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SCIENTIFIC ARTICLE

Cytotoxic effects of local anesthesia through lidocaine/ropivacaine on human melanoma cell lines

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KEYWORDS
Lidocaine;
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Cytotoxicity;
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Melanoma cell lines;
Flow cytometry

Abstract
Background: Local anesthetics (LAs) are generally considered as safe, but cytotoxicity has been reported for several local anesthetics used in humans, which is not well investigated. In the present study, the cytotoxicity of lidocaine, ropivacaine and the combination of lidocaine and ropivacaine were evaluated on human melanoma cell lines. Melphalan, a nitrogen mustard alkylating agent, was used as a control agent for comparison of cytotoxic activity.
Methods: Melanoma cell lines, A375 and Hs294T, were exposed to 1 h to different concentrations of above agents. Cell-viability after exposure was determined by flow cytometry.
Results: Investigated LAs showed detrimental cytotoxicity on studied melanoma cell lines in time- ($p < 0.001$), concentration- ($p < 0.001$), and agent dependant. In both A375 and Hs294T cell lines, minimum cell viability rates were found after 72 h of exposure to these agents. Lidocaine 2% caused a reduction of vital cells to $10\% \pm 2\%$ and $14\% \pm 2\%$ in A375 and Hs294T, respectively after 72 h of exposure. Ropivacaine 0.75% after 72 h reduced viable cells to $15\% \pm 3\%$ and $25\% \pm 3\%$ in A375 and Hs294T, respectively. Minimum cell viability after 72 h exposure to the combination was $10\% \pm 2\%$ and $18\% \pm 2\%$ in A375 and Hs294T, respectively. Minimum cell viability after 72 h exposure to melphalan was $8\% \pm 1\%$ and $12\% \pm 2\%$, in A375 and Hs294T, respectively.
Conclusion: LAs have cytotoxic activity on human melanoma cell lines in a time-, concentration- and agent-dependant manner. Apoptosis in the cell lines was mediated through activity of caspases-3 and caspases-8.

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PALAVRAS-CHAVE
Lidocaina;
Ropivacaína;
Citotoxicidade;
Anestésicos locais do grupo amino-amida;
Linhagens celulares de melanoma;
Citometria de fluxo

Efeitos citotóxicos de anestesia local com lidocaína/ropivacaína em linhagens celulares de melanoma humano

Resumo
Justificativa: Os anestésicos locais (ALs) são geralmente considerados como seguros, mas citotoxicidade foi relatada em vários anestésicos locais usados em seres humanos, a qual não é bem investigada. No presente estudo, a citotoxicidade de lidocaína e ropivacaína e da combinação de lidocaína e ropivacaína foi avaliada em linhagens celulares de melanoma humano. Melfalano, um agente alquilante de mostarda nitrogenada, foi usado como um agente de controle para a comparação da atividade citotóxica.
Métodos: Linhagens celulares de melanoma, A375 e Hs294T, foram expostas por 1 hora a concentrações diferentes dos agentes mencionados acima. A viabilidade celular após a exposição foi determinada por citometria de fluxo.
Resultados: Os ALs investigados mostraram citotoxicidade prejudicial nas linhagens celulares de melanoma estudadas dependente do tempo ($p < 0{,}001$), da concentração ($p < 0{,}001$) e do agente. Em ambas as linhagens de células A375 e Hs294T, níveis mínimos de viabilidade celular foram encontrados após 72 horas de exposição a esses agentes. Lidocaína a 2% provocou uma redução das células vitais para $10\% \pm 2\%$ e $14\% \pm 2\%$ em A375 e Hs294T, respectivamente, após 72 horas de exposição. Ropivacaína a 0,75% após 72 horas reduziu as células viáveis para $15\% \pm 3\%$ e $25\% \pm 3\%$, em A375 e Hs294T, respectivamente. A viabilidade celular mínima após exposição de 72 horas para a combinação foi de $10\% \pm 2\%$ e $18\% \pm 2\%$ em A375 e Hs294T, respectivamente. A viabilidade celular mínima após exposição de 72 horas ao melfalano foi de $8\% \pm 1\%$ e 12 ± 2, em A375 e Hs294T, respectivamente.
Conclusão: Os ALs possuem atividade citotóxica em linhagens de celulares de melanoma humano de modo dependente do tempo, da concentração e do agente. A apoptose nas linhagens celulares foi mediada por meio da atividade das caspases-3 e caspases-8.

Introduction

A little is known about chemotherapy and anesthetics in general. In perioperative and ambulatory settings local anesthetics are administered as intra-articular injections.[1] Aminoamide local anesthetics exhibit their activity mainly by blocking the impulse conduction of nerve axons in a reversible manner. Lidocaine and ropivacaine both belong to amino amide class of local anesthetics with the former having antiarrhythic activity (class-1b). In general, local anesthetics (LAs) prevent or relieve pain by binding to specific receptor sites on the sodium (Na+) channels in nerves and blocking the movement of ions through these pores. Both the chemical and pharmacologic properties of individual local anesthetic drugs determine their clinical properties.[2] Local anesthetics including lidocaine applied topically have been shown to produce good pain control in patients with oral or rectal cancer. Lidocaine and ropivacaine are used at a concentration of 1.5% or 2.0% and 0.5% or 0.75%, respectively, for surgical anaesthesia with reduced cardiotoxicty and CNS toxicity.[1,3,4] These LAs are widely used for pain control in patients with head and neck cancer, to inhibit the metastasis and relapse of tumours and to reduce surgical stress induced inhibition of natural killer (NK) cell activity. However, both *in vitro* and *in vivo* studies have shown cytotoxicity towards several cultured cells.[5,6] Previous publications have reported that single dose injection of 1% lidocaine may have significant chondrotoxic activity. Cytotoxicity of lidocaine on mesenchymal stem cells, human oral and tumour cells, corneal endothelial cells and rotor cuff tenofibroblasts has been reported previously.[7–13] Ropivacaine, a long-acting aminoamide local anesthetic, inhibits influx of sodium ions reversibly and thereby blocks impulse conduction in nerve fibres. Ropivacaine has been reported to demonstrate reduced potential for CNS and cardiotoxicity and is used more frequently for local anesthesia and in the management of labour pain and postoperative pain. In addition to local anesthetic activity, ropivacaine has also been reported to inhibit platelet aggregation and antibacterial activity in *in vitro* studies. Ropivacaine was also reported to be cytotoxicity on mesenchymal cell lines at a concentration of 0.5%. Neurotoxicity of local anesthetics was associated with their apoptosis.[14–17,10,18] Despite the long use of aminoamide local anesthetics in several complications, there is inadequate information about cytotoxic activity of these agents and this need to be studied in detail. The cytotoxic activity of these commonly used LAs on melanoma has not yet been studied extensively. We hypothesized that these regularly used amino amide LAs have cytotoxic effects on human melanoma, the most deadly form of skin cancer, in a dose-, time-, and agent-specific manner. In this study, besides evaluating different concentrations of the above stated local anesthetics individually we have also investigated the combination of both the

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