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Análise de genotoxicidade de compostos 4-amino-2,6-diaril-5-carbonitrila-pirimidínicos

Vitória de Santo Antão

Ano 2014

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Orientador: Prof. Dr. Cristiano Aparecido Chagas

Coorientador: Prof. Dr. Emerson Peter da Silva Falcão

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“Foi o tempo que dedicaste à tua rosa

que a fez tão importante” (Saint-Exupéry – O pequeno príncipe).

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SUMÁRIO

RESUMO

Os derivados pirimidínicos compreendem um diverso e interessante grupo de compostos. O anel pirimidínico está presente na estrutura de vários produtos farmacêuticos, tais como drogas para hipertensão, ansiolíticos, bem como em agentes antimicrobianos e antitumorais. Em estudos anteriores, foi realizada a síntese do composto Análise de genotoxicidade de compostos 4-amino-2,6-diaril-5-carbonitrila-pirimidínicos, os derivados 5 a-d foram testados em relação a atividades antiinflamatórias com resultados entusiasmantes. Porém seus efeitos na molécula de DNA são desconhecidos. Assim, o objetivo deste trabalho foi avaliar a atividade mutagênica dessos compostos 5 a-d em cultura de células. A metodologia utilizada foi o teste do micronúcleo *in vitro,* que é referência para a avaliação de atividade mutagênica, com células das linhagens HeLa e HepG2. Os compostos foram dissolvidos em DMSO e depois diluídos em meio de cultura até atingirem as concentrações testadas: 364 µg/mL, 61 µg/mL, 10 µg/mL e 1,7 µg/mL. As frequências de micronúcleos nas culturas com cada concentração foram comparadas com culturas que receberam apenas DMSO e serviram como controles negativos em cada experimento. As análises estatísticas foram realizadas utilizando-se a análise de variância com pós-teste de Tukey. Não houve diferença significativa na frequência de micronúcleos entre qualquer dos compostos, em nenhuma das concentrações, e os controles negativos. Estes resultados demonstram que, nestas concentrações, os compostos pirimidínicos testados não apresentam atividade mutagênica. Mais testes de segurança devem ser feitos com tais compostos antes de testá-los efetivamente como fármacos, mas danos ao DNA podem ser descartados dos riscos que tais compostos oferecem.

Palavras-Chave: Derivados pirimidínicos, mutagênese, micronúcleo

ABSTRACT

Pyrimidine derivatives comprise an interesting group of compouds and may have diverse pharmaceutical activities. Pyrimidinic ring is part of the structure of many pharmaceutical products, like anxiolytic, hypertension, antitumor and anti-inflammatory drugs. In a previous study, the compounds 4-amine-2,6 diaryl-5-carbonitrile-Pyrimidines, 5a-d were synthesized and evaluated against inflammation, showing antiiflammatory activities. However, it was not determined if the compounds have mutagenic activities and mutagenic tests are mandatory for drug candidates. This paper presents an *in vtiro* micronucleus test with the compounds N-phthalimido pyrimidine 5A (m-nitro- pyrimidine), 5B (p-nitro- pyrimidine), 5C (p-ethoxy-pyrimidine) e 5D (p-bromine/p-chlorine-pyrimidine). The tests were performed in HeLa and HepG2 cell lineages and four different concentrations of each compounds was tested: 364 µg/mL, 61 µg/mL, 10 µg/mL, 1,7 µg/mL. The compounds was first dissolved in DMSO and then, the solution was diluted in Dulbecco's modified Eagle medium (DMEM) to the final concentrations. The micronucleus frequency in cell cultures treated with each compound, in different concentrations, was compared with micronucleus frequency in cell cultures treated with DMSO in same concentrations (negative control) of that ones used to dissolve the compounds. None of the compounds, at none of the concentrations, showed significance increase in micronucleus frequency when compared with negative control. Our results show that the four pyrimidinic compounds have not mutagenic activities, at the tested concentrations. More safety tests must be performed with such compounds before use then for pharmaceutical purpose, but DNA damage is not a concern.

Keywords: Pyrimidine derivatives, mutagenesis, micronuclei

CAPÍTULO 1

1.1 Introdução

Compostos heterocíclicos de pirimidina são extensivamente estudados devido a suas propriedades farmacológicas e biológicas (LUNA et al., 2011, SILVA et al., 2008). Pirimidinas em geral apresentam importantes atividades biológicas. Por exemplo, algumas são agentes antivirais (NASR e GINEINAH, 2002), outras são antagonistas seletivos dos receptores da colecistoquinina (BARTOLOME-NEBREDA et al., 2001), e algumas são anti-inflamatórias (FALCÃO et al., 2006).

O anel pirimidínico está presente na estrutura de vários produtos farmacêuticos, tais como drogas para hipertensão, ansiolíticos, bem como em outros agentes antimicrobianos e antitumorais (LUNA et al., 2011). Segundo Falcão et al (2006), quando unimos a ftalimida à pirimidina, a atividade antiinflamatória é ainda superior. Falcão et al (2006) realizaram a síntese do composto, 4-amino-2,6-diaril-5-carbonitrila-pirimidínicos. Foram sintetizados os derivados 5 a-d, que apresentaram atividade contra a inflamação, além de apresentarem baixa toxicidade. No entanto, seus efeitos na molécula de DNA são desconhecidos.

Lesões persistentes no DNA levam a um aumento da mutagênese pela maior probabilidade de ocorrerem erros durante uma replicação. Com o objetivo de manter o genoma íntegro, as células desenvolveram mecanismos de reparo ao DNA (ALBERTS et al, 2010). No entanto, estes mecanismos estão sujeitos a erros e mutações genéticas em geral se acumulam em células somáticas e, eventualmente, em células germinativas.

Mutações acumuladas em células somáticas são importantes no processo de carcinogênese quando ocorrem em genes críticos do câncer (DE ROBERTIS 2006). Portanto, ensaios de genotoxicidade e mutagênese possibilitam identificar substâncias que apresentam riscos à saúde (CARDOSO, 2006). A avaliação da genotoxicidade de vários compostos fornece informações de grande interesse, permitindo o conhecimento do risco em potencial a população humana (AQUINO, 2010).

Um agente mutagênico é capaz de produzir diferentes eventos mutacionais em organismos geneticamente distintos. Contudo, a maior parte destes agentes exibem diferentes mutações, e isso depende de vários fatores, incluindo a natureza das alterações primárias no DNA (por ex.: modificações de base, de resíduos de açúcar fosfato, quebras de filamentos, ou incorporações de bases modificadas), e os efeitos secundários causados pela resposta do organismo a estas modificações (PRESTON et al., 1987**;** AQUINO, 2010). Esses agentes podem interagir diretamente com a molécula de DNA, ou podem se tornar mutagênicos ao sofrerem reações metabólicas no organismo vivo (AQUINO, 2010). A determinação do efeito genotóxico ou mutagênico de substâncias que têm potencial para ser usadas como medicamentos é obrigatória. O teste do micronúcleo é um método de referência para a avaliação da atividade mutagênica (MARZIN, 1997).

1.2 OBJETIVOS

1.2.1 Objetivo geral

Avaliar a ação mutagênica dos derivados 5 a-d, do composto 4-amino-2,6-diaril-5-carbonitrila-pirimidínicos, em cultura de células.

1.2.2 Objetivo específico

- Avaliar a ação mutagênica dos derivados 5 a-d, em culturas de células HeLa e HepG2 através do teste do micronúcleo *in vitro*.

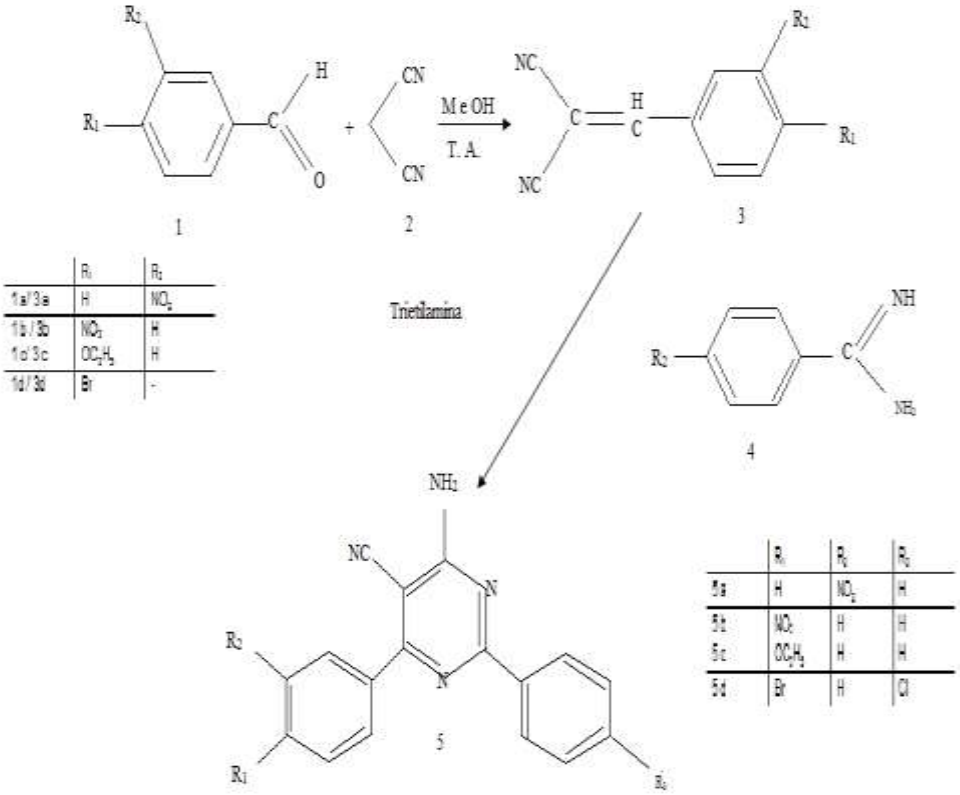
1.3 REVISÃO DA LITERATURA

1.3.1 Ácidos Nucléicos

Os ácidos nucléicos são macromoléculas responsáveis pelo armazenamento e transmissão da informação genética entre as células. Existem dois tipos de ácidos nucléicos: o ácido desoxirribonucléico (DNA) e o ácido ribonucléico (RNA), ambos constituídos pela polimerização de nucleotídeos. Cada nucleotídeo é composto por um grupo fosfato, um açúcar (pentose) e uma base nitrogenada. As bases nitrogenadas que compõem os ácidos nucléicos podem ser de dois tipos: purinas, compostas por dois anéis aromáticos, e pirimidinas, compostas por apenas um anel aromático (ALBERTS et al, 2010).

1.3.2 Derivados Pirimidínicos

Os anéis de pirimidina constituem o núcleo de ácidos nucléicos, antibióticos, co-enzimas e vitaminas, e são importantes para a regulação dos sistemas biológicos (BOOYSEN; GERBER; MAYER, 2008). Os derivados pirimidínicos compreendem um diverso e interessante grupo de drogas (FALCÃO et al, 2006). Compostos contendo pirimidina foram extensivamente estudados devido a suas propriedades biológicas e farmacológicas, por exemplo: atividades antifúngica, antiviral, antimicrobiana, antitumorais e anti-inflamatórias. O anel de pirimidina está presente na estrutura química de vários produtos farmacêuticos, como medicamentos para hipertensão e ansiolíticos, bem como em outros agentes antimicrobianos e antitumorais (LUNA et al, 2011).



Esquema 1- Síntese dos compostos 4-amino-2-aril-5-ciano-6-{3 e 4-(N-ftalimidofenil)} pirimidina

Os Compostos são sintetizados seguindo-se o esquema 1. Inicialmente reagem, a temperatura ambiente e em metanol aldeídos aromáticos substituídos com malononitrila. O produto, bisnitrílas, reage em seguida com amidinas aromáticas originando os compostos 5.

Falcão et al (2006) incorporou um grupo ftalimido em um dos aneis fenil desta molécula na intenção de se potencializar os efeitos antinflamatórios dos compostos 4-amino-2,6-diaril-5-carbonitrilicos-bissubstituidos. Seus resultados comprovaram que o processo de hibridização molecular aumentou o efeito antinflamatório da série de compostos sintéticos com ação superior *in vivo* ao ibuprofeno.

Estes resultados incentivaram novas investigações envolvendo derivados pirimidínicos, e nos últimos anos pesquisadores têm tentado sintetizar novos compostos pirimidínicos para avaliar suas atividades (SILVA et al., 2008), quantificar seus efeitos e

prever a síntese de novos análogos sintéticos mais potentes através de relações quantitativas entre as propriedades físico-químicas e a atividade biológica (QSAR)

Ainda no trabalho de Falcão et al (2006), foi realizada a síntese do composto 4-amino-2,6-diaril-5-carbonitrila-pirimidínicos. Os resultados sugeriram que os derivados são muito ativos contra a inflamação. Além disso, eles apresentam baixa ou nenhuma toxicidade após a administração intraperitoneal em ratos albinos (SILVA et al, 2008). Não há referências quanto à atividade genotóxica ou antigenotóxica de tais compostos.

1.3.3 Efeito Mutagênico

Para garantir a segurança terapêutica de um agente natural ou sintético, deve ser comprovado que o seu uso não traz agravos à saúde humana por meio de estudos farmacológicos, histopatológicos e toxicológicos, sendo então necessária a avaliação de uma possível atividade genotóxica e/ou mutagênica deste agente (AQUINO, 2010).

O efeito mutagênico é a conseqüência de danos genéticos causados por agentes físicos, químicos e biológicos, induzido por mutações nas células de um organismo. Já o efeito antimutagênico é o contrário, tem ação inversa impedindo que haja mutações causadas por um determinado agente mutagênico. Para avaliar este efeito são utilizados testes como do micronúcleo (GAMEIRO, 2005).

A exposição a agentes genotóxicos em nosso ambiente pode causar uma variedade de efeitos sobre a saúde humana. Alguns efeitos se expressam imediatamente, enquanto outros levam anos para se manifestar. Efeitos tardios bem conhecidos incluem a indução de câncer, notadamente as leucemias, doenças genéticas nas gerações seguintes, desordens de desenvolvimento, entre outros (NASRALLA, 2008).

Os testes de genotoxicidade/mutagenicidade são, portanto, de particular importância, pois são capazes de detectarem compostos que induzem danos genéticos direta (metabolização) ou indiretamente (biotransformação) por diversos mecanismos (AQUINO, 2010).

1.3.4 Micronúcleos

Para avaliar a segurança no uso dessas substâncias, são utilizados testes de genotoxicidade, como o Ensaio cometa ou Single-Cell Gel Electrophoresis (SCGE) e mutagenicidade, como o Teste do Micronúcleo, *in vivo*, utilizando, em geral, células de mamíferos, geralmente camundongos, ou *in vitro*, quando se pode utilizar toda uma gama de linhagens celulares, de roedores e humanos (metabolizadoras, como HTC e HepG2; ou não metabolizadoras, como as de ovário, CHO e OVCAR) (AQUINO, 2010).

Quando as anormalidades genéticas ocorridas são bastante evidentes, são chamadas de instabilidade cromossômica e podem ser evidenciadas por estudos citogenéticos, incluindo a análise de micronúcleos (DUESBERG et al., 1998). Micronúcleos são estruturas visualizadas, ao microscópio óptico, como pequenos corpos de DNA, encontrados no citoplasma de células em divisão. Estas estruturas são formadas a partir de fragmentos cromossômicos e/ou cromossomos inteiros que não foram incluídos nos núcleos filhos durante a divisão celular (NERSESYAN E ADAMYAN, 2004). Também podem se originar a partir de fragmentos acêntricos, cromatínicos cêntricos, ou ainda de quebras de cromossomos multicêntricos (LOHMANN, 1995), apresentando características cromatínicas semelhantes às do núcleo principal da célula (CARVALHO, 2002). Tais estruturas são, portanto, utilizadas como biomarcadores de danos genéticos e toxicidade genética em indivíduos expostos a agentes genotóxicos (CAO, 2003).

A indução de micronúcleos nas células em cultura tem sido proposta como uma alternativa para a análise biológica de risco químico. O teste de micronúcleo é o único teste *in vitro* capaz de detectar as aberrações numéricas e cromossomais, dos mecanismos envolvidos no risco genético e cancerígeno (MARZIN, 1997).

A vantagem da utilização do teste do micronúcleo é o resultado rápido, sem custos excessivos e produtos químicos prejudiciais. É importante como um teste de triagem para a avaliação inicial de risco químico (MARZIN, 1997).

1.3.5 Células HeLa

As células HeLa são provenientes de um cancro epitelial do colo do útero humano, são as primeiras células humanas a partir da qual foi estabelecida uma linhagem de células permanentes. Em 9 de Fevereiro 1951, o cirurgião Lawrence Wharton Jr. removeu tecido da paciente Henrietta Lacks, uma mulher americana de 31 anos de idade, na Clínica da Mulher do Hospital Johns Hopkins. A paciente morreu oito meses depois da descoberta da doença. Uma porção de células da biópsia foram enviados para George Gey, o então chefe do laboratório de cultura celular na Universidade Johns Hopkins Hospital. As células foram cultivadas e propagadas em cultura celular tão bem que até hoje são largamente utilizadas em pesquisa.

As células Hela são uma linhagem celular aderente que cresce agarrada ao fundo do frasco de cultura de célula. Elas são capazes de crescer rapidamente até que as células entram em contato uns com os outros e, em seguida, eles param de crescer. Elas costumam se espalhar por toda a superfície do frasco e quando duas células adjacentes entram em contato umas com as outras, elas param de crescer, um fenômeno chamado de "inibição por contato". O tempo de duplicação para as células Hela são cerca de 24 horas. (TAVEIRA 2010).

1.3.6 Células Hep G2

Hep G2 é uma linhagem celular permanente que foi derivado do tecido do fígado de um rapaz americano caucasiano de 15 anos de idade, com um carcinoma hepatocelular bem diferenciado. Estas células são de morfologia epitelial, e não são tumorgênico em camundongos. As células secretam uma variedade de proteínas plasmáticas, por exemplo, albumina , transferrina , e as proteínas de fase aguda fibrinogênio , alfa 2-macroglobulina , alfa 1-antitripsina , transferrina , e plasminogênio . Elas foram cultivadas com sucesso em sistemas de cultivo em larga escala. As células HepG2 são adequados *in vitro* do sistema modelo para o estudo de hepatócitos humanos polarizados. (Outra linha de células de hepatócitos polarizada bem caracterizada é a linha de células híbridas derivadas de hepatoma de rato WIF-B). Com as condições de cultivo adequadas, células HepG2

exibem diferenciação robusto morfológica e funcional, com uma formação controlável de domínios de superfície celular apical e basolateral (KALINAUSKIENE et al., 2009).

CAPÍTULO 2

ENVIRONMENTAL TOXICOLOGY AND PHARMACOLOGY

ANÁLISE DE GENOTOXICIDADE DE DERIVADOS DO COMPOSTO 4-AMINO-2,6-DIARIL-5-CARBONITRILA-PIRIMIDÍNICOS.

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RESUMO

O anel pirimidínico está presente na estrutura de vários produtos farmacêuticos. Em estudos anteriores, foi realizada a síntese do composto 4-amino-2,6-diaril-5-carbonitrila-pirimidínicos, com atividade antiinflamatória positiva. Porém seus efeitos no DNA são desconhecidos. O objetivo deste trabalho foi avaliar a atividade mutagênica desse composto em cultura de células. A metodologia utilizada foi o teste do micronúcleo *in vitro,* com células HeLa e HepG2. Os compostos foram dissolvidos em DMSO e meio de cultura até atingirem as concentrações: 364 µg/mL, 61 µg/mL, 10 µg/mL e 1,7 µg/mL. As frequências de micronúcleos nas culturas foram comparadas com culturas que receberam apenas DMSO. As análises estatísticas foram realizadas por ANOVA com pós-teste de Tukey. Não houve diferença na frequência de micronúcleos, em nenhuma das concentrações, e os controles negativos. Isso demonstra que nestas concentrações, os compostos pirimidínicos não apresentam atividade mutagênica. Mais testes de segurança devem ser feitos,porém danos ao DNA podem ser descartados.

Palavras-Chave: Derivados pirimidínicos, mutagênese, micronúcleo

2. 1 INTRODUÇÃO

Os derivados de pirimidina compreendem um interessante grupo de drogas que estão sendo muito estudadas (CHABNER et al. 2001; HARDMAN et al. 2001). Possuem uma série de atividades biológicas como, antifúngica, antiviral, antimicrobiana, e antiinflamatória (LUNA et al. 2011). Falcão et al (2006) realizaram a síntese do composto 4-amino-2,6-diaril-5-carbonitrila-pirimidínicos, sintetizando os derivados 5a – h e 7a – e. Tais compostos apresentaram atividade contra a inflamação, além de apresentarem baixa toxicidade. Entretanto, seus efeitos na molécula de DNA são desconhecidos.

A avaliação da genotoxicidade dos compostos fornecem informações de grande interesse, garantindo uma maior segurança terapêutica e permitindo o conhecimento do risco em potencial a população humana (AQUINO 2010). Para avaliar a segurança no uso dessas substâncias, são utilizados testes de mutagênese, como o teste do micronúcleo In vitro, utilizando, em geral, uma gama de linhagens celulares (AQUINO 2010). Micronúcleos são estruturas visualizadas, ao microscópio óptico, como pequenos corpos de DNA, encontrados no citoplasma de células em divisão (Nersesyan e Adamyan 2004). Tais estruturas são, portanto, utilizadas como biomarcadores de danos genéticos e toxicidade genética em organismos expostos a agentes genotóxicos (CAO, 2003).

O objetivo deste estudo foi avaliar a ação mutagênica dos derivados 5 a-d, da 4-amino-2,6-diaril-5-carbonitrila-pirimidínicos, em cultura de células HeLa e HepG2, uma vez que tais compostos apresentam importantes atividades biológicas com potencial aplicação farmacológica.

2.2 MATERIAIS E MÉTODOS

2.2.1 Cultura de células e Compostos Testados

Os ensaios foram realizados com culturas de células das linhagens Hela (carcinoma epidermóide de colón de útero) e HepG2 (Hepatocarcinoma humano) em fase exponencial de crescimento, obtidas do Banco de células do Rio de Janeiro. Para manutenção das células e realização dos ensaios in vitro, foi utilizado o meio de cultura DMEM, suplementado com 10% (v/v) de Soro Fetal Bovino (SFB), 1% (v/v) de Glutamina 200 mM, 1% de solução de antibióticos (100.000 UI/mL de penicilina G-potássica e 25 mg/mL de Sulfato de streptomicina).

Os compostos N-ftalimido-pirimidínicos 5A (meta-nitro-pirimidina), 5B (para-nitro-pirimidina), 5C (para-etoxi-pirimidina) e 5D (para-bromo-para-cloro-pirimidina) foram sintetizados e disponibilizados pelo grupo de pesquisa em novos fármacos e produtos naturais bioativos do Centro Acadêmico de Vitória (CAV) da Universidade Federal de Pernambuco (FALCAO et al. 2006).

2.2.2 Tratamento

Previamente, os compostos foram todos dissolvidos em dimetil sulfóxido (DMSO) na concentração de 2 mg/mL. Para atingir-se as concentrações finais, cada solução referente aos compostos 5 a-d foi novamente diluída em meio de cultura DMEM, de modo a atingir as concentrações finais de 364 µg/mL, 61 µg/mL, 10 µg/mL e 1,7 µg/mL. Assim, cada composto foi testado nestas quatro concentrações.

As células foram cultivadas em placas de 12 poços sobre lamínula por 48 h em estufa a 37ºC, antes de receberem o tratamento. Em cada placa, além dos compostos testados nas diferentes concentrações, havia um poço destinado a um tratamento sem composto algum que servia como controle negativo (meio de cultura); um poço destinado a um tratamento apenas com DMSO a 10 µg/mL, que servia de controle negativo com o diluente; um poço destinado a um controle positivo com 0,05 mg/mL de ciclofosfamida, uma droga mutagênica que necessita ser metabolizada; e um poço com 0,05 mg/mL de colchicina, droga mutagênica que não precisa ser metabolizada.

2.2.3 Teste de micronúcleo

Assim que o composto a ser testado foi aplicado, cada poço recebeu 5 µl do inibidor de citocinese citocalasina B (Sigma – C 6762) e a placa voltou para a estufa, onde permaneceu por 48 h. Após esse período, o sobrenadante foi retirado e as células foram fixadas com 1 ml de solução composta por 25ml de metanol e 1ml de ácido acético por 5 min. Essa solução foi retirada do poço e as lamínulas foram deixadas para secar ao ar. Previamente, lâminas foram preparadas com laranja de acridina segundo (HAYASHI, 1990). Depois da secagem das lâminas, 10 µL de soro fisiológico foram colocados sobre cada lâmina que recebeu, então, a lamínula com a cultura de células fixada.

Para a análise dos micronúcleos, as lâminas previamente codificadas foram analisadas em teste cego, em microscópio de fluorescência, com aumento de 1000 vezes. Os critérios para a seleção das células foram: células binucleadas (1000 células/lâmina); núcleos intactos, com tamanhos iguais, mesmo padrão de coloração e dentro do limite citoplasmático; membrana celular intacta; célula claramente distinguível das células adjacentes. Para a análise da frequência de micronúcleos e de células micronucledas, não foram incluídas na amostra de células analisadas aquelas com um ou com mais de dois núcleos, as células necróticas e aquelas em apoptose.

A análise estatística foi realizada através do teste ANOVA, e pós teste de TUKEY utilizando-se o nível de significância de 5%, com a utilização do software SPSS for Windows versão 12.0. O trabalho foi realizado nos Laboratórios de Biotecnologia e Fármacos, e NANOBIOCEL do CAV, UFPE.

2.3 RESULTADOS E DISCUSSÃO

O teste do micronúcleo (Figura 1), não demonstrou diferença significativa entre qualquer um dos compostos e o controle negativo DMSO em nenhuma das linhagens

celulares testadas. Deste modo, nenhum dos compostos foi mutagênico em qualquer das concentrações. Estes compostos pirimidínicos tiveram ação antiinflamatória demonstrada por Silva e colaboradores (2008), evidenciando o potencial farmacológico de tais componentes. A falta de ação genotóxica dos compostos é um fator importantes em relação à segurança na sua utilização como fármaco, conquanto mais testes de segurança biológica devam ser feitos. Não há na literatura testes mutagênicos ou genotóxicos que sirvam de parâmetros de comparação com os testes apresentados neste artigo.

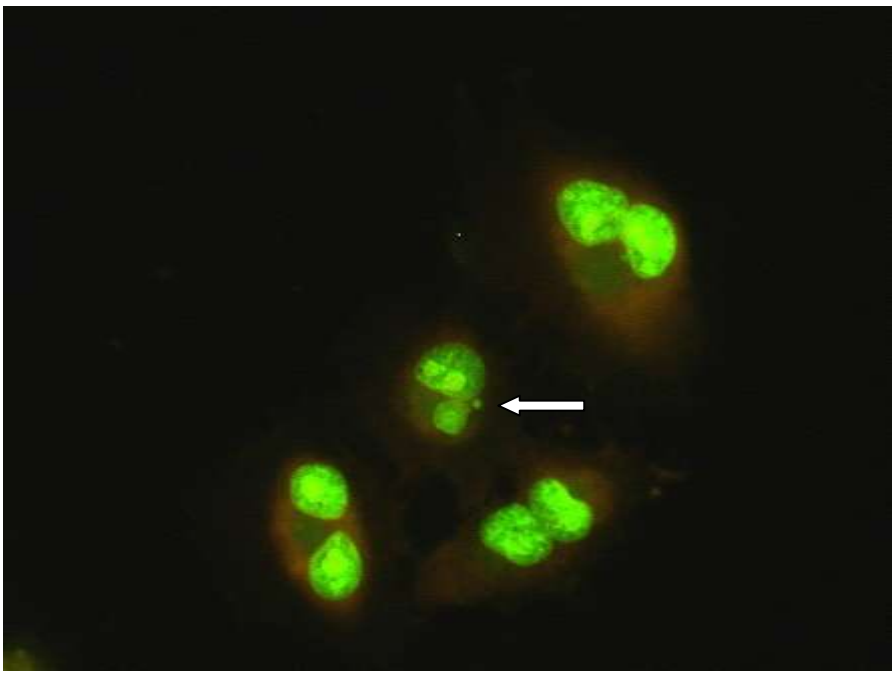

Figura 1: Micronúcleo em células HeLa em cultivo após tratamento.

Houve diferença significativa entre alguns dos tratamentos e as células que estavam somente em meio de cultura (tabela 1). No entanto, acreditamos que tais diferenças sejam resultado de efeito genotóxico causado pelo DMSO no qual todos os compostos foram dissolvidos previamente. Houve diferença significativa entre o DMSO e o Meio de Cultura em todas as placas de culturas testadas. Tal efeito demonstra que o DMSO apresenta alguma atividade mutagênica. O DMSO é largamente utilizado como solvente em estudos

químicos, pois tem baixa toxicidade, e boa miscibilidade com diversos compostos. Existem poucos dados sobre a toxicidade do DMSO, mas foi demonstrado que tem efeito mutagênico em algumas estirpes utilizadas no teste de Ames quando em altas concentrações (FIORE; ZANIER; DEGRASSI, 2002). Rosin e Stich (2006),o DMSO, quando usado como um solvente, pode afetar significativamente a eficiência de um antioxidante como um inibidor de mutagênese induzida por agente carcinogênico. Deste modo, o DMSO pode ser considerado uma substância que potencializa a mutagênese ao inibir antioxidantes intracelulares.

Tabela 1: Compostos e concentrações que apresentaram diferença significativa com o meio de cultura, de acordo com o pós teste de Tukey ($p<0,05$).

Composto	**Linhagem**	**Concentração**
5B	(HeLa)	Todas
5A	(HeLa)	10 µg/mL
		1,7 µg/mL
5B	(HepG2)	Todas
5A	(HepG2)	Todas
5C	(HepG2)	Todas
5F	(HepG2)	Todas

Neste artigo, utilizamos as linhagens celulares HeLa e HepG2 para o teste do micronúcleo. Ambas as linhagens são utilizadas com frequência para estudos de genotoxicidade e mutagênese em métodos como o ensaio cometa e o teste do micronúcleo. As células HepG2 foram escolhidas porque, além de demonstrarem sensibilidade no teste do micronúcleo (LAMY et al., 2004) (VALENTIN-SEVERIN et al., 2003), são capazes de metabolizar substâncias químicas xenobiontes de modo que, ao utilizarmos tais células, estávamos testando se os metabólitos das pirimidinas tinham ação mutagênica; nossos resultados demonstram que não. Por sua vez, as células da linhagem HeLa demonstram não serem capazes de metabolizar substâncias xenobiontes, além de, como HepG2,

apresentarem sensibilidade no teste do micronúcleo (JAGETIA; NAYAK, 1996a, 2000b). Ao utilizarmos tais células, testamos a mutagênese dos compostos em sua forma original, não metabolizados; tais testes demonstraram que os compostos não são mutagênicos.

2.4 CONCLUSÃO

Este estudo demonstrou que os derivados 5 a-d do 4-amino-2,6-diaril-5-carbonitrila-pirimidínicos não apresentam atividade mutagênica significativa nas condições testadas. É relevante salientar os efeitos do DMSO nas células HeLa e HepG2; mais testes de mutagênese com estes compostos devem ser feitos para determinar se este seria o melhor agente de dissolução de compostos a serem testados em meio de cultura. Os testes realizados neste trabalho demonstram certa segurança dos compostos do ponto de vista da mutagênese, conquanto mais testes de segurança biológica devam ser feitos. Mais testes farmacológicos e de segurança, como o teste do micronúcleo *in vivo,* devem ser realizados no futuro com vistas a determinar se tais compostos podem ser usados como fármacos.

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DISCUSSÃO GERAL E CONCLUSÕES

Em nenhuma das linhagens celulares, houve diferença significativa entre os compostos e o controle negativo, descartando a mutagenicidade dos derivados nas diversas concentrações como um problema na utilização destes compostos. E isso é importante em relação à segurança desses compostos e na sua utilização como um fármaco.

Detectamos diferença significativa entre a maioria dos compostos nas diferentes concentrações e o controle negativo meio de cultura, o que pode sugerir a atividade mutagênica do DMSO, já que este era usado como diluente, além do que, existiu diferença significativa entre o DMSO e o meio de cultura. O DMSO foi escolhido como diluente por que ele tem boa miscibilidade e baixa toxicidade (FIORE; ZANIER; DEGRASSI, 2002). Porém existem poucos estudos sobre o tema.

As células HeLa e HepG2, usadas neste estudo, demonstraram resultados satisfatórios na obtenção dos dados, e essas linhagens já são utilizadas com frequência em estudos de mutagenicidade como no teste de micronúcleo.

No futuro, os compostos devem passar por mais testes de eficiência farmacológica e testes de segurança para fins de utilização terapêutica, mas os resultados do teste do micronúcleo *in vitro* são promissores no que se refere à segurança em relação à mutagênese.

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GUIDE FOR AUTHORS

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(a) The total length of a mini-review should be about 30 double-spaced typewritten pages, including references, tables and figures.
(b) A brief introduction to the subjects, comprising not more than 500 words, in which reference is made to recent key articles or reviews on the topic should be given.
(c) Conclusions should be summarized in 500-1000 words at the end of this article. They should be preferentially outlined in a flow diagram or a graph.
(d) A maximum of 40 references may be used.

Assessments

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The total length of a letter should not exceed one page. Please name three potential reviewers making sure to include their name, affiliation and email address.

AFTER ACCEPTANCE

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