REVIEW

Toxicity of Five Local Anesthesia Drugs on Cells and Multipotent Stem Cells

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ABSTRACT

Objectives: Mesenchymal stem cells (MSCs) play an important role in treating damaged tissues, growing and developing body tissues. Nowadays, the injection of stem cells has been considered for therapeutic purposes. Some substances which can be effective in the success rate of treatment are injected with the stem cells in the stem cell therapy. Anesthetics are a group of them. Local anesthetics toxicity on tissues such as nerve, cartilage, muscle and tendon are well described in many studies. Studies show local anesthesia can be toxic for stem cells too, and induce MSCs apoptosis and necrosis As a result, repairing of tissue by stem cells can be in trouble in damaged tissue which exposure to LAs. According to this, it is important to find the appropriate LA which has the least toxic effect on stem cells. In this study, we have considered the effects of LA such as lidocaine, bupivacaine, ropivacaine and mepivacaine on MSCs. **Literature review:** Local anesthetics toxicity has been described on chondrocytes by several studies. In this study, we have tried to find the effects of these drugs on mesenchymal stem cells. We have arranged local anesthetics for toxic effects to MSCs from high to low. According to this arrangement bupivacaine is the first drug, after that there are mepivacaine, lidocaine and ropivacaine, respectively. This sequence can be true for increasing the cellular metabolism, adhesive cells adhesion and also cellular appendages. **Conclusion:** The studies have indicated that MSCs is more sensitive to local anesthetics in comparison with chondrocytes. In addition to type of LAs, exposure time and drug dose play an important role in damaging to the MSCs. In other word, LAs effects are dose-dependent and time-dependent. however, The studies consider lesser neurotoxicity and longer local anesthesia effect for bupivacaine in comparison with other LAs such as lidocaine but it is recommended to use drugs which are safer (such as ropivacaine) in procedures including stem cell therapy, prolonged anesthesia and tissues are repairing. Because bupivacaine has high toxicity effect on mesenchymal stem cells.

Keywords: Mesenchymal Stem Cell, Bupivacaine, Ropivacaine, Lidocaine, Mepivacaine.

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Introduction

Stem cells are a group of body cells playing role in restoring damaged tissues, growing and developing body tissues [1]. These cells have two main characteristics which distinguish them from another type of cells: self-renewal and differentiation potential [2].

A sort of stem cells called mesenchymal stem cells (MSCs), located in connective tissues and play an important role in tissue regeneration in the injuries. These cells, which are multipotent, can differentiate into adipocyte, cartilage, bone, tendon, nerve tissue and muscle [3]. This differentiation greatly depends on the niche and growth factors in the stem cells environment [4, 5]. Nowadays, the injection of stem cells has been considered for therapeutic purposes. They are used in treating some diseases such as diabetes, heart failure and diseases associated with bone marrow [6-8]. Also, stem cells are used in the orthopedic field for the purposes such as repairing damaged cartilage and scrappy ligaments [9]. Some substances which can be effective in the success rate of treatment are injected with the stem cells in the stem cell therapy. Anesthetics are a group of them [10].

Using of local anesthetics (LA) is widely common in controlling post-operative pain and damaged tissue [11, 12]. These drugs reduce the sense of pain in patients by blocking signals. All of the local anesthetics using clinically are specific sodium channel inhibitors and prevent electrical activity in peripheral nerves [13]. Local anesthetics despite benefits in reduction of pain can cause damage, too. Arrhythmias and cardiac arrest, seizures, stroke and respiratory system depression are some of these complications in some patients [14-19]. Also, local anesthetics toxicity on tissues such as nerve, cartilage, muscle and tendon are well described in many studies [20-23]. For example, Negative effects of bupivacaine on various cells are confirmed [19, 24].

Stem cells play a major role in regenerating damaged tissues. Local anesthetics use in operations to reduce pain widely. Investigation of effects of these drugs on stem cells helps anesthesiologists to select better LA which has lesser toxicity on repairing injuries by stem cells. LAs and Stem cells are used in some remedies, which are done with the help of stem cells, at the same time. Understanding the effects of local anesthetics on MSCs is helpful to find LAs which have lesser negative effects and to increase the success of stem cell therapy.

In this study, we have first considered the influence of local anesthetics on cells, are outcomes of differentiation of MSCs, then the effects of LA such as lidocaine, bupivacaine, ropivacaine, mepivacaine and morphine on MSCs, separately.

Part 1: Local anesthesia and cells

Anesthetics are fat soluble and can penetrate into cells and their organelles easily [25]. Therefore, they can affect different tissues by influence potassium and calcium channels (in addition to sodium channels) [26]. But the exact mechanism is not fully understood. Some of these cells are mentioned here:

Adipocytes

Local anesthetics (LA) strongly prevent glucose transport, lipolysis in fat cells and also their growth in culture. However, these effects persist only as long as they are present. After washing, the cells return to their original state and regain their growth and normal function. Local anesthetics may halt cell growth and metabolism [27]. It's noteworthy; the risks of local anesthetics are lesser than general anesthetics [28].

Bone cells

Regional anesthetics are usually safe for bones and show a little complication [29]. Although, there are some ways to form new bones, adding local anesthetics specially bupivacaine help to achieve the aim [30].

Muscle cells

However about muscle, local anesthetics can damage muscular fibers. LAs ,including bupivacaine and lidocaine have direct cytotoxicity on myocyte [21]. Bupivacaine induce releasing of Ca^{2+} from sarcoplasmic reticulum [SR] and prevent Ca^{2+} uptake by the SR, finally its intracellular level increases [31].

In addition, the deranged energy balance is exacerbated by suppressing mitochondrial function. Then cell viability will be decreased. However, it seems cytotoxicity to lidocaine is minimal at a physiologic concentration in vitro [13].

Tendon cells

LAs also have adverse effects on tendons. They decrease cell viability which can be dropped by N-acetyl-L-cysteine or reduction of extracellular calcium [32]. Bupivacaine for example, applies a severe reaction oxygen species-mediated effect on tendon cell viability in vitro, depending on extracellular calcium concentration [33]. Anesthetics by influence on cell metabolism induce apoptosis and increase of pro-matrix metalloproteinase [34]. However, these effects are impermanent [23].

Chondrocytes

About cartilage, chondrocyte viability will be decreased in contact with Las [35]. Chondro-toxicity did not correlate with potency of local anesthetics [36]. Bupivacaine chondro-toxicity is much more than lidocaine and ropivacaine and significantly causes fewer vital cells [37, 38]. Bupivacaine is used for the goals of infiltration, nerve block, epidural, and intrathecal anesthesia [39].

Bupivacaine leads to histopathologic change and chondrotoxic effect in animal models [40]. Glycosaminoglycan (GAG) accumulation/tissue volume decreases and apoptosis increases as the concentration of lidocaine increases [41].

Repeated joint injection of lidocaine speed up cartilage decadence [38] Its intra-articular use in any concentration in clinical process should be dissuaded. Ropivacaine may be a safer intraarticular anesthetic [42].

As mentioned, LAs can reach organelles such as mitochondria, which play a vital role in cell metabolism, then lead to cell death [43]. These drugs selectively decrease pro-inflammatory cytokines such as TNF-α (Tumor Necrosis Factor-α) and increase anti-inflammatory cytokines [44]. After both type of cell death, necrosis or apoptosis, necrosis can occur [38]. The increase in cell death is more related to cell necrosis rather than cell apoptosis [45].

Preoperatively, LAs can be used both alone and in combination with other pharmaceuticals to reduce pain and narcotic character [21].

A group of these pharmaceuticals are steroids such as methylprednisolone and triamcinolone which are commonly used with anesthetics in some procedures to reduce pain associated with inflammation by their anti-inflammatory effects [44]. However, it has been shown the methylprednisolone has an additive toxicity with lidocaine and caution is warranted. Also, combination of triamcinolone and bupivacaine caused an intrinsic loss of chondrocyte viability but did not show a synergistic chondrocidal effects [46-48].

In addition of steroid agents, there is another substance, magnesium sulfate, which can increase analgesic character and also decrease toxicity of local anesthetics, if not combination of ropivacaine and magnesium sulphate [47]. It has been shown adding magnesium to LA decreases its toxicity on articular chondrocyte.

It seems location and manner of anesthetics injection have influence on potency of their effects. Maybe peri-capsular incisional injections reduce the adverse effects of LAs on articular cartilage [49]. Another limitation of a study is the lack of a demonstration and identification of the absorption of anesthetics into joint tissues (i.e. articular cartilage)

At last, it is important to consider that almost all of local anesthetics are dose- and time-dependent [36, 50, 51].

Part 2: Local anesthesia and stem cells
Mepivacaine

This drug is a category of amide-type-local anesthesia's which block pain receptors and reduce sense of pain like other members of the group. The survival rate of MSCs exposed to mepivacaine greatly depends on the concentration. Studies were designed in vitro and in the one-dimensional medium to examine the effect of mepivacaine on these cells. In these studies, the MSCs were first exposed to mepivacaine for 120 minutes. Then after 24 hours, its effects on the cells were analyzed. These studies indicated that exposure to concentrations up to 1% of mepivacaine have significant effects on MSCs [21, 52]. In other hand, a study indicated that mepivacaine does not have effect significantly on viability and or proliferation of stem cell. However, in most studies mepivacaine has the toxic effect on stem cells among local anesthesia [53].

Apoptosis in these cells increased and the necrotic phase rose (but not as much as apoptosis). Also, the metabolic rate of the cells decreased in the mentioned condition. And adhesion and cell appendages in adherent mesenchymal stem cells were significantly increased. In addition to the concentration factor, duration of exposure is important, too. As the

duration of exposure to mepivacaine is changed from 120 min to 40 min in above conditions, cell death rate is also decreased. Even cell death will not be significant in concentration of 1%, while increasing the time to 6 hours causes cell death at low concentrations like 0.5% [21, 52].

Morphine

The effects of morphine, as a local anesthetic, have not been greatly studied. But according to a study done on this subject, it can be concluded that morphine may be the safest LA for stem cells. In this study, the morphine 0.25%, which is widely used in local anesthesia of joints, was applied. What is clear is that morphine does not have much negative effect on stem cells, even after 6 hours of the exposure and the analysis after 24 hours. So that it is not much different from the effects of saline on Stem Cells. Also, morphine has no remarkable morphological changes in the cells. It should be noted that the study was conducted on tendon stem/progenitor cells or TSPC which are a kind of cells located in tendons [10]. Previous studies have also expressed that morphine does not have toxic effect on chondrocytes derived from mesenchymal cells [54].

Lidocaine

Among local anesthesia lidocaine have most usage. Lidocaine is used to reduce pain in damaged tissues and its effects on stem cells that are responsible for tissue repair is important [55].
The effects of Lidocaine on mesenchymal stem cells are dose-dependent like other similar medicines. In different studies, the effects of various concentrations (0.125%, 0.25%, 0.5%, 1%, and 2%) of lidocaine on mesenchymal stem cells have been examined. In these studies, MSCs have been exposed to lidocaine for about 2 hours. The amount of viable cells in the MSCs sample has been studied. Among these concentrations, all concentrations up to 0.25% of lidocaine were significantly caused reduction of MSCs. The adhesion and cell appendages have also significantly decreased at concentrations up to 0.25%.
Increasing the annexin-v^+ levels indicate that cell death occurs more through the apoptosis [56]. Necrosis is also seen but not as much as apoptosis. [45, 52, 57]. A study showed that lidocaine can change expression of 4 miRNA (miR-9*, 29a, 296-5p and 37) in stem cells that can cause apoptosis in cells [58]. in another hand, in a study, level of annexin-v^+ had no significant difference between lidocaine group and control Therefore, this study suggests that lidocaine causes cell necrosis and, apoptosis pathway is not activated [59].
In two separate studies, it was found that cellular metabolism and content ATP have decreased in MSCs in above condition). In another study, adipogenic differentiation of stem cells was measured by expression of FABP4. This protein is only express by adipocyte and stem cells cannot expressed it. In this study, differentiation of stem cells had no significant difference between lidocaine 1% group and control [45, 52]. Also, Girad and et al claimed that in low concentration and short exposure (<2 h) of lidocaine did not effect on stem cell. It seemed differences in dose and time and some methodology can cause difference results in studies [59].
The exposure time of MSCs to lidocaine is another factor of increasing cell death rate. As the exposure time of MSCs to anesthetics increase from 120 min to 360 min, the cellular death increase by lidocaine at lower concentrations such as 0.25%, [45, 52] therefore it seems to reduce of period time of exposure can increase viability and function of stem cells and in clinic, it recommended that remove residual lidocaine from site of injury [59]. In conclusion it can be said lidocaine is safer than mepivacaine and bupivacaine, and more toxic in comparison with ropivacaine for mesenchymal stem cells; nevertheless, it recommended that fat-graft should wash with PBS before implanting to reduce negative effects of lidocaine [45, 52, 56].

Ropivacaine

Among amide-type local anesthetics, ropivacaine is the safest drug for MSCs. According to the studies conducted on this topic, this drug has a negative effect on MSCs only at concentrations up to 0.75%. In these studies, MSCs were exposed to ropivacaine for about 2 hours, and afterward, its effects have been analyzed after 24 hours. At last, it was concluded that ropivacaine does not have significant adverse effects on MSCs at concentration under 0.75 of ropivacaine. The adherent MSCs adhesion significantly decreases at concentrations up to 0.125%, however, the rate of reduction is substantially lesser than other drugs such as lidocaine, mepivacaine and bupivacaine. [10, 45, 60]; However, some studies indicated that generally

opium (like fentanyl and morphine) have less toxic effect rather than local anesthesia. [61]
Cellular metabolism is not changed much in cells which are exposed to ropivacaine And least damage to the endoplasmic reticulum and the least increase in intracellular calcium are observed among anesthetic drugs. Like other drugs discussed, the exposure time of MSCs to ropivacaine affect the rate of cell death (apoptosis) induction.
As the exposure time extend from 2 hours to 6 hours, apoptosis is significantly increased in MSCs at concentration under 0.75% videlicet 0.5%. It can be concluded the toxicity of ropivacaine on mesenchymal stem cells is lesser than bupivacaine, lidocaine and mepivacaine [10, 45, 60].

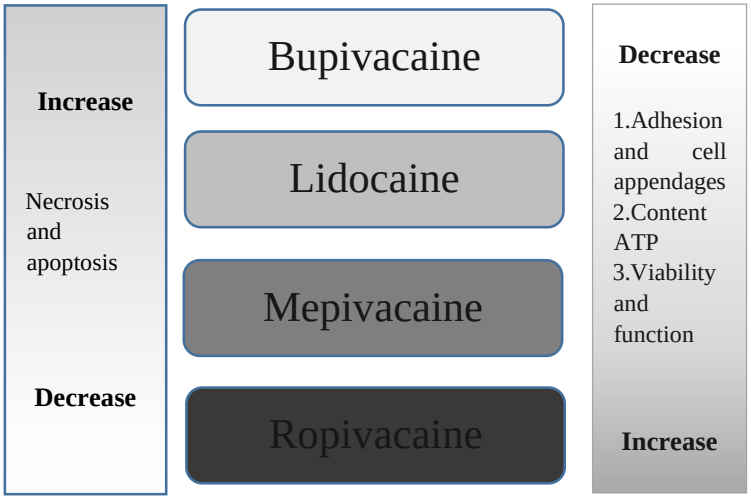

Figure 1: Summery of effects of local anesthesia on mesenchymal stem cells

Bupivacaine

Bupivacaine may be present as the most harmful and the most dangerous drug for MSCs among local anesthetics which are known. Bupivacaine is the only drug that can cause cell death in monolayer medium at concentration of about 0.125% and 2-hour exposure time over 24 hours. In the same concentration, adherent MSCs adhesion substantially decreases, too. Necrosis and apoptosis induction to the cells can be observed at small doses like 0.0625% when the exposure time of MSCs to bupivacaine extend from 2 hours to 6 hours [10, 45, 52, 60].
The notable reasons for high toxicity of bupivacaine on MSCs are numerous. For example, it seems the interaction between bupivacaine and sodium-potassium pump is one of these reasons [62]. Or according to conducted studies, it has been observed that bupivacaine causes destruction of endoplasmic reticulum and increase the intracellular calcium. This leads to the induction of apoptosis and cell death [52].
In a study, it has mentioned that Nitric Oxide synthesis increase in astrocytes and glial cells by bupivacaine [63]. This may lead to inflammatory processes and consequently causes cell death. Recent studies show toxicity of bupivacaine is more than lidocaine; however, one study has shown that lidocaine toxicity is more and this confirms the importance of further study on bupivacaine.

Conclusion

Local anesthetic drugs can affect almost every tissues and body cells because of their fat soluble property. Some of them include fat tissue, bone, muscle, tendon and the most important chondrocytes.
LAs affect cell growth, metabolism and finally the cell viability and change them. These decrease cell viability and induce cell death. It occurs through the apoptosis rather than necrosis. These effects usually are temporary and the cells return to their normal state after washing.
According to the studies, bupivacaine is the most toxic drug among local anesthetics, however, it seems it would be safe for bones. And ropivacaine has the least toxicity, especially for chondrocytes.
Adding some substances such as triamcinolone can reduce LAs toxicity.
Local anesthetics toxicity has been described on chondrocytes by several studies [64-67]. In this study, we have tried to find the effects of these drugs on mesenchymal stem cells. We have arranged local anesthetics for toxic effects to MSCs from high to low. According to this arrangement, bupivacaine is the first drug, after that there are mepivacaine, lidocaine, ropivacaine and morphine, respectively. This sequence can be true for increasing the cellular metabolism, adhesive cells adhesion and also cellular appendages.
The studies have indicated that MSCs is more sensitive to local anesthetics in comparison with chondrocytes. In addition to the type of LAs, exposure time and drug dose play an important role in damaging to the MSCs. In another word, LAs effects

are dose-dependent and time-dependent. The studies consider lesser neurotoxicity and longer local anesthesia effect for bupivacaine in comparison with other LAs such as lidocaine [68], however, it is recommended to use drugs which are safer (such as ropivacaine and morphine) in procedures including stem cell therapy, prolonged anesthesia and tissues are repairing. Because bupivacaine has high toxicity effect on mesenchymal stem cells.

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