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Formulations of intra-articular injections for the treatment of knee osteoarthritis

Osteoarthritis (OA) is a progressive degenerative multifactorial disease affecting many joints including the knee, hip, ankle, thumb base, shoulder and temporomandibular joint and may lead to disabilities. The inflammatory reaction is involved during the disease progression leading to activation of degradative enzymes and production of chemokines and cytokines like tumor necrosis factor (TNFα) and interleukin-1 (IL-1) that leads to cartilage destruction and changes in the joint components by decreasing viscosity of the hyaluronic acid and increasing the synovial fluid (SF) volume. OA cause could be either idiopathic or secondary to trauma. Its prevalence increases with aging and the incidence is higher in women.

Introduction

OA can be classified based on the severity of the pain that might differ from one patient to another (mild or moderate to severe) depending on the cause and how far the disease has progressed. Patients with OA present with joint pain that worsens when the patient is involved in activities and stiffness (1, 2).

The changes that happen in the knee joint during OA are mainly located in the cartilage (3). The crucial cause is the chondrocyte because once it is activated, cytokines are released triggering the release of metalloproteinases (MMPs) resulting in reduction of proteoglycan and collagen loss (4, 5).

TNFα and IL-1 are considered the main mediators responsible for articular cartilage destruction, inflammation, and synovitis, for that they might be associated with OA and synovial fibroblast and chondrocyte activation (6). The pain and inflammation associated with OA is due to leukotrienes, prostaglandins, cytokines, and MMPs production (7).

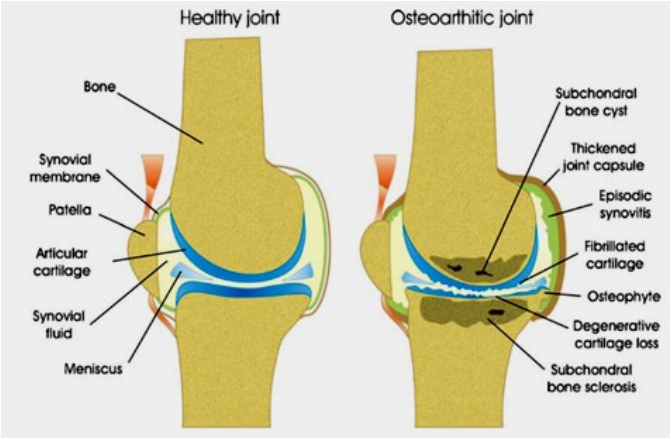

Fig 1. The difference between a healthy joint and osteoarthritic joint (8)

Modifiable risk factors

- Trauma
- Occupation: standing or bending the knee for long time
- Muscle weakness or imbalance
- Increased weight (obesity)
- Metabolic syndrome

Non-modifiable risk factors

- Gender (higher incidence of OA in female patients)
- Age (OA risk increases with aging)
- Genetics (there is no single gene that associated with OA development)
- Race

Intra-articular knee injections, whether corticosteroids or hyaluronic acid, are being used to delay the need of surgical intervention, which is the last resort for patients with knee osteoarthritis.

Different intra-articular (IA) injections are available like hyaluronic acid (HA) and corticosteroids (CS). The latter gives only short-term relief compared to HA that has longer but delayed effect. HA formulations differ in their molecular weight which affects the residence time and the dosing frequency. IA CS injection dosage forms are available; suspensions, solutions & emulsions. IA CS and HA injections are localised types of intervention that have drawbacks of rapid clearance and short resident time.

In this issue

Cochrane review showed that IA CS injections give symptomatic relief of pain up to 3 weeks starting one week after injection, but there is little evidence to support its long-lasting effect beyond 3 weeks. Also, there is lack of evidence in the ability of IA CS injections to improve knee function. To overcome the problem of rapid clearance of IA injections from the joint, several drug delivery systems are being developed, including micro- and nanoparticles and liposomes.

Treatment options

Knee OA treatment is based on the clinical manifestations that a patient presents with, and whether the patient has mild or moderate to severe OA.

Treatment options (9) can be classified into

i. Non-pharmacological measures

- Heat or cold compression.
- Low impact exercises like walking.
- Weight loss

ii. Pharmacological measures

- Acetaminophen
- NSAIDS (topical or oral)
- Tramadol
- Intra-articular knee injections (corticosteroids or hyaluronic acid)

Intra-articular corticosteroid injections (IA CS injections)

Intra articular corticosteroids are being widely used due to their ability to work as anti-inflammatory and immunosuppressive agents, by breaking the inflammatory process and retarding the progress of the immune cascade (10,11). As a result, the production of pro-inflammatory mediators like cytokines (IL1, IL6, AND IL17, and TNFα) which are responsible for cartilage destruction during OA and chemokines will be interrupted (11, 12).

The anti-inflammatory action of IA CS injections can be translated into their ability to reduce pain, redness, and swelling, and this can be achieved with minimal systemic side effects (11).

The complications that are associated with IA CS injections are rare like discomfort and pain at the site of injection but one of the most serious complications is joint infection (13).

IA CS injections are contraindicated in patients with periarticular infection or fracture, septic arthritis, joint instability, and juxta-articular osteoporosis (14). Their frequency should be limited due to the risk of cartilage damage.

Intra-articular hyaluronic acid injections (IA HA injections)

Normal knee has the synovial fluid that contains hyaluronic acid (Fig 2.), also called hyaluronan, which is an anionic and non-sulfated compound made from repeated units of *N*-acetyl-D-glucosamine and D-glucuronic acid. It is found in the synovial fluids in normal knee in concentrations of 2.5 to 4 mg/ml and it has a molecular weight of 4-10 million Da. The volume of the synovial fluids in a normal knee is 2 ml (8, 15).

HA can work either as a lubricant or as shock absorber and the behavior will differ depending on the applied force (6). HA is responsible for providing viscosity and elasticity to the knee joint. However, the previous functions might be affected in patients with knee OA because the concentration of the synovial fluid content is decreased and the chain length, which reflects the molecular weight is also reduced (16). Both the concentration and the molecular weight of the HA will be reduced by (33-50%) due to the increase of the SF volume that will lead to diluting the HA concentration (15, 17). Intra-articular hyaluronic acid injections will sufficiently coat the articular cartilage and ensure its protection (18, 19).

HA injections are more effective in reducing pain than oral NSAIDS and they are safer because they do not have gastrointestinal related problems that non-selective NSAIDS like ibuprofen have, or cardiovascular related issues of the selective ones (COX2 agents, e.g. celecoxib) (20, 21).

HA injections can be classified into low and high molecular weight hyaluronic acid (LMWHA and HMWHA respectively). Both have disease modifying effects, however the latter is preferred (11). HMWHA is more efficacious because it stays longer to provide an anti-inflammatory effect (19).

Based on Cochrane studies, the effect on pain and improving function will be seen starting from 5 to 14 weeks after the injection (22). In addition, literature reviews suggest that Hylan GF-20,which is a cross linked hyaluronan with molecular weight of 6 million Da, is safe and effective in reducing pain (23).

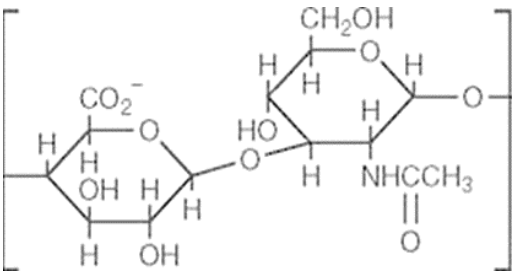

Fig 2. Structure of hyaluronic acid

In contrast to corticosteroid injections, HA injections do not provide immediate analgesic effect. However, the effect will start after four weeks, but it may have long lasting benefit unlike IA CS injections (10, 24). The response to HA injections depends on the grade of the disease, and since when the patient was diagnosed with OA (20). So, HA injection, which is also called viscosupplementation, is used to treat mild to moderate cases of knee OA (24).

Table 3. Available intra-articular injection dosage forms

Non-particulate (Solution)

- Betamethasone sodium phosphate
- Dexamethasone acetate

Particulate (Suspension)

- Betamethasone acetate
- Methylprednisolone acetate
- Triamcinolone hexacetonide
- Triamcinolone acetonide

Emulsion

- Dexamethasone-21-palmitate

IA CS injections (Table 3) can be classified into suspensions, solutions, and emulsions. They include:

I. poorly soluble CS (called particulate CS and ends with"lone" like methylprednisolone acetate, triamcinolone acetate and triamcinolone hexacetonide).
II. soluble CS (called non-particulate CS) and which ends with "sone" like betamethasone sodium phosphate (4).
III. Dexamethasone-21-palmitate (emulsion formulation) where the active ingredient is dissolved in the lipid component of the emulsion, so the drug will be released slowly post IA injection. Dexamethasone structure is modified by adding a fatty acid chain that forms a covalent bond. This addition will result in reducing the solubility of the modified form (8).

Intra-articular HA injection formulations

IA HA injections can be derived from fermentation with streptococcus equii, or obtained from rooster combs. If a patient has allergy to eggs or chicken, the latter type is contraindicated (8, 25).

Advantages and disadvantages of each formulation type

IA injections are appropriate for the joints that are frequently affected by OA, which are the knee, hand and foot. IA CS (Table 3) or HA (Table 4) injections will be localised at the injection site providing the needed concentration to achieve the desired effect, and this might be accomplished with low doses (8).

On the other hand, IA injections are associated with infection risk and the patient might complain from discomfort and pain at the site of injection. The infection risk and the local adverse effects can be reduced by using the prefilled syringe form of IA injections and providing aseptic conditions (6, 8). One more problem is that after injecting in the knee, the drug will flow out and be cleared from the joint cavity rapidly, which will decrease the efficacy of the IA injections (27).

The effectiveness of IA CS injections has been proven only for short term relief due to the short half-life resulting from their quick uptake by the circulation (8, 11, 27). As a result, multiple injections during the year might be needed to overcome this problem. But multiple injections during the year cannot be done due to pain at the injection site and high risk of infection (8). Also, recent studies showed that IA CS injections are associated with chondrotoxicity if given in high doses and for long duration. For this reason, the number of injections are limited to 3-4 per year for a maximum of two years (21, 24, 28).

HA injections are safe and cost effective (11). The cross linked hyaluronic acid injections will overcome the problem of short half-life that low molecular weight injections have, as they will stay longer in the joint, so the number of injections is one injection per week for 3 weeks, which is less compared to LMWHA injections that are administered as one injection per week for five weeks. Besides that, the viscoelastic properties and the molecular weight of cross-linked hyaluronic acid injections are higher, so they might be more effective compared to LMWHA injections (12,15, 29).

Formulation factors that affect the efficacy and safety of intra-articular injections

Several factors affect the transport of the drug and its residence time; drug $t_{1/2}$, drug solubility and dissolution, lipophilicity, charge, pH and isotonicity of the formulation (30).

The available IA formulations should be optimally designed to produce preparations that are effective and safe to the patient. To achieve that, solubility properties should be managed by choosing a suitable vehicle, suitable particle size, and by maintaining formulation stability during storage to have a better shelf life by adding a buffer system to reduce the possibility of degradation due to hydrolysis or oxidation. In addition, additives are added to maintain sterility and avoid pain post injection.

Table 4. Available HA formulations in the international market (26)

Formulation type	Agent and MW	Available conc/ml	Dose and Frequency
Gel (cross-linked hyaluronate)	**Gel-One**	10 mg	Inject 30 mg once (3 mL)
Solution [sodium hyaluronate]	**Gelsyn-3** 1.1MD	8.4 mg	Inject 16.8 mg (2 mL) once weekly for 3 weeks (total of 3 injections)
	Euflexxa 2.400-3.600 KDa	10 mg	Inject 20 mg (2 mL) once weekly for 3 weeks (total of 3 injections)
	Hyalgan 500-730 KDa	10 mg	Inject 20 mg (2 mL) once weekly for 5 weeks (total of 5 injections); some patients may benefit from a total of 3 injections
	Visco-3 0.62-1.17 MDa	10 mg	Inject 25 mg (2.5 mL) once weekly for 3 weeks (total of 3 injections)
	GenVisc 850 0.85 MDa	10 mg	Inject 25 mg (2.5 mL) once weekly for 5 weeks (total of 5 injections); some patients may benefit from a total of 3 injections
Solution (hyaluronan)	**Monovisc** 1-2.9 MDa	22 mg	Inject 88 mg (4 mL) once
	Hymovis 0.5-0.73 MDa	8mg	Inject 24 mg (3 mL) once weekly for 2 weeks (total of 2 injections)
	Orthovisc 2.900 KDa	15mg	Inject 30 mg (2 mL) once weekly for 3 to 4 weeks (total of 3 to 4 injections)
Solution [hylan polymers A and B]	**Synvisc-One** 6000 KDa	8 mg	Inject 48 mg (6 mL) once
	Synvisc Injec 6000 KDa	8 mg	16 mg (2 mL) once weekly for 3 weeks total of 3 injections)

Formulation excipients

Excipients include (8, 31):

* Water for injection as solvent
* Isotonic agents, such as sodium chloride, mannitol, and/or sorbitol
* pH modifiers, such as sodium hydroxide, sodium hydrogen phosphate, hydrochloric acid, or citric acid. The preparations should be maintained isotonic to minimize irritation.
* Cathepsins are proteolytic enzymes that might be activated under non-physiological pH. To prevent that, the pH of the formulation should not reach below 5.5 and maintained close to the SF pH (7.4). pH should be selected also to maintain the active ingredient stability and to avoid formulation instability during storage.
* Formulations may contain polysorbate 80 (as surfactant, solubiliser).
* Sodium edetate (as stabiliser, make a complex with metal ions that catalyze oxidation).
* Propylene glycol or polyethylene glycol (as co-solvents for solution formulations).
* Benzyl alcohol, methyl- and propyl-4-hydroxybenzoate, or cetylpyridinium chloride (antimicrobial agents) to ensure sterility and improve formulation shelf-life.

Suspension dosage forms need additional agents to prevent or minimize sedimentation as much as possible, like carmellose sodium, hypromellose or gelatin that work as stabilizers by increasing viscosity. Stabilizers ensure that the particles in the suspension formulations are re-dispersed adequately before administration (8).

Particle size, solubility, and dissolution rate

IA CS injections are of two types: the non-particulate and the particulate. The latter needs less frequent injections because they are long acting compared to the non-particulate one (5).

If the IA CS injection formulations are soluble (solution) like betamethasone sodium phosphate, this will result in high clearance rate from the injection site. However, the particles in the suspension formulations will take time to dissolve. This means that the particles will stay longer in the joint before being cleared (8) . The determinate of the extent of presence of poorly soluble glucocorticoids (suspension depot formulations) in SF is the dissolution rate that should be adjusted based on the particle size, because as the particle size decreases the total surface area increases and based on Noyes Whitney equation the dissolution rate is directly proportional to the surface area and to the saturated solubility of the particles (3). It can be concluded that the residence time of the IA CS injection formulations depends on two factors: the solubility of the active ingredient and its dissolution rate (8).

The suspension dosage forms contain suspended particles that should be maintained less than 10 μm, because particle size larger than that will interfere with the biocompatibility of IA CS injections (8).
Table 5 shows the relation between the potency of the product and its solubility and duration of action. As drug solubility decreases, the duration will increase. Solubility is a determinant of particles diffusion rate and residence time (28).

Normal type HA (the unmodified form) use is limited due to high clearance and degradation rate. Its half-life is 1-2 days and it has poor mechanical properties. To overcome that problem, the cross-linked form of hyaluronic acid is formed. This formulation has a better residence time and it is both biodegradable and biocompatible (32, 33).

Table 5. Relation between drug solubility and duration of action (28)

Drug	Potency*	Solubility in H_2O (mg/ml)	Duration action (h)
Methylprednisolone	5	120	12-36
Triamcinolone	5	80	18-36
Betamethasone	25	30/acetate 58/ phosphate	36-54

Protein binding in the synovial fluid

When the amount of drug that binds to protein increases, the half-life will increase (3). The injected drugs could be of two types; either the free or the bound-to protein form. The latter one has a longer residence time and slower absorption as it crosses the synovial membrane slowly compared to the first type (30).

Lipophilicity and charge

The charge of the particles has a big impact on the penetration and the residence time in the cartilage matrix, which is negatively charged, so the cationic drugs have a better distribution to the knee cartilage compared to the anionic drugs (3, 30). Lipophilicity

plays also a role in clearance rate; lipophilic drugs showed slower clearance rate compared to polar hydrophilic ones (3).

A lot of investigations are in progress to develop IA depot formulations (like liposomes, microparticles, nanoparticles, and hydrogels), and to check the appropriateness of drug formulations in overcoming the problem of the conventional dosage forms like rapid clearance and short residence time (3).

Drug delivery systems are designed to improve the efficacy of IA injections and to reduce the systemic side effects, by enhancing residence time and reducing clearance, which are a big concern that results from the dense structure of the cartilage extracellular matrix (ECM) that will hinder the entry of the drug particles, resulting in rapid clearance and interfering with drug bioavailability (34). Carrier based drug formulations must be appropriately designed and studied in terms of size, shape, and charge to be effective, and to avoid undesired effects like inflammation and immune cell activation (27).

Nanoparticles and Microparticles

Nanoparticles generally have particle size less than 1 μm, while microparticles are larger than 1 μm (up to tens of μm). Both types of depot formulations are made of either lipids or polymers. Among the widely used polymers are the albumin and the polylactic co-glycolic acid (PLGA) as they are biodegradable and biocompatible (8). PLGA also has no immunogenicity and the metabolite that forms after its degradation post IA injection already exists naturally (32).

Nanoparticles

Drugs are either adsorbed on or loaded in the nanoparticles. Nanospheres contain the drug dispersed in the carrier, however, in nano-capsules, the drug will be present in the core, encapsulated by a polymer or lipid shell (34). Nanoparticles incorporate many compounds, including poorly soluble ones, to provide better release of the drug and avoid the problem of rapid clearance (32).

In formulating nanoparticles, two aspects should be considered; the particle size and the retention time. Particles should be small enough so the entry to the articular cartilage is assured and they stay sufficiently. Once they enter the cartilage, they face compression by the normal joint movement, facing attraction toward the cartilage extracellular matrix, then they reside there (33). There is no specific size that is agreed on that would be most appropriate for drug formulations. Some studies have shown that using formulations with nanoparticle size less than 60 nm gives the advantage of reaching the cartilage matrix easily, particularly the collagen (30). In another study, the author made a conclusion based on the size of the collagen network (60 nm), that any particles above this size will not enter and by choosing the correct nanoparticle size, the cartilage will turn from being a barrier to a reservoir (33).

Microparticles

They are used to increase the residence time of the drug in the joint and to provide sustained release of the drug. PLGA microspheres are biocompatible and provide sustained drug release to manage pain. However, they did not show additional benefit on targeting chondrocytes except the case with very high doses. The polymer-based drug delivery system could be of either a natural or a synthetic origin (7, 32).

Microspheres elimination is size dependent. Particles that are 10μm and specially between 1-4 μm face phagocytosis (7). Microparticles engulfed by macrophages can be used as a depot. They reach the synovial membrane then the drug is liberated once the carrier breaks, so the time needed for the drug to be released provides the potential to increase the residence time. The release time should be optimized to ensure maintaining an effective therapeutic drug concentration for sufficient time (7).

The shape of the particle affects the efficacy and safety of the IA injections, as injecting irregular shape microspheres showed immune cell activation resulting in inflammation in the injected tissue, however the round shape type did not. Round shape particles are thus preferred (27).

Liposomes

Are a biocompatible carrier made from one or more phospholipid bilayers (where lipophilic drugs are inserted) and an aqueous core (where water soluble drugs are localized), that are investigated to achieve longer residence time of the drug in the knee joint (3,7). It was demonstrated that the most suitable lipid for liposomal formulation is dipalmitoyl phosphatidyl choline (DPPC), due to its transition properties of liquid-gel at 41ºC and its presence in the joint as endogenous material that counts for 45% of SF lipid composition. Studies have proven the efficacy of liposomes on animals and on humans as local IA formulations (27). Liposomes were associated with less incidence of inflammatory reactions compared to glucocorticoid suspensions (35).

Hydrogels

They are macromolecules (polymers of either natural or synthetic origin) dispersed in water or aqueous

solutions. The natural polymers include polysaccharides (e.g. alginate or starch) and proteins (e.g. gelatin or collagen) (8, 33).

New studies aim to improve the hydrogel formulation by preparing hybrid hydrogels, by adding PEG. The synthesis of cartilage extracellular matrix (ECM) is not enhanced by PEG. To have better regeneration of ECM and better chondroprotection, kartogenin (KGN) is combined with PEG to form biodegradable PEG/KGN HA (32). A copolymer hydrogel (PEG-PCLA) with a thermosensitive property was loaded with celecoxib. The hydrogel at room temperature is in solution form, however, it is converted to gel at 37ºC. The formulation showed gradual hydrolysis post IA injection providing sustained release of celecoxib for more than 90 days. In another study on rabbits with knee OA, an anti-inflammatory drug was inserted in chitosan microspheres dispersed in chitosan hydrogel using the spray-drying method. The study showed controlled release of the drug that lasted more than 7 days (33).

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TEST YOUR KNOWLEDGE

Answers on back page

1. ***Which of the following is a component of the synovial fluid of a normal knee?***

a) Erythrocytes
b) Hyaluronic acid
c) Osteoblasts
d) Uric acid

2. ***Which of the following IA CS injections is classified as an emulsion?***

a) Betamethasone sodium phosphate
b) Dexamethasone acetate
c) Betamethasone acetate
d) Dexamethasone-21-palmitate

3. ***The analgesic effect of hyaluronic acid injection will start after***

a) 30 min
b) 12 h
c) 4 weeks
d) 60 min

Is there a problem?
A patient is given the prescription for the treatment of his iron deficiency anemia. Is there any major error with the prescription?

RSB HOSPITAL

Patient Name: Hasan Ali Age: 45 years
Address: Street No: 45

Rx

Ferrous Sulfate 200mg tablet
1 tablet once daily

Dr. Fahad
Signature Date: 11/12/19

Answer (Prescription Exercise)
The frequency is wrong. For the treatment of iron deficiency anemia the dose should be 200mg 2 to 3 three time daily. Once daily dose is for prohylaxis of iron deficiency anemia.

Source: British National Formulary

TOPICAL ISSUES AND CONTROVERSIES

Gut microbes boost flu vaccine

Antibiotics disrupt the immune response to the influenza vaccine in people who haven't recently had exposure to the virus or immunisation.

Evidence in animal models, as well as correlative studies in humans, indicate that microbes present in the gut can shape immune responses. In a study published in *Cell,* researchers have confirmed that link in humans. They showed that, for people who hadn't had a flu shot or hadn't contracted the infection in the previous three years, a course of antibiotics just before a flu shot led to fewer antibodies produced in response to the immunisation than among study participants who didn't take antibiotics.

In 2011, an immunologist now at Stanford University, and colleagues, monitored gene expression in people who received the seasonal influenza vaccine in order to explore how the flu shot works and why it varies in efficacy in different people.

While most of the changes made sense, one stuck out: an increased activity of the gene encoding a receptor in the innate immune system that recognises the bacterial protein flagellin, the core part of flagella that many microbes use to move. This indicated that perhaps the immune system's recognition of bacteria- potentially the gut microbes- was somehow playing a role in the its response to the vaccine.

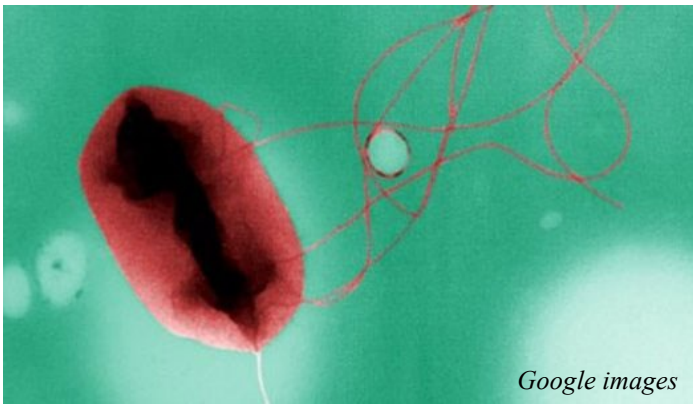
Then in 2014, the researchers gave mouse knockouts of the receptor for bacterial flagellin the flu shot. These mice made fewer antibodies to the flu

than their littermate controls. The research team suspected that this reduction was mediated by the receptor sensing flagellin present in the animals' gut microbes, so in separate experiments they depleted mice's microbiota with antibiotics before vaccination and gave germ-free mice the vaccine. Both groups of mice did not respond as well to the flu vaccine as controls that had their microbiomes intact. They concluded that the gut microbiota plays a role in generating an optimal antibody response against the flu shot, and the next step was to bring the work back to humans.

In the current study, the researchers designed a Phase 1 clinical trial to test the influence of gut microbes on flu vaccine–induced immunity. First, they treated 11 healthy adults with broad-spectrum antibiotics- metronidazole, neomycin, and vancomycin- for five days. On day four of the trial, their subjects and 11 untreated controls received the seasonal influenza vaccine. The people who received antibiotics had a corresponding drop in gut microbe diversity and bacterial load. But when the researchers monitored antibody generation in response to the vaccine, they didn't see much difference in the treated and untreated groups.

Because the flu antibody levels in both groups were quite high to start with, next the authors recruited 11 people who hadn't had the seasonal flu shot or been sick from the virus in the previous three years. Five of these subjects received the five -day course of broad-spectrum antibiotics, and six served as untreated controls. Everyone got the flu vaccine on day four. This time, the research team observed a marked difference in vaccine-induced immunity between the two groups. Treated subjects made far fewer flu-specific antibodies than their untreated peers, an effect that persisted through the 90 days of monitoring.

The researchers suggested that "If the immune system has very poor immune memory or imprinting, then it is far more susceptible to the effects of the perturbation of the gut microbiota by antibiotics".


Google images

It is possible that people's immune systems are sensing bacterial flagellin, just like the researchers found in mice. Directly translating the mechanism is difficult, though, because influenza was new to the animals' immune systems, which is almost never the case for people.

Because they saw such a striking difference in the role for the microbiome in people lacking immune memory of the flu, the team are investigating the relationship between gut bacteria, vaccines, and a completely new immune challenge such as a virus the immune system's never seen before. They concluded that "the results of this relatively small Phase 1 study demonstrate that the gut microbiota has an important influence on immune physiology in humans and, in particular, the immune response to vaccination, but this needs to be explored more broadly in the context of other vaccines and in the context of different populations".

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Blood thinner delays Alzheimer's disease in mice

Alzheimer's disease is the most common form of dementia, a neurodegenerative condition in which people experience progressive memory loss. Although some treatments can help people with Alzheimer's disease to manage this symptom to a certain extent, there is currently neither a cure nor a tried and true method of preventing the condition. So researchers worldwide continue to search for strategies and therapies that could at least delay the onset of Alzheimer's symptoms.

An existing blood thinner, used to prevent the formation of blood clots in people at risk of stroke, could help delay the development of Alzheimer's disease, according to a new study, as individuals with this condition also tend to have poor circulation in the brain.

In their paper in the Journal of the American College of Cardiology this team explained that just one year's treatment with this drug resulted in no memory loss and no reduction in cerebral blood flow in a mouse model of the disease. In the current study, the researchers worked with female mice that they had bioengineered to become prone to developing Alzheimer's-like symptoms later in life. To

these mice and a control group, the investigators administered either a placebo or dabigatran etexilate, a blood thinning drug, mixed with regular chow over a period of 1 year. The researchers calculated that each mouse in the treatment group received an average dose of around 60 mg dabigatran per kg body weight over 24h. Mice that received this treatment for 1 year developed no memory loss and maintained normal cerebral blood flow. Moreover, the researchers found a significant reduction in typical biological markers of Alzheimer's disease in the mice that had received the drug. Specifically, these mice had a 24% reduction in the extent of amyloid protein plaques, typical of the Alzheimer's condition. The researchers also found a 31% reduction in aggressive immune brain cells called phagocytic microglia and a 32% reduction in infiltrated T cells, another type of immune cell. These reductions indicate lower rates of inflammation and blood vessel injury in the brain, as well as less protein buildup that disrupts normal communication between brain cells.

To effectively combat Alzheimer disease, individualised combination therapy targeting the various processes that contribute to this disease is required. One of the goals is to improve the cerebral circulation. The study shows that treatment with oral anticoagulants has the potential to be an effective approach in Alzheimer patients who have a tendency for coagulation. Dabigatran is all the more promising as a potential new treatment for Alzheimer's because it has already been approved as a treatment for other conditions and health events, and it reportedly has fewer side effects than other anticoagulant drugs.

The researchers recommend that future studies should develop better diagnostic tools to determine which patients with Alzheimer's disease are also prone to developing blood clots. This cohort, they explain, may benefit most from a treatment that includes anticoagulants such as dabigatran.

Sources

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Metabolic biomarker "score" may predict death in next 5–10Y

According to a newly developed tool described in August in *Nature Communications*, it is claimed that it may be possible to use the metabolites in blood samples to predict the likelihood of a person surviving another five to 10 years, The authors of the report say the information may be useful in helping decide whether or not to do surgery on patients who are frail or could serve as endpoints in new clinical trials.

A biostatistician at Boston University who was not involved in the study said it showed the potential usefulness of metabolomic biomarkers but added that the field will need longitudinal studies in the future to assess the biomarkers' clinical usefulness.

According to the senior author on the study the team's goal was to find blood-based biomarkers that can "indicate risk of vulnerability, especially if that information provides opportunities for an improvement in lifestyle or better treatment,

Doctors often use functionality measures such as grip strength and gait to determine an elderly patient's health status, but these measures are imprecise. Other traditional biomarkers don't necessarily apply to patients who hit a certain age. Working with biobanks from all over the world for three years this group undertook the largest study of its kind to detect blood-based biomarkers of metabolism to reach their conclusions that "for example, a somewhat higher weight, blood pressure, or cholesterol level is not as bad for individuals over 80 years of age as compared to younger individuals,"

The team used data from 12 cohorts of individuals of European descent, a total of 44,168 people aged 18–109 years, to identify 14 metabolites that they could use to develop a "score" to evaluate a person's risk for mortality at five and 10 years out. During the study's follow up, which ranged from 3-17 years, depending on the cohort, 5,512 of the participants died.

Most of the biomarkers, which are involved in a variety of physiological processes such as fatty acid metabolism, fluid balance, and inflammation, have previously been associated with mortality on their own, but never been combined to form a single

predictive score. Moreover, unlike traditional measures of weight and cholesterol, the biomarkers consistently predicted mortality in all participants rather than only among the younger ones.

A single point added to the score was associated with a 2.73-fold increased risk of mortality during the course of the study. In one of the cohorts of 7,603 individuals (including 1,213 who died), the team compared the accuracy of the metabolic score and of traditional biomarkers in predicting mortality. The metabolic score was about 83% accurate, whereas the traditional scores were about 78% accurate.

The association of their biomarker score with mortality was surprisingly strong, given that it is only based on 14 metabolic markers in blood measured at a single point in the life of individuals.

Despite the study's impressive size, the authors caution that the information can't yet be used to estimate an individual person's risk of mortality. And outside experts also warned about over-interpreting the study's results.

The scientists used nuclear magnetic resonance (NMR) to analyze the samples because it is inexpensive and allowed them to process a large number of samples, but this strategy can lead to less reliable results than newer techniques such as mass spectrometry for detecting metabolites.

The team are beginning to test the validity of the biomarker score in a range of existing studies to determine when the measurement might be most useful. They are considering whether it could be used for elderly patients who enter the hospital with hip fractures or if the score could be useful to determine if a novel medication improves the risk of mortality in older patients. However, the team have expressed concern that the people most interested in the ability to predict the likelihood of five and 10 year mortality may not be healthcare providers and patients, but instead, their insurers.

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New immunotherapy against brain tumors

Brain cancers account for a disproportionate number of deaths, even though they represent only a small percentage of cancer cases. According to the National Cancer Institute, an estimated 23,820 people in the U.S will find out that they have brain cancer in 2019, and 17,760 will die of the disease in this same year. These figures show that while brain cancer will only account for 1.4% of cancer cases, it will be responsible for more than double this percentage of cancer deaths (2.9%) in the U.S in 2019. Between 2009 and 2015, fewer than one-third of people with brain cancer in the U.S survived 5 years or more following diagnosis.

About 15% of primary brain tumours are glioblastomas, which are particularly aggressive and fast-growing because a large number of their cells are replicating at any given time. These tumours readily invade neighboring regions of the brain. One of the features that make brain tumours aggressive is their ability to suppress attack from anti-cancer cells in the local immune system. These tumours use immune cells, such as special macrophages and T regulatory cells, as shields against anti-cancer cells.

For the first time, it has been shown that a new type of immunotherapy can reach and treat brain cancer from the bloodstream in mice. The nano-immunotherapy stopped brain tumor cells multiplying and increased survival.

The researchers believe that the new treatment could be the key to improving survival in people with glioblastoma. A recent paper describes how they combined advances in nanotechnology and immunotherapy to deliver checkpoint inhibitors across the blood-brain barrier. In the new immunotherapy, the drugs can remove a mechanism that enables the brain tumor to withstand attack from cancer-killing cells.

The blood-brain barrier (BBB) is a unique feature of the vessels that supply blood to the brain and the rest of the central nervous system. The barrier stops potentially harmful toxins and pathogens from entering brain tissue from the bloodstream. To date, promising types of immunotherapy that have passed clinical trials have not been very successful at crossing the barrier.

They developed a nano-immunotherapy that can carry checkpoint inhibitors across the BBB. Checkpoint inhibitors are drugs that help the immune system fight cancer. The new study is also the first to describe an immunotherapy that can stimulate immune systems both throughout the body and local to the tumor in mice. Without the protection of their shielding cells, the tumour cells are vulnerable to attack by lymphocytes and microglial cells that can eliminate cancer cells. The checkpoint inhibitors can

then block the T regulatory cells and macrophages, allowing the local immune cells to get activated and destroy the tumour.

They used a versatile drug carrier, poly (β-L-malic acid) (PMLA) (a natural polymer obtained from the slime mold Physarum polycephalum), to deliver covalently conjugated CTLA-4 and PD-1 antibodies (a-CTLA-4 and a-PD-1) to brain tumour cells, which resulted in the activation of the local immune system and prolongation of the survival of intracranial GBM GL261-bearing mice. The PMLA-based nanotherapeutics cross the BBB using transferrin receptor (TfR)-mediated transcytosis and target brain tumours. They also used an alternative delivery mechanism through BBB with PMLA-conjugated Angiopep-2 (AP-2) peptide, which is a synthetic low-density lipoprotein receptor-related protein 1 (LRP-1) ligand.

Although the findings were not made in humans the results bring us closer to developing a treatment that might effectively attack brain tumours with systemic drug administration. Using drugs that can treat the brain systemically, by using the bloodstream to deliver them, would be an advantage over treatments that only work when injected directly into brain tissue.

The new immunotherapy has to undergo further tests before it is ready for human trials. The authors hope that by delivering multifunctional new-generation drugs through the BBB, they can explore new therapies for many neurological conditions.

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Fighting cancer with anthrax

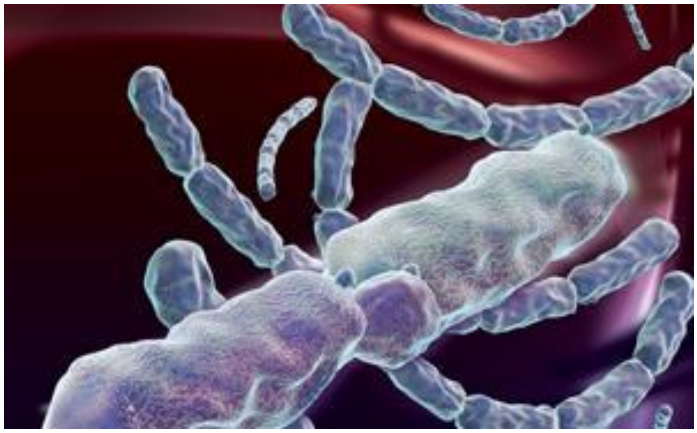
According to the Centers for Disease Control and Prevention (CDC), about 74000 people develop bladder cancer each year in the United States, and close to 17,000 die of the disease. Additionally, bladder cancer often returns following surgery, making repeat treatments a common feature of the disease. Standard treatments for bladder cancer are invasive and time consuming. The person undergoing treatment must sit for at least 2 h with a bladder full of cancer-killing drugs.

Researchers from Purdue University are looking in unlikely places for a solution. Currently, they are investigating the use of anthrax toxin produced bacterium Bacillus anthracis.

So-called umbrella cells protect bladder cells from coming into contact with urine, doing this in several different ways. Firstly, they form tight junctions, where the cell membranes of adjacent cells connect to form a barrier.

Secondly, they produce a protective coat comprising a protein called uroplakin, and thirdly, they develop an insulating layer of glycosaminoglycans. In contrast, bladder cancer cells do not form tight junctions. They also have virtually no uroplakin, and their layer of glycosaminoglycans is poorly assembled. These differences present a great opportunity to treat tumours with minimal effects on normal cells.

However, because fluid moves rapidly through the bladder, it is important that any cancer medication is targeted and works quickly. The increased number of epidermal growth factor receptors



(EGFR) on bladder cancer cells represents a potentially important target.

Unfortunately, previous attempts to target EGFR to treat bladder cancer have not been very successful. This is partly because these cancer drugs rely on EGFR to work correctly and take up the drug. However, in bladder cancer cells, these receptors do not always function normally, and the toxic agents may not enter their intended target. To bypass this, the scientists combined epidermal growth factor with anthrax toxin, which can enter cells independently. As the authors explain, it can "induce its own internalisation."

With this combination, the authors targeted efficiently; and human, mouse and canine bladder tumor cells were eliminated. Importantly, the beneficial effects occurred within minutes, rather than hours. They have effectively come up with a promising method to kill the cancer cells without harming the normal cells in the bladder.

Importantly, the authors explained that because

only tiny amounts of anthrax toxin are necessary, even if some did leak from the bladder into the blood supply, infection risk would be minimal. They explain that the component of each agent would be independently diluted, making toxin re-assembly virtually impossible.

In fact, they believe that their approach is safer than any other toxin-based approach reported in the literature; and that this discovery could be a turning point in the treatment of bladder cancer. Moreover, the authors hope that in the future, this technique could be used to treat other types of cancer such as lung and skin.

The study concluded that the approach employed in this study can transform the treatment of superficial bladder cancer because of its high efficacy *in vitro* and *in vivo* and its quick action. The study also speculates that its high *in vivo* efficiency can make it a feasible substitute for cystectomy in the early stages of invasive bladder cancer.

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In the NEWS

New WHO prevention guidelines for dementia evaluate 12 risk factors

As many as 50 million people across the world have dementia, an umbrella term for a series of neurodegenerative conditions that cause memory loss. In the next 30 years, this number is expected to triple. These conditions can become severe enough to impair a person's ability to continue their normal daily activities. The most common form of dementia is Alzheimer's disease, which affects 5.8 million people in the USA alone, according to the Alzheimer's Association.

Dementia changes the lives of so many people and their families around the globe, but the causes remain elusive. However, there is much information on the risk factors that may contribute to its development. Some of these are lifestyle-related and, as such, modifiable. Therefore, with adequate information, people may be able to learn how to adapt to their condition.

The World Health Organisation (WHO) has recently published a new set of guidelines (downloadable from their website) that seeks to advise governments, policymakers and healthcare providers on how best to tackle dementia prevention. These guidelines review existing evidence on the most significant lifestyle-related risk factors for dementia and take each one into account when issuing recommendations for prevention.

They evaluate 12 possible risk factors and offer advice on how to address each of them. These possible factors are: low levels of physical activity, smoking, a poor diet, alcohol misuse, insufficient or impaired cognitive reserve (the brain's ability to compensate for neural problems), lack of social activity, unhealthy weight gain, hypertension, diabetes, dyslipidemia (unhealthy cholesterol levels), depression and hearing loss.


Google images

While the WHO used the guidelines primarily to issue recommendations on how to address each of these potential factors, they also considered whether there is sufficient, strong evidence that tackling these risk factors can help stave off dementia. In doing so, they found that there is moderate evidence in support of the notion that being more physically active and following a Mediterranean-style diet can play a protective role against cognitive decline. The same goes for cutting down alcohol consumption. Currently, there is insufficient evidence that engaging in more social activities, taking anti-depressants, or wearing hearing aids can reduce the risk of dementia. However, the WHO emphasise that being socially involved, adequately treat-

ing depression, and managing hearing loss are, nevertheless, important.

The existence of potentially modifiable risk factors means that prevention of dementia is possible through a public health approach, including the implementation of key interventions that delay or slow cognitive decline or dementia. The official WHO document lists dementia management as a top priority to improve the lives of people with dementia, their carers and families, and decrease its impact on communities and countries.

Source:
https://www.medicalnewstoday.com/articles/325206.php

NEWS from the FDA

New concern over N-nitrosodimethylamine contamination in key drugs

Dec 6, 2019; Levels of possible cancer-causing chemicals in some key drugs has caused recent concern and at least two are under investigation by the U.S FDA and other non-US authorities.

The FDA had earlier released a statement stating that it had learned that some ranitidine medicines, including Zantac, contain low levels of N-nitrosodimethylamine (NDMA) which has been classified as a probable genotoxic substance that may increase the risk of cancer if above acceptable levels over long periods. These impurities can be present in drugs due to several reasons, including manufacturing processes or even the conditions in which they are packaged or stored.

In the weeks following that statement, retailers pulled Zantac and other over-the-counter ranitidine medications from their shelves and Sanofi voluntarily recalled the medication.

Now it turns out that although many of these levels of NDMA observed through FDA testing are much lower than the levels some third-party scientists first claimed, some levels still exceed what the FDA considers acceptable for these medicines. The "acceptable limits" of NDMA are 96 ng per day or 0.32 ppm. if the FDA or manufacturers find NDMA levels above that limit, companies will be asked to inform the agency and to voluntarily recall their ranitidine and nizatidine products before making them available to consumers.

An investigation was launched to understand the cause of this impurity in these drugs and to provide information for patients and consumers who take them. As part of this investigation, manufacturers were asked to conduct their own laboratory testing to examine levels of NDMA in ranitidine and nizatidine and to send samples to be tested by scientists.

FDA announced that they have asked manufacturers of ranitidine and nizatidine products to expand their testing for NDMA to include all lots of the medication.

On the other hand, FDA scientists have determined ranitidine does not form NDMA in typical stomach conditions. However, further investigation is needed to fully test how ranitidine and nizatidine behave in the human body. There is also some evidence that there may be a link between the presence of nitrites and the formation of NDMA in the body if ranitidine or nizatidine is also present. Because of this, consumers who wish to continue taking these drugs should consider limiting their intake of nitrite-containing foods, e.g. processed meats and preservatives like sodium nitrite.

Consumers may also consider alternative treatments that are approved for the same or similar uses as ranitidine and nizatidine. To date, FDA's testing has not found NDMA in Pepcid (famotidine), Tagamet (cimetidine), Nexium (esomeprazole), Prevacid (lansoprazole), or Prilosec (omeprazole).

Some metformin medicines in countries other than USA were also reported to have low levels of NDMA but these levels were noted to be within the range that naturally occurs in some foods and in water. Nonetheless, regulators in these countries are recalling certain metformin drugs. No metformin

recalls affect the U.S. market at the moment. The FDA will also work with companies to test samples of metformin sold in the U.S. and will recommend recalls as appropriate if high levels of NDMA are found.

If as part of the investigation, metformin drugs are recalled, the FDA will provide timely updates to patients and health care professionals. Meanwhile, patients who have been prescribed metformin should continue taking it to keep their diabetes under control. Also, during the investigation, the FDA recommends clinicians continue to prescribe metformin when appropriate because no alternative medications treat the condition in the same way.

STATE OF KUWAIT
Pharmaceutical & Herbal Medicines Control and Registration Administration

New pharmaceutical products approved from August to December 2019

Emgality Solution for Injection120mg/ml; Galcanezumab (rDNA) – 120mg; Bader Sultan; Eli Lilly and Company/USA.
Kepcitam Tablets 1000mg; Levetiracetam –1000mg; Safwan; Hexal AG/Germany.
Myomax Tablets 150m; Tolperisone HCl – 150mg; Palestine Pharmacy; The United Pharmaceutical Manufacturing Co. Ltd./Jordan.
Progesta Pessaries 400mg; Progesteron – 400mg; Palestine Pharmacy; Jordan River Pharm. Ind. L.L.C./ (Joriver)/Jordan.
Qall Tablets 4mg; Ondansetron (as hydrochloride – 4mg; International United for Medical Commerce Co; Quickmed Biotech & Research Lab Pvt. Ltd./India.
Tango Film Coated Tablets 20mg; Tadalafil – 20mg; Palestine Pharmacy; The United Pharmaceutical Manufacturing Co. Ltd./Jordan.
Vitadad D3 5,000, 10,000 and 50,000 IU/Capsules; Vitamin D3 (Cholecalciferol) – 5,000, 10,000 and 50,000IU; Safwan; Dar Al Dawa Development & Investment Co. Ltd./Jordan.
Zetex Tablets 10mg; Ezetimibe – 10mg; Al-Mojil; Tabuk Pharmaceuticals Man. Co./Saudi Arabia.
Zetmol Tablets 10mg; Ezetimibe –10mg; Ali Abdulwahab; Laboratories Cinfa, S.A./Spain.

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Answers to: Test your knowledge

Correct answers:
1-B; 2-D; 3-C

The *Kuwait Pharmacy Bulletin* (ISSN 1028-0480) is published quarterly by the Faculty of Pharmacy, Kuwait University, and includes a list of recently approved drugs from the MOH. It aims to provide instructive reviews and topical news items on a range of drug related issues. It is widely distributed free within the university, to hospitals, polyclinics & private pharmacies as well as to other universities within the Gulf & Middle East region.

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