumentor (L-Pix Chemi Molecular Imaging - Loccus biotecnologia, Cotia, São Paulo, Brasil). As imagens obtidas foram medidas no programa ImageJ.

2.2 Considerações éticas na utilização dos animais

O estudo desenvolvido seguiu a Lei 11.794 de 8 de outubro de 2008, que estabelece procedimentos para o uso científico de animais e as Diretrizes da Prática de Eutanásia do CONCEA (2013). O projeto foi aprovado na Comissão de Ética no Uso de Animais do Hospital de Clínicas de Porto Alegre sob o número 11-0202.

2.3 Análise Estatística

A distribuição das variáveis foi avaliada pelo teste de Kolmogorov-Smirnov e o teste-t foi aplicado na comparação entre os grupos. O teste ANOVA foi utilizado para análise de medidas repetidas com o pós-teste de Tukey. Um $P < 0,05$ foi considerado significante.

3 RESULTADOS

Nos dados adquiridos pelo ecocardiograma nos 28 dias de acompanhamento foi possível identificar diferença estatisticamente significativa entre o diâmetro do ventrículo esquerdo em sístole e diástole, fração de encurtamento, fração de ejeção e tamanho do infarto entre os grupos (Tabela 1).

Tabela 1 – Dados do ecocardiograma do grupo sham e IAM em 28 dias.

	Sham **(n=8)**	**IAM (n=9)**	**p**
DS (mm)	3,98 ± 0,09	8,66 ± 0,09	<0,001
DD (mm)	7,45 ± 0,05	9,98 ± 0,08	<0,001
PP (mm)	1,66 ± 0,04	1,63 ± 0,04	0,904
FS (%)	46,99 ± 10,35	13,4 ± 3,76	<0,001
FE (%)	83,4 ± 9,21	34,7 ± 8,16	<0,001
IAM (%)	0 ± 0	34,5 ± 6,55	<0,001

Valores expressos em média ± desvio padrão. DS, diâmetro do ventrículo esquerdo em sístole; DD, diâmetro do ventrículo esquerdo em diástole; PP, espessura da parede posterior; FS, fração de encurtamento; FE, Fração de ejeção; IAM (%), tamanho da área do IAM.

3.1 *Browning* e Fluorescência

Não foi encontrada diferença significativa nos níveis de AGEs entre o grupo *sham* e o grupo IAM ao longo do tempo de seguimento comparando os valores obtidos tanto na formação de *browning* quanto na fluorescência (Figura 1- A e C). Com o objetivo de corrigir eventuais variações individuais, os valores obtidos nos tempos 2 (48h), 7 e 28 dias foram ponderados pelo valor obtido no plasma coletados 12 horas após a cirurgia. Ainda assim, os níveis de AGEs não diferiram entre os grupos *sham* e IAM em nenhum tempo avaliado (Figura 1 – B e D). Entretanto, encontrou-se um aumento persistente no browning do plasma do grupo IAM (Figura 1A e 1B).

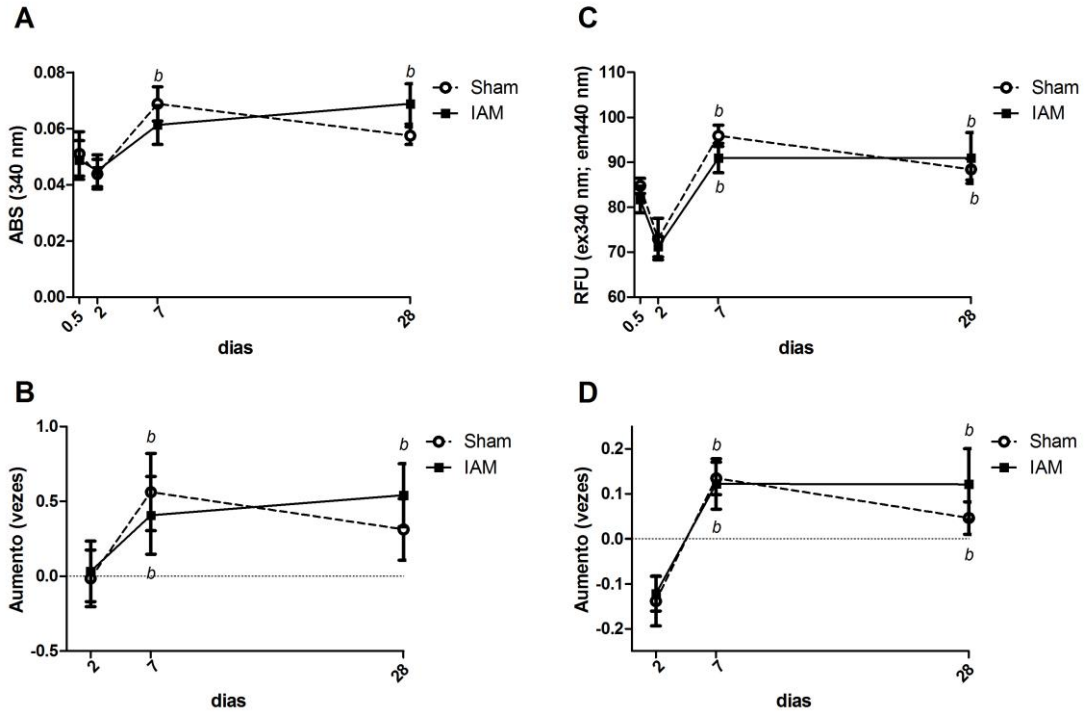

Figura 1 - Cinética de formação de AGEs plasmáticos. A - Valores de absorvância em 340 nm no plasma completo; B - Mudança (em vezes) da absorvância em relação aos valores obtidos para o plasma coletado 12 horas após a cirurgia (indicado pela linha pontilhada) ; C - Valores de fluorescência no plasma completo ; D - Mudança (em vezes) da fluorescência em relação aos valores obtidos para o plasma coletado 12 horas após a cirurgia (indicado pela linha pontilhada) . *Sham*, n = 8; IAM, n = 8. ; a, b indicam comparações entre os tempos e dentro do grupo, sendo: a - diferença em relação ao valor no tempo 0,5 dias; b - diferença em relação ao valor no tempo 2 dias; P < 0,05. Valores de Média ± Erro Padrão.

3.2 *Browning* e Fluorescência na fração de baixo peso molecular

A avaliação do *browning* na fração de baixo peso molecular não apresentou leituras, possivelmente devido à baixa sensibilidade da técnica (dados não mostrados). A avaliação da fluorescência na fração de baixo peso molecular mostrou que não há diferença nas quantidades de AGEs entre os grupos *sham* e IAM (Figura 2), mas há diferença estatística ao longo do tempo nos dois grupos.

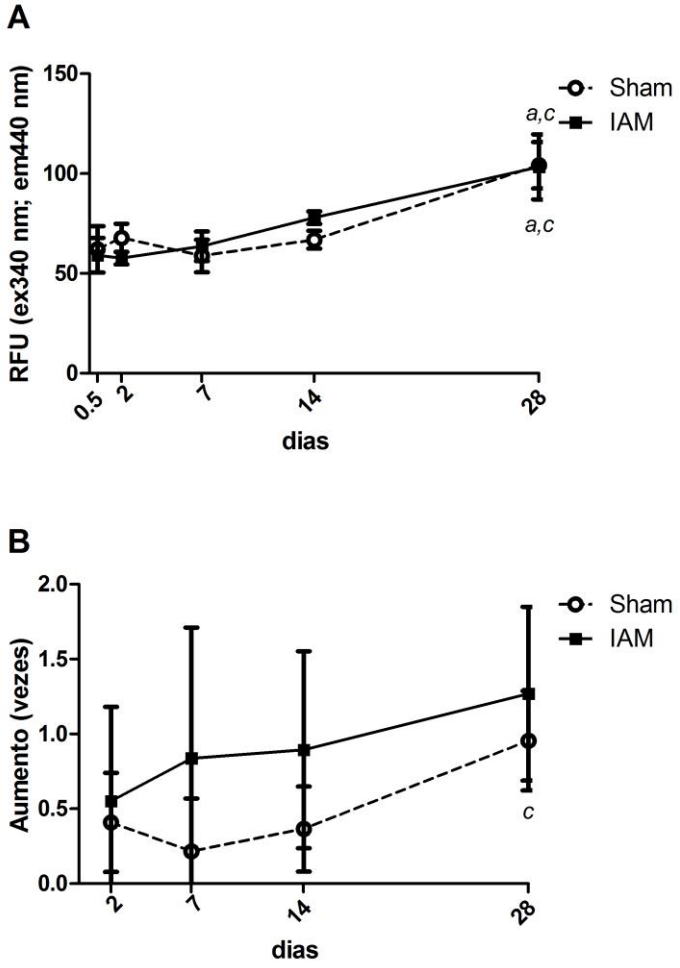

Figura 2 - Cinética de formação de AGEs em plasma desproteinizado. A - Valores de fluorescência em plasma desproteinizado; B - Mudança (em vezes) da fluorescência em relação aos valores obtidos com o plasma coletado em 12 horas. *Sham*, n = 8; IAM, n = 8. a, c indicam comparações entre os tempos e dentro do grupo, sendo: a - diferença em relação ao valor no tempo 0,5 dias; c - diferença em relação ao valor no tempo 7 dias; $P < 0,05$. Valores de Média ± Erro Padrão.

3.3 Imunodetecção de AGEs – dot blot

Utilizando uma técnica de imudodetecção para a CML, encontramos diferença significativa entre os grupos *sham* e IAM 12 horas e 2 dias após a cirurgia (Figura 3). Apesar desse aumento inicial, os grupos não diferiram nos tempos mais tardios.

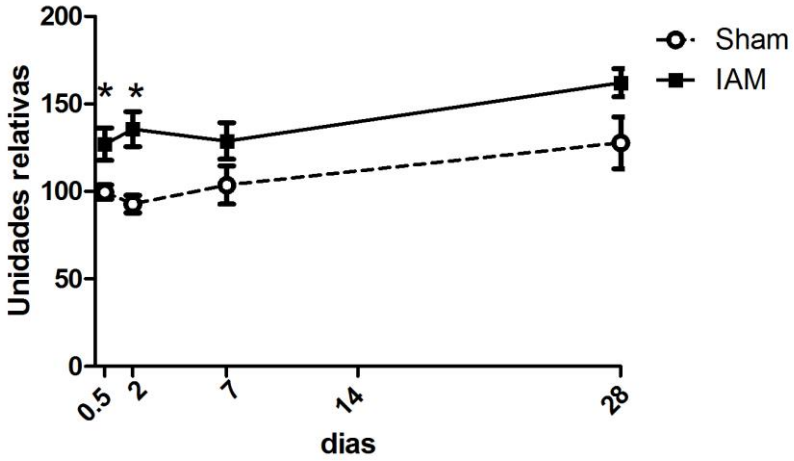

Figura 3 – Cinética de formação de AGEs plasmáticos por dot blot usando anticorpo anti-CML. Valores do dot blot do plasma, comparação entre os grupos. *Sham*, n = 8; IAM, n = 9. *p<0,05, em relação ao *sham* no mesmo tempo. Valores de Média ± Erro Padrão.

4 DISCUSSÃO

A utilização de modelos experimentais na área de cardiologia, especialmente do modelo utilizado nesse estudo, tem fornecido informações importantes sobre a morfologia, bioquímica, fisiologia e propriedades mecânicas do miocárdio infartado [23]. O presente trabalho descreve, pela primeira vez, o comportamento de formação de AGEs em um modelo animal representativo de isquemia do miocárdio que leva ao estabelecimento da IC. Apesar de preliminar, as nossas análises apontam para um perfil diferente de AGEs no plasma de ratos Wistar infartados em relação ao grupo *sham.*

O modelo animal mais empregado nos estudos de IC é feito pela interrupção cirúrgica do fluxo sanguíneo nas coronárias. Esse modelo encontra-se paralelo em todos os passos da doença no ser humano, pois apresenta necrose, inflamação, remodelamento ventricular (hipertrofia compensatória seguida de afinamento das paredes do ventrículo) e, finalmente, insuficiência cardíaca [24]. O modelo empregado nesse estudo mimetiza o processo de remodelamento ventricular desencadeado pelo IAM, gerando uma extensa área de fibrose, com afinamento parietal, alargamento ventricular e fração de ejeção prejudicada, conforme descrito anteriormente [18]. Com isso as modificações funcionais e morfológicas ocorridas nos animais infartados podem caracterizar o desenvolvimento de IC [25].

O processo de remodelamento que se segue a um evento isquêmico do coração envolve ativação de metaloproteinase de matriz, ativação inflamatória via NF-κB e fibrose, processos ligados à sinalização por AGEs [4, 26]. Nesse sentido, já foi descrito que camundongos submetidos à lesão por isquemia-reperfusão apresentaram aumento de CML e do receptor para AGE (RAGE) no tecido cardíaco [27]. A concentração dos AGEs após a ocorrência de um IAM e no curso da IC tem se mostrado importante, inclusive sendo preditora de eventos adversos [4, 12], indicando que abordagens farmacológicas anti-AGE podem ter papel terapêutico importante.

Os AGEs são formados a partir de moléculas variadas e vias distintas. Por isso, deve ser utilizado mais de um tipo de técnica para identificar a presença dessas substâncias. A formação de *browning*, realizado através da absorbância, é utilizada devido à característica dessas moléculas de adquirir coloração amarronzada quando formadas [20, 28]. Entretanto, essa técnica é mais utilizada na mensuração de *browning* em alimentos [29-31] e pouco explorada em amostras biológicas [32].

Monier et al. (1984) demonstrou que os valores de fluorescência e *browning* no colágeno de diabéticos eram o dobro daqueles encontrados em pessoas sadias. No nosso trabalho, apesar de não detectarmos diferença nos níveis de AGEs entre os grupos *sham* e IAM utilizando a técnica de *browning*, observamos que a quantidade de AGEs no grupo *sham* está retornando aos valores basais no 28º dia, enquanto que o grupo IAM aumenta progressivamente com diferença significativa ao longo do tempo.

A fluorescência é outro método também utilizado para detecção de AGEs e mais difundido do que o *browning*. Um estudo comparando a presença de AGEs em pacientes em tratamento com hemodiálise e indivíduos saudáveis, por meio da fluorescência e por ELISA, mostrou que o grupo controle tinha níveis de AGEs menores do que o grupo com o tratamento [19]. Apesar disso, no nosso estudo não foi evidenciada diferença significativa nos níveis de AGEs entre os grupos no tempo de seguimento. No entanto, as medidas adquiridas com a fluorescência apresentaram o mesmo perfil que os resultados do *browning*, ocorrendo aumento dos níveis no IAM e retorno aos valores basais (12h) no grupo *sham*.

Para maiores comparações também foi aplicado o método de imunodetecção por dot blot. Nesta técnica utilizou-se o anticorpo monoclonal para a molécula carboximetil lisina (CML). A CML é o AGE melhor caracterizado na literatura [33], possui relação com diversos episódios patológicos, mas não emite fluorescência. A presença de CML já foi encontrada em placas de aterosclerose em aorta de humanos [34], está associado com a vasoconstrição de artérias coronarianas [35] e sua concentração se encontra aumentada em diabéticos, sendo relacionado com piora de prognóstico da doença causando rigidez vascular [36] e auxiliando no desenvolvimento de doenças cardíacas [37]. Em Hartog et al. 2007 [38], foi relacionado o aumento da

concentração de CML plasmática com piora do quadro clínico do paciente de acordo com a classe funcional de IC. Além disso, no mesmo estudo, foi relatada diminuição da sobrevida dos pacientes com maiores níveis de CML. Pacientes infartados também apresentam níveis elevados de CML tanto no tecido cardíaco como em pequenos vasos sanguíneos intramiocardíacos em relação a indivíduos normais [39]. No modelo animal avaliado no nosso estudo verificamos apenas um aumento transitório de CML plasmática em 12 e 48 horas após o IAM.

Algumas limitações devem ser consideradas. Primeiro, as avaliações apresentadas aqui não são definitivas. As técnicas de fluorescência e *browning* não são capazes de detectar toda a variedade de AGEs existentes, apesar de serem amplamente utilizadas e encontrarem correlação com outras técnicas de avaliação [19, 20, 40]. Segundo, a detecção de moléculas fluorescentes utiliza parâmetros ótimos para a excitação/emissão de AGEs, mas não podemos excluir a interferência de outras moléculas fluorescentes que mascarem uma diferença entre os grupos. Por fim, o nosso estudo avaliou apenas AGEs plasmáticos. Apesar de a IC apresentar-se como uma condição sistêmica, não podemos excluir que haja uma modulação mais acentuada no tecido cardíaco. Portanto, a avaliação tecidual deve ser considerada em um próximo estudo. Além disso, provavelmente o motivo de não haver diferença nos níveis de AGEs plasmáticos entre os grupos estudados no trabalho seja ao fato do grupo *sham* também ser submetido a um trauma cirúrgico e esse processo irá estimular a produção dos AGEs, uma vez que os produtos finais de glicação avançada estão relacionados com vias pró-inflamatórias.

5 CONCLUSÃO

O modelo de ligadura da artéria coronária descendente anterior em rato Wistar é um modelo representativo de desenvolvimento de IC por evento isquêmico onde há um aumento transitório de CML plasmática precocemente. Técnicas de avaliação de AGEs, como a detecção de *browning e* fluorescência plasmática não são capazes de identificar diferença entre os grupos *sham* e IAM em um seguimento de 28 dias. Considerando em conjunto, os resultados sugerem que o modelo empregado aqui pode ser adequado para o estudo de terapias anti-AGE. Contudo, a mensuração dos AGEs deve ser avaliado em tempos maiores do que 28 dias.

6 REFERÊNCIAS

[1] Vlassara H, Cai W, Crandall J, Goldberg T, Oberstein R, Dardaine V, et al. Inflammatory mediators are induced by dietary glycotoxins, a major risk factor for diabetic angiopathy. Proc Natl Acad Sci U S A. 2002;99:15596-601.

[2] Anderson MM, Requena JR, Crowley JR, Thorpe SR, Heinecke JW. The myeloperoxidase system of human phagocytes generates Nepsilon-(carboxymethyl)lysine on proteins: a mechanism for producing advanced glycation end products at sites of inflammation. J Clin Invest. 1999;104:103-13.

[3] Lapolla A, Flamini R, Dalla Vedova A, Senesi A, Reitano R, Fedele D, et al. Glyoxal and methylglyoxal levels in diabetic patients: quantitative determination by a new GC/MS method. Clin Chem Lab Med. 2003;41:1166-73.

[4] Hartog JW, Voors AA, Bakker SJ, Smit AJ, van Veldhuisen DJ. Advanced glycation end-products (AGEs) and heart failure: pathophysiology and clinical implications. Eur J Heart Fail. 2007;9:1146-55.

[5] Nagai R, Hayashi CM, Xia L, Takeya M, Horiuchi S. Identification in human atherosclerotic lesions of GA-pyridine, a novel structure derived from glycolaldehyde-modified proteins. J Biol Chem. 2002;277:48905-12.

[6] Andrades ME, Lorenzi R, Berger M, Guimaraes JA, Moreira JC, Dal-Pizzol F. Glycolaldehyde induces fibrinogen post-translational modification, delay in clotting and resistance to enzymatic digestion. Chem Biol Interact. 2009;180:478-84.

[7] Zeng J, Dunlop RA, Rodgers KJ, Davies MJ. Evidence for inactivation of cysteine proteases by reactive carbonyls via glycation of active site thiols. The Biochemical journal. 2006;398:197-206.

[8] Kislinger T, Fu C, Huber B, Qu W, Taguchi A, Du Yan S, et al. N(epsilon)-(carboxymethyl)lysine adducts of proteins are ligands for receptor for advanced glycation end products that activate cell signaling pathways and modulate gene expression. J Biol Chem. 1999;274:31740-9.

[9] Vouillarmet J, Maucort-Boulch D, Michon P, Thivolet C. Advanced glycation end products assessed by skin autofluorescence: a new marker of diabetic foot ulceration. Diabetes Technol Ther. 2013;15:601-5.

[10] Meerwaldt R, Hartog JW, Graaff R, Huisman RJ, Links TP, den Hollander NC, et al. Skin autofluorescence, a measure of cumulative metabolic stress and advanced glycation end products, predicts mortality in hemodialysis patients. Journal of the American Society of Nephrology : JASN. 2005;16:3687-93.

[11] Braunwald E. Heart failure: an update. Clinical pharmacology and therapeutics. 2013;94:430-2.

[12] Raposeiras-Roubin S, Rodino-Janeiro BK, Paradela-Dobarro B, Grigorian-Shamagian L, Garcia-Acuna JM, Aguiar-Souto P, et al. Predictive value of advanced glycation end products for the development of post-infarction heart failure: a preliminary report. Cardiovasc Diabetol. 2012;11:102.

[13] Willemsen S, Hartog JW, van Veldhuisen DJ, van der Meer P, Roze JF, Jaarsma T, et al. The role of advanced glycation end-products and their receptor on outcome in heart failure patients with preserved and reduced ejection fraction. American heart journal. 2012;164:742-9 e3.

[14] Chang KC, Hsu KL, Chou TF, Lo HM, Tseng YZ. Aminoguanidine prevents age-related deterioration in left ventricular-arterial coupling in Fisher 344 rats. Br J Pharmacol. 2004;142:1099-104.

[15] Alderson NL, Chachich ME, Youssef NN, Beattie RJ, Nachtigal M, Thorpe SR, et al. The AGE inhibitor pyridoxamine inhibits lipemia and development of renal and vascular disease in Zucker obese rats. Kidney Int. 2003;63:2123-33.

[16] Balakumar P, Rohilla A, Krishan P, Solairaj P, Thangathirupathi A. The multifaceted therapeutic potential of benfotiamine. Pharmacological research : the official journal of the Italian Pharmacological Society. 2010;61:482-8.
[17] Voziyan PA, Hudson BG. Pyridoxamine: the many virtues of a maillard reaction inhibitor. Annals of the New York Academy of Sciences. 2005;1043:807-16.
[18] Pfeffer MA, Pfeffer JM, Fishbein MC, Fletcher PJ, Spadaro J, Kloner RA, et al. Myocardial infarct size and ventricular function in rats. Circulation research. 1979;44:503-12.
[19] Munch G, Keis R, Wessels A, Riederer P, Bahner U, Heidland A, et al. Determination of advanced glycation end products in serum by fluorescence spectroscopy and competitive ELISA. European journal of clinical chemistry and clinical biochemistry : journal of the Forum of European Clinical Chemistry Societies. 1997;35:669-77.
[20] Valencia JV, Weldon SC, Quinn D, Kiers GH, DeGroot J, TeKoppele JM, et al. Advanced glycation end product ligands for the receptor for advanced glycation end products: biochemical characterization and formation kinetics. Analytical biochemistry. 2004;324:68-78.
[21] Wrobel K, Garay-Sevilla ME, Nava LE, Malacara JM. Novel analytical approach to monitoring advanced glycosylation end products in human serum with on-line spectrophotometric and spectrofluorometric detection in a flow system. Clinical chemistry. 1997;43:1563-9.
[22] Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 1970;227:680-5.
[23] Doggrell SA, Brown L. Rat models of hypertension, cardiac hypertrophy and failure. Cardiovascular research. 1998;39:89-105.
[24] Hasenfuss G. Animal models of human cardiovascular disease, heart failure and hypertrophy. Cardiovascular research. 1998;39:60-76.
[25] Sjaastad I, Sejersted OM, Ilebekk A, Bjornerheim R. Echocardiographic criteria for detection of postinfarction congestive heart failure in rats. J Appl Physiol (1985). 2000;89:1445-54.
[26] Zhang F, Banker G, Liu X, Suwanabol PA, Lengfeld J, Yamanouchi D, et al. The novel function of advanced glycation end products in regulation of MMP-9 production. J Surg Res. 2011;171:871-6.
[27] Bucciarelli LG, Kaneko M, Ananthakrishnan R, Harja E, Lee LK, Hwang YC, et al. Receptor for advanced-glycation end products: key modulator of myocardial ischemic injury. Circulation. 2006;113:1226-34.
[28] Poulsen MW, Hedegaard RV, Andersen JM, de Courten B, Bugel S, Nielsen J, et al. Advanced glycation endproducts in food and their effects on health. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association. 2013;60:10-37.
[29] Mosneaguta R, Alvarez V, Barringer SA. The effect of antibrowning agents on inhibition of potato browning, volatile organic compound profile, and microbial inhibition. Journal of food science. 2012;77:C1234-40.
[30] Nayak B, Berrios Jde J, Powers JR, Tang J. Effect of extrusion on the antioxidant capacity and color attributes of expanded extrudates prepared from purple potato and yellow pea flour mixes. Journal of food science. 2011;76:C874-83.
[31] Cämerer B, Wedzicha BL, Kohn LW. Nonenzymatic browning reactions of retro-aldol degradation products of carbohydrates. Eur Food Res Technol. 1999;209: 261–5.
[32] Monnier VM, Kohn RR, Cerami A. Accelerated age-related browning of human collagen in diabetes mellitus. Proc Natl Acad Sci U S A. 1984;81:583-7.
[33] Ikeda K, Higashi T, Sano H, Jinnouchi Y, Yoshida M, Araki T, et al. N (epsilon)-(carboxymethyl)lysine protein adduct is a major immunological epitope in proteins modified with advanced glycation end products of the Maillard reaction. Biochemistry. 1996;35:8075-83.
[34] Kume S, Takeya M, Mori T, Araki N, Suzuki H, Horiuchi S, et al. Immunohistochemical and ultrastructural detection of advanced glycation end products in atherosclerotic lesions of human aorta with a novel specific monoclonal antibody. Am J Pathol. 1995;147:654-67.

[35] Kamata K, Ozawa Y, Kobayashi T, Matsumoto T. Effect of N-epsilon-(carboxymethyl)lysine on coronary vasoconstriction in isolated perfused hearts from control and streptozotocin-induced diabetic rats. Journal of smooth muscle research = Nihon Heikatsukin Gakkai kikanshi. 2009;45:125-37.
[36] Schleicher ED, Wagner E, Nerlich AG. Increased accumulation of the glycoxidation product N(epsilon)-(carboxymethyl)lysine in human tissues in diabetes and aging. J Clin Invest. 1997;99:457-68.
[37] Ahmed KA, Muniandy S, S. II. Role of N ε-(Carboxymethyl)Lysine in the Development of Ischemic Heart Disease in Type 2 Diabetes Mellitus. J Clin Biochem Nutr. 2007;41:97-105.
[38] Hartog JW, Voors AA, Schalkwijk CG, Scheijen J, Smilde TD, Damman K, et al. Clinical and prognostic value of advanced glycation end-products in chronic heart failure. European heart journal. 2007;28:2879-85.
[39] Baidoshvili A, Krijnen PA, Kupreishvili K, Ciurana C, Bleeker W, Nijmeijer R, et al. N(epsilon)-(carboxymethyl)lysine depositions in intramyocardial blood vessels in human and rat acute myocardial infarction: a predictor or reflection of infarction? Arteriosclerosis, thrombosis, and vascular biology. 2006;26:2497-503.
[40] Taneda S, Monnier VM. ELISA of pentosidine, an advanced glycation end product, in biological specimens. Clinical chemistry. 1994;40:1766-73.

ANEXO 1

Normas da revista: Journal of Molecular and Cellular Cardiology

JOURNAL OF MOLECULAR AND CELLULAR CARDIOLOGY

AUTHOR INFORMATION PACK

TABLE OF CONTENTS

ISSN: 0022-2828

DESCRIPTION

The *Journal of Molecular and Cellular Cardiology* publishes work advancing knowledge of the mechanisms responsible for both normal and diseased cardiovascular function. To this end papers are published in all relevant areas. These include (but are not limited to): structural biology; genetics; proteomics; morphology; stem cells; molecular biology; metabolism; biophysics; electrophysiology; pharmacology and physiology. Papers are encouraged with both basic and translational approaches. The journal is directed not only to basic scientists but also to clinical cardiologists who wish to follow the rapidly advancing frontiers of basic knowledge of the heart and circulation.

US National Institutes of Health (NIH) voluntary posting ("Public Access") policy

Journal of Molecular and Cellular Cardiology and Elsevier facilitate the author's response to the NIH Public Access Policy. For more details please see the Guide for authors

IMPACT FACTOR

2012: 5.148 © Thomson Reuters Journal Citation Reports 2013

ABSTRACTING AND INDEXING

EMBASE
EMBiology
Scopus

EDITORIAL BOARD

Consulting Editors

GUIDE FOR AUTHORS

Your Paper Your Way
ypyw-gfa-banner.gifyour paper your way

INTRODUCTION

Journal Categories

Regular Articles
To accelerate publication, preference is given to manuscripts no longer than approximately 6000 words containing 6-8 Figures and/or Tables. Regular articles are reviewed on average within 23 days of receipt by the editorial office.

Rapid communication
To provide for the rapid publication of data of special interest (including preliminary data and novel methods), short papers may be submitted for review within seven days of receipt by the editorial office. Rapid Communication manuscripts should not exceed 2000 words plus no more than 15 references. Results and Discussion sections may be combined . No more than 2 Figures and/or Tables should be included. A brief statement explaining the general importance of the results and why rapid publication is desired must accompany the manuscript.Decisions on Rapid Communications may be on a 'yes-no' basis and detailed referee's opinion may not be obtained.

Letters to the Editor
These will normally be comments on a paper previously published in JMCC and should be no more than 2000-3000 words in length including references. Letters will be reviewed by the Editor and the author of the original paper may be invited to respond.

Review Articles
Review Articles may be requested by the Editor, but authors are encouraged to submit Review Articles after consultation with the Associate Editor for Special Issues and Review Articles (Donald Bers). All such articles are subject to normal review process for regular articles. Review Articles should generally be no longer than 6000 words and should include an abstract.

BEFORE YOU BEGIN

Ethics in Publishing
Reporting standards

Authors of reports of original research should present an accurate account of the work performed as well as an objective discussion of its significance. Underlying data should be represented accurately in the paper. A paper should contain sufficient detail and references to permit others to replicate the work. Fraudulent or knowingly inaccurate statements constitute unethical behavior and are unacceptable.

Review and professional publication articles should also be accurate and objective, and editorial *opinion* works should be clearly identified as such.

Data Access and Retention

Authors may be asked to provide the raw data in connection with a paper for editorial review, and should be prepared to provide public access to such data (consistent with the ALPSP-STM Statement on Data and Databases), if practicable, and should in any event be prepared to retain such data for a reasonable time after publication.

Originality and Plagiarism

The authors should ensure that they have written entirely original works, and if the authors have used the work and/or words of others, that this has been appropriately cited or quoted.

Plagiarism takes many forms, from passing off another paper as the author(s) own paper, to copying or paraphrasing substantial parts of another(s) paper (without attribution), to claiming results from research conducted by others. Plagiarism in all its forms constitutes unethical publishing behavior and is unacceptable.

Multiple, Redundant or Concurrent Publication

An author should not in general publish manuscripts describing essentially the same research in more than one journal or primary publication. Submitting the same manuscript to more than one journal concurrently constitutes unethical publishing behaviour and is unacceptable.

In general, an author should not submit for consideration in another journal a previously published paper. Publication of some kinds of articles (eg, clinical guidelines, translations) in more than one journal is sometimes justifiable, provided certain conditions are met. The authors and editors of the journals concerned must agree to the secondary publication, which must reflect the same data and interpretation of the primary document. The primary reference must be cited in the secondary publication. Further detail on acceptable forms of secondary publication can be found at http://www.icmje.org/

Acknowledgement of Sources

Proper acknowledgment of the work of others must always be given. Authors should cite publications that have been influential in determining the nature of the reported work. Information obtained privately, as in conversation, correspondence, or discussion with third parties, must not be used or reported without explicit, written permission from the source. Information obtained in the course of confidential services, such as refereeing manuscripts or grant applications, must not be used without the explicit written permission of the author of the work involved in these services.

Authorship of the Paper

Authorship should be limited to those who have made a significant contribution to the conception, design, execution, or interpretation of the reported study. All those who have made significant contributions should be listed as co-authors. Where there are others who have participated in certain substantive aspects of the research project, they should be acknowledged or listed as contributors.

The corresponding author should ensure that all appropriate co-authors and no inappropriate co-authors are included on the paper, and that all co-authors have seen and approved the final version of the paper and have agreed to its submission for publication.

Hazards and Human or Animal Subjects

If the work involves chemicals, procedures or equipment that have any unusual hazards inherent in their use, the author must clearly identify these in the manuscript. If the work involves the use of animal or human subjects, the author should ensure that the manuscript contains a statement that all procedures were performed in compliance with relevant laws and institutional guidelines and that the appropriate institutional committee(s) have approved them. Authors should include a statement in the manuscript that informed consent was obtained for experimentation with human subjects. The privacy rights of human subjects must always be observed.

Disclosure and Conflicts of Interest

All authors should disclose in their manuscript any financial or other substantive conflict of interest that might be construed to influence the results or interpretation of their manuscript. All sources of financial support for the project should be disclosed.

Examples of potential conflicts of interest which should be disclosed include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. Potential conflicts of interest should be disclosed at the earliest stage possible.

Fundamental errors in published works

When an author discovers a significant error or inaccuracy in his/her own published work, it is the authors obligation to promptly notify the journal editor or publisher and cooperate with the editor to retract or correct the paper. If the editor or the publisher learn from a third party that a published work contains a significant error, it is the obligation of the author to promptly retract or correct the paper or provide evidence to the editor of the correctness of the original paper.

Conflict of interest

All authors are requested to disclose any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, their work. See also http://www.elsevier.com/conflictsofinterest. Further information and an example of a Conflict of Interest form can be found at: http://help.elsevier.com/app/answers/detail/a_id/286/p/7923.

Submission declaration

Submission of an article implies that the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder. To verify originality, your article may be checked by the originality detection software iThenticate. See also http://www.elsevier.com/editors/plagdetect.

Changes to authorship

This policy concerns the addition, deletion, or rearrangement of author names in the authorship of accepted manuscripts:

Before the accepted manuscript is published in an online issue: Requests to add or remove an author, or to rearrange the author names, must be sent to the Journal Manager from the corresponding author of the accepted manuscript and must include: (a) the reason the name should be added or removed, or the author names rearranged and (b) written confirmation (e-mail, fax, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed. Requests that are not sent by the corresponding author will be forwarded by the Journal Manager to the corresponding author, who must follow the procedure as described above. Note that: (1) Journal Managers will inform the Journal Editors of any such requests and (2) publication of the accepted manuscript in an online issue is suspended until authorship has been agreed.

After the accepted manuscript is published in an online issue: Any requests to add, delete, or rearrange author names in an article published in an online issue will follow the same policies as noted above and result in a corrigendum.

Copyright

This journal offers authors a choice in publishing their research: Open Access and Subscription.

For Subscription articles

Upon acceptance of an article, authors will be asked to complete a 'Journal Publishing Agreement' (for more information on this and copyright, see http://www.elsevier.com/copyright). An e-mail will be sent to the corresponding author confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form or a link to the online version of this agreement.

Subscribers may reproduce tables of contents or prepare lists of articles including abstracts for internal circulation within their institutions. Permission of the Publisher is required for resale or distribution outside the institution and for all other derivative works, including compilations and translations (please consult http://www.elsevier.com/permissions). If excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article. Elsevier has preprinted forms for use by authors in these cases: please consult http://www.elsevier.com/permissions.

For Open Access articles

Upon acceptance of an article, authors will be asked to complete an 'Exclusive License Agreement' (for more information see http://www.elsevier.com/OAauthoragreement). Permitted reuse of open access articles is determined by the author's choice of user license (see http://www.elsevier.com/openaccesslicenses).

Retained author rights
As an author you (or your employer or institution) retain certain rights. For more information on author rights for:
Subscription articles please see http://www.elsevier.com/journal-authors/author-rights-and-responsibilities.
Open access articles please see http://www.elsevier.com/OAauthoragreement.

Role of the funding source
You are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement then this should be stated. Please see http://www.elsevier.com/funding.

Funding body agreements and policies
Elsevier has established agreements and developed policies to allow authors whose articles appear in journals published by Elsevier, to comply with potential manuscript archiving requirements as specified as conditions of their grant awards. To learn more about existing agreements and policies please visit http://www.elsevier.com/fundingbodies.

Open access
This journal offers authors a choice in publishing their research:

Open Access
• Articles are freely available to both subscribers and the wider public with permitted reuse
• An Open Access publication fee is payable by authors or their research funder
Subscription
• Articles are made available to subscribers as well as developing countries and patient groups through our access programs (http://www.elsevier.com/access)
• No Open Access publication fee

All articles published Open Access will be immediately and permanently free for everyone to read and download. Permitted reuse is defined by your choice of one of the following Creative Commons user licenses:
Creative Commons Attribution (CC BY): lets others distribute and copy the article, to create extracts, abstracts, and other revised versions, adaptations or derivative works of or from an article (such as a translation), to include in a collective work (such as an anthology), to text or data mine the article, even for commercial purposes, as long as they credit the author(s), do not represent the author as endorsing their adaptation of the article, and do not modify the article in such a way as to damage the author's honor or reputation.
Creative Commons Attribution-NonCommercial-ShareAlike (CC BY-NC-SA): for non-commercial purposes, lets others distribute and copy the article, to create extracts, abstracts and other revised versions, adaptations or derivative works of or from an article (such as a translation), to include in a collective work (such as an anthology), to text and data mine the article, as long as they credit the author(s), do not represent the author as endorsing their adaptation of the article, do not modify the article in such a way as to damage the author's honor or reputation, and license their new adaptations or creations under identical terms (CC BY-NC-SA).
Creative Commons Attribution-NonCommercial-NoDerivs (CC BY-NC-ND): for non-commercial purposes, lets others distribute and copy the article, and to include in a collective work (such as an anthology), as long as they credit the author(s) and provided they do not alter or modify the article.

To provide Open Access, this journal has a publication fee which needs to be met by the authors or their research funders for each article published Open Access.
Your publication choice will have no effect on the peer review process or acceptance of submitted articles.

The publication fee for Open Access in this journal is **$1,800**, excluding taxes. Learn more about Elsevier's pricing policy: http://www.elsevier.com/openaccesspricing.

Language (usage and editing services)

Please write your text in good English (American or British usage is accepted, but not a mixture of these). Authors who feel their English language manuscript may require editing to eliminate possible grammatical or spelling errors and to conform to correct scientific English may wish to use the English Language Editing service available from Elsevier's WebShop (http://webshop.elsevier.com/languageediting/) or visit our customer support site (http://support.elsevier.com) for more information.

Submission

Submission to this journal proceeds totally online and you will be guided stepwise through the creation and uploading of your files. The system automatically converts source files to a single PDF file of the article, which is used in the peer-review process. Please note that even though manuscript source files are converted to PDF files at submission for the review process, these source files are needed for further processing after acceptance. All correspondence, including notification of the Editor's decision and requests for revision, takes place by e-mail removing the need for a paper trail.

PREPARATION

NEW SUBMISSIONS

Submission to this journal proceeds totally online and you will be guided stepwise through the creation and uploading of your files. The system automatically converts your files to a single PDF file, which is used in the peer-review process.
As part of the Your Paper Your Way service, you may choose to submit your manuscript as a single file to be used in the refereeing process. This can be a PDF file or a Word document, in any format or lay-out that can be used by referees to evaluate your manuscript. It should contain high enough quality figures for refereeing. If you prefer to do so, you may still provide all or some of the source files at the initial submission. Please note that individual figure files larger than 10 MB must be uploaded separately.

References

There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/book title, chapter title/article title, year of publication, volume number/book chapter and the pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct.

Formatting requirements

There are no strict formatting requirements but all manuscripts must contain the essential elements needed to convey your manuscript, for example Abstract, Keywords, Introduction, Materials and Methods, Results, Conclusions, Artwork and Tables with Captions.
If your article includes any Videos and/or other Supplementary material, this should be included in your initial submission for peer review purposes.
Divide the article into clearly defined sections.

Figures and tables embedded in text

Please ensure the figures and the tables included in the single file are placed next to the relevant text in the manuscript, rather than at the bottom or the top of the file.

REVISED SUBMISSIONS

Use of word processing software

Regardless of the file format of the original submission, at revision you must provide us with an editable file of the entire article. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier: http://www.elsevier.com/guidepublication). See also the section on Electronic artwork.
To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

Article structure

Subdivision - numbered sections
Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

Introduction
State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods
Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.

Results
Results should be clear and concise.

Discussion
This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Conclusions
The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Glossary
Please supply, as a separate list, the definitions of field-specific terms used in your article.

Appendices
If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

Essential title page information

• ***Title.*** Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
• ***Author names and affiliations.*** Where the family name may be ambiguous (e.g., a double name), please indicate this clearly. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.
• ***Corresponding author.*** Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. **Ensure that phone numbers (with country and area code) are provided in addition to the e-mail address and the complete postal address. Contact details must be kept up to date by the corresponding author.**
• ***Present/permanent address.*** If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Abstract

A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

Graphical abstract

A Graphical abstract is optional and should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership online. Authors must provide images that clearly represent the work described in the article. Graphical abstracts should be submitted as a separate file in the online submission system. Image size: Please provide an image with a minimum

of 531 × 1328 pixels (h × w) or proportionally more. The image should be readable at a size of 5 × 13 cm using a regular screen resolution of 96 dpi. Preferred file types: TIFF, EPS, PDF or MS Office files. See http://www.elsevier.com/graphicalabstracts for examples.
Authors can make use of Elsevier's Illustration and Enhancement service to ensure the best presentation of their images also in accordance with all technical requirements: Illustration Service.

Highlights

Highlights are mandatory for this journal. They consist of a short collection of bullet points that convey the core findings of the article and should be submitted in a separate file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point). See http://www.elsevier.com/highlights for examples.

Keywords

Immediately after the abstract, provide a maximum of 6 keywords, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Abbreviations

Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

Acknowledgements

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Nomenclature and units

Follow internationally accepted rules and conventions: use the international system of units (SI). If other quantities are mentioned, give their equivalent in SI. You are urged to consult IUPAC: Nomenclature of Organic Chemistry: http://www.iupac.org/ for further information.

Database linking

Elsevier encourages authors to connect articles with external databases, giving their readers one-click access to relevant databases that help to build a better understanding of the described research. Please refer to relevant database identifiers using the following format in your article: Database: xxxx (e.g., TAIR: AT1G01020; CCDC: 734053; PDB: 1XFN). See http://www.elsevier.com/databaselinking for more information and a full list of supported databases.

Footnotes

Footnotes should be used sparingly. Number them consecutively throughout the article. Many wordprocessors build footnotes into the text, and this feature may be used. Should this not be the case, indicate the position of footnotes in the text and present the footnotes themselves separately at the end of the article. Do not include footnotes in the Reference list.
Table footnotes
Indicate each footnote in a table with a superscript lowercase letter.

Artwork

Electronic artwork
General points
• Make sure you use uniform lettering and sizing of your original artwork.
• Preferred fonts: Arial (or Helvetica), Times New Roman (or Times), Symbol, Courier.
• Number the illustrations according to their sequence in the text.
• Use a logical naming convention for your artwork files.
• Indicate per figure if it is a single, 1.5 or 2-column fitting image.
• For Word submissions only, you may still provide figures and their captions, and tables within a single file at the revision stage.
• Please note that individual figure files larger than 10 MB must be provided in separate source files.
A detailed guide on electronic artwork is available on our website:
http://www.elsevier.com/artworkinstructions.
You are urged to visit this site; some excerpts from the detailed information are given here.
Formats

Regardless of the application used, when your electronic artwork is finalized, please 'save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):
EPS (or PDF): Vector drawings. Embed the font or save the text as 'graphics'.
TIFF (or JPG): Color or grayscale photographs (halftones): always use a minimum of 300 dpi.
TIFF (or JPG): Bitmapped line drawings: use a minimum of 1000 dpi.
TIFF (or JPG): Combinations bitmapped line/half-tone (color or grayscale): a minimum of 500 dpi is required.
Please do not:
• Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); the resolution is too low.
• Supply files that are too low in resolution.
• Submit graphics that are disproportionately large for the content.

Color artwork
Please make sure that artwork files are in an acceptable format (TIFF (or JPEG), EPS (or PDF), or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures then Elsevier will ensure, at no additional charge, that these figures will appear in color on the Web (e.g., ScienceDirect and other sites) regardless of whether or not these illustrations are reproduced in color in the printed version. **For color reproduction in print, you will receive information regarding the costs from Elsevier after receipt of your accepted article.** Please indicate your preference for color: in print or on the Web only. For further information on the preparation of electronic artwork, please see http://www.elsevier.com/artworkinstructions.
Please note: Because of technical complications which can arise by converting color figures to 'gray scale' (for the printed version should you not opt for color in print) please submit in addition usable black and white versions of all the color illustrations.

Color artwork
The Publisher will not charge authors for color figures where its use is integral to the useful illustration of the data.

Manipulation and Editing of Figures
It is important that any editing of figures (particularly gels and fluorescence images) be done in a way that does not distort the meaning of the results. The same processing must be performed on all parts of the image or gel. Any cropping of images to selectively remove parts of gels or blots should be explicitly noted in the legend and discontinuities should be visibly apparent in the figure. It is not permissible to add or remove data from figures. Any image processing used in preparing the figure (other than simple changes of brightness and contrast) should be described in the legend. Finally, the Editors may request copies of original, unprocessed data.

Illustration services
Elsevier's WebShop (http://webshop.elsevier.com/illustrationservices) offers Illustration Services to authors preparing to submit a manuscript but concerned about the quality of the images accompanying their article. Elsevier's expert illustrators can produce scientific, technical and medical-style images, as well as a full range of charts, tables and graphs. Image 'polishing' is also available, where our illustrators take your image(s) and improve them to a professional standard. Please visit the website to find out more.

Figure captions
Ensure that each illustration has a caption. A caption should comprise a brief title (**not** on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

Tables
Number tables consecutively in accordance with their appearance in the text. Place footnotes to tables below the table body and indicate them with superscript lowercase letters. Avoid vertical rules. Be sparing in the use of tables and ensure that the data presented in tables do not duplicate results described elsewhere in the article.

References
Citation in text
Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the

journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

Web references
As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

References in a special issue
Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

Reference management software
This journal has standard templates available in key reference management packages EndNote (http://www.endnote.com/support/enstyles.asp) and Reference Manager (http://refman.com/support/rmstyles.asp). Using plug-ins to wordprocessing packages, authors only need to select the appropriate journal template when preparing their article and the list of references and citations to these will be formatted according to the journal style which is described below.

Reference style
Text: Indicate references by number(s) in square brackets in line with the text. The actual authors can be referred to, but the reference number(s) must always be given.
List: Number the references (numbers in square brackets) in the list in the order in which they appear in the text. Please note that a reference should include a minimum 6 authors before the use of "et al."
Examples:
Reference to a journal publication:
[1] Van der Geer J, Hanraads JAJ, Lupton RA. The art of writing a scientific article. J Sci Commun 2000;163:51–9.
Reference to a book:
[2] Strunk Jr W, White EB. The elements of style. 3rd ed. New York: Macmillan; 1979.
Reference to a chapter in an edited book:
[3] Mettam GR, Adams LB. How to prepare an electronic version of your article. In: Jones BS, Smith RZ, editors. Introduction to the electronic age, New York: E-Publishing Inc; 1999, p. 281–304.
Note shortened form for last page number. e.g., 51–9, and that for more than 6 authors the first 6 should be listed followed by "et al." For further details you are referred to "Uniform Requirements for Manuscripts submitted to Biomedical Journals" (J Am Med Assoc 1997;277:927–934) (see also http://www.nlm.nih.gov/bsd/uniform_requirements.html).

Journal abbreviations source
Journal names should be abbreviated according to the
List of title word abbreviations: http://www.issn.org/2-22661-LTWA-online.php.

Video data

Elsevier accepts video material and animation sequences to support and enhance your scientific research. Authors who have video or animation files that they wish to submit with their article are strongly encouraged to include links to these within the body of the article. This can be done in the same way as a figure or table by referring to the video or animation content and noting in the body text where it should be placed. All submitted files should be properly labeled so that they directly relate to the video file's content. In order to ensure that your video or animation material is directly usable, please provide the files in one of our recommended file formats with a preferred maximum size of 50 MB. Video and animation files supplied will be published online in the electronic version of your article in Elsevier Web products, including ScienceDirect: http://www.sciencedirect.com. Please supply 'stills' with your files: you can choose any frame from the video or animation or make a separate image. These will be used instead of standard icons and will personalize the link to your video data. For more detailed instructions please visit our video instruction pages at http://www.elsevier.com/artworkinstructions. Note: since video and animation cannot be embedded in the print version of the journal, please provide text for both the electronic and the print version for the portions of the article that refer to this content.

AudioSlides

The journal encourages authors to create an AudioSlides presentation with their published article. AudioSlides are brief, webinar-style presentations that are shown next to the online article on ScienceDirect. This gives authors the opportunity to summarize their research in their own words and to help readers understand what the paper is about. More information and examples are available at http://www.elsevier.com/audioslides. Authors of this journal will automatically receive an invitation e-mail to create an AudioSlides presentation after acceptance of their paper.

Supplementary Data

Elsevier accepts electronic supplementary material to support and enhance your scientific research. Supplementary files offer the author additional possibilities to publish supporting applications, high-resolution images, background datasets, sound clips and more. Supplementary files supplied will be published online alongside the electronic version of your article in Elsevier Web products, including ScienceDirect: http://www.sciencedirect.com. In order to ensure that your submitted material is directly usable, please provide the data in one of our recommended file formats. Authors should submit the material in electronic format together with the article and supply a concise and descriptive caption for each file. All supplementary material except videos, audio slides and 3D molecular models should be combined into a single PDF. The Supplemental PDF file should be in a "presentation style" (i.e. not double-spaced) with Figures and their legends integrated within the text. For more detailed instructions please visit our artwork instruction pages at http://www.elsevier.com/artworkinstructions.

3D molecular models

You can enrich your online articles by providing 3D molecular models (optional) in PDB, PSE or MOL/MOL2 format, which will be visualized using the interactive viewer embedded within the article. Using the viewer, it will be possible to zoom into the model, rotate and pan the model, and change display settings. Submitted models will also be available for downloading from your online article on ScienceDirect. Each molecular model will have to be uploaded to the online submission system separately, via the '3D molecular models' submission category. For more information see: http://www.elsevier.com/3DMolecularModels.

Submission checklist

The following list will be useful during the final checking of an article prior to sending it to the journal for review. Please consult this Guide for Authors for further details of any item.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:

- E-mail address
- Full postal address
- Telephone

All necessary files have been uploaded, and contain:

- Keywords
- All figure captions
- All tables (including title, description, footnotes)

Further considerations

- Manuscript has been 'spell-checked' and 'grammar-checked'
- All references mentioned in the Reference list are cited in the text, and vice versa
- Permission has been obtained for use of copyrighted material from other sources (including the Web)
- Color figures are clearly marked as being intended for color reproduction on the Web (free of charge) and in print, or to be reproduced in color on the Web (free of charge) and in black-and-white in print
- If only color on the Web is required, black-and-white versions of the figures are also supplied for printing purposes

For any further information please visit our customer support site at http://support.elsevier.com.

AFTER ACCEPTANCE

Use of the Digital Object Identifier

The Digital Object Identifier (DOI) may be used to cite and link to electronic documents. The DOI consists of a unique alpha-numeric character string which is assigned to a document by the publisher upon the initial electronic publication. The assigned DOI never changes. Therefore, it is an ideal medium for citing a document, particularly 'Articles in press' because they have not yet received their full bibliographic information. Example of a correctly given DOI (in URL format; here an article in the journal *Physics Letters B*):

http://dx.doi.org/10.1016/j.physletb.2010.09.059
When you use a DOI to create links to documents on the web, the DOIs are guaranteed never to change.

Proofs

One set of page proofs (as PDF files) will be sent by e-mail to the corresponding author (if we do not have an e-mail address then paper proofs will be sent by post) or, a link will be provided in the e-mail so that authors can download the files themselves. Elsevier now provides authors with PDF proofs which can be annotated; for this you will need to download Adobe Reader version 7 (or higher) available free from http://get.adobe.com/reader. Instructions on how to annotate PDF files will accompany the proofs (also given online). The exact system requirements are given at the Adobe site: http://www.adobe.com/products/reader/tech-specs.html.
If you do not wish to use the PDF annotations function, you may list the corrections (including replies to the Query Form) and return them to Elsevier in an e-mail. Please list your corrections quoting line number. If, for any reason, this is not possible, then mark the corrections and any other comments (including replies to the Query Form) on a printout of your proof and return by fax, or scan the pages and e-mail, or by post. Please use this proof only for checking the typesetting, editing, completeness and correctness of the text, tables and figures. Significant changes to the article as accepted for publication will only be considered at this stage with permission from the Editor. We will do everything possible to get your article published quickly and accurately – please let us have all your corrections within 48 hours. It is important to ensure that all corrections are sent back to us in one communication: please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is solely your responsibility. Note that Elsevier may proceed with the publication of your article if no response is received.

Authors have 48 hours to respond to proofs unless otherwise requested. If no response occurs within this time, the manuscript will be published exactly as the proof reads.

Offprints

The corresponding author, at no cost, will be provided with a PDF file of the article via e-mail (the PDF file is a watermarked version of the published article and includes a cover sheet with the journal cover image and a disclaimer outlining the terms and conditions of use). For an extra charge, paper offprints can be ordered via the offprint order form which is sent once the article is accepted for publication. Both corresponding and co-authors may order offprints at any time via Elsevier's WebShop (http://webshop.elsevier.com/myarticleservices/offprints). Authors requiring printed copies of multiple articles may use Elsevier WebShop's 'Create Your Own Book' service to collate multiple articles within a single cover (http://webshop.elsevier.com/myarticleservices/offprints/myarticlesservices/booklets).

Additional information

The directives for preparing the paper in the style of the journal as set out in the Instructions for Authors must be followed; i.e. ensure the document is in the following order: Title; Authors; Addresses; Short title; Abstract; Introduction; Materials and Methods; Results; Discussion; Acknowledgements; References. Tables, Figure legends and Footnotes, should be saved in a separate file. Use two carriage returns to end headings and paragraphs. Type text without end of line hyphenation, except for compound words. Do not use lower case "I" for "1" (one) or "O" for "0" (zero). (They have different typesetting values.) Footnotes, Abbreviations, Tables and Figure legends should be saved in a separate file from the main text. Be consistent with punctuation and only insert a single space between words and after punctuation. Please include a list of any special characters you have had to use, e.g. Greek, maths.

AUTHOR INQUIRIES

For inquiries relating to the submission of articles (including electronic submission) please visit this journal's homepage. For detailed instructions on the preparation of electronic artwork, please visit http://www.elsevier.com/artworkinstructions. Contact details for questions arising after acceptance of an article, especially those relating to proofs, will be provided by the publisher. You can track accepted articles at http://www.elsevier.com/trackarticle. You can also check our Author FAQs at http://www.elsevier.com/authorFAQ and/or contact Customer Support via http://support.elsevier.com.