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Somatic Gene Therapy in the Prevention of Toxic Effects of Organophosphate Agents

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Medical intervention in poisoning by organophosphate toxic agents (OPA) using atropine sulfate, 2-pyridinaldoxymethyl chloride (2-PAM), diazepam and other similar drugs can prevent the fatal outcome of poisoning. These drugs do not protect in case of sudden chemical attack and against post-exposure complications associated with permanent brain damage. The U.S. Department of Defense is funding research that can significantly simplify the protection of military personnel from OPA damage in the future. Their essence is in the use of gene therapy technologies, which allow experimental animals to produce their own proteins that destroy OPA and provide them with protection for several months. ***The aim of the work*** is to identify the achieved level of knowledge in the research using gene therapy technologies to create living objects resistant to OPA. ***The research method*** is analytical. ***The source base of the research*** are publications in scientific journals and descriptions of patents. ***Discussion of the results.*** As an enzyme that breaks down OPA in such experiments, genetically modified paraoxanase 1 (PON1) showed the greatest efficiency. PON1 hydrolyzes G-type OPAs, paraoxone, chlorpyrifosoxone, diazoxone and several other organophosphates. Adeno-associated virus vectors (AAV8, etc.) were used to introduce the gene encoding PON1 into the animal's body. A single injection of AAV8 carrying the recombinant *PON1-IF11* gene (AAV8-PON1-IF11) resulted in high expression and secretion of the recombinant PON1-IF11 protein into the bloodstream and provided asymptomatic protection against multiple lethal doses of G-type OPA for at least 5 months. These studies are still in their early stage. An analysis of the affiliation of the authors of publications and patents showed a high involvement of the U.S. military department and its cooperating organizations (DTRA, etc.) in such research. ***Conclusion.*** Given the fascination in the West with the ideas of human modification using gene therapy methods, this direction will be intensively developed for military purposes. At the same time, the idea of pre-created resistance to OPA is in demand by the widespread use of organophosphates in agriculture. The author believes that it would be safer to use allogeneic mesenchymal stem cells transfected with genetically modified PON1 variants with enhanced enzyme activity. This resistance to OP agents can be health protective and lifesaving in soldiers in real combat when the enemy uses these agents. However, this approach must be based on a strong experimental background. The door is open, the technologies are available.

Keywords: *adeno-associated virus; chemical weapons; gene therapy; genetic modification; nerve agents; organophosphates; paraoxonase 1; PON1; protect soldiers; rePON1.*

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Соматическая генная терапия и предотвращение токсического действия фосфорорганических отравляющих веществ и токсичных химикатов

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Медицинское вмешательство при поражениях фосфорорганическими отравляющими веществами (ФОВ) с использованием сульфата атропина, 2-пиридинальдоксимметилхлорида (2-ПАМ), диазепама и других аналогичных препаратов способно предотвратить смертельный исход отравления. Однако эти средства не защищают от внезапного химического нападения и от постконтактных осложнений, связанных с необратимым повреждением головного мозга. Военное ведомство США финансирует исследования, способные в будущем значительно упростить защиту военнослужащих от поражения ФОВ. Их суть заключается в использовании технологий генной терапии, в ходе которой организм экспериментальных животных вырабатывает собственные белки, разрушающие ФОВ, что обеспечивает защиту на несколько месяцев. ***Цель работы*** – выявить достигнутый уровень исследований, использующих технологии генной терапии для создания устойчивых к ФОВ живых объектов. ***Метод исследования*** – аналитический. ***Источниковая база исследования*** – публикации в научных журналах и описания к патентам. ***Обсуждение результатов.*** В качестве фермента, расщепляющего ФОВ в таких экспериментах, наибольшую эффективность показала генно-модифицированная параоксаназа 1 (PON1). PON1 обладает способностью гидролизовать ФОВ G-типа, параоксон, хлорпирифосоксон, диазоксон и ряд других органофосфатов. Для введения в организм животного гена, кодирующего PON1, используются векторы на основе аденоассоциированного вируса (AAV8 и др.). Однократное введение AAV8, несущего рекомбинантный ген *PON1-IF11* (AAV8-PON1-IF11), приводило к высокой экспрессии и секреции рекомбинантного белка PON1-IF11 в кровеносное русло и давало бессимптомную защиту от нескольких смертельных доз ФОВ G-типа в течение как минимум 5 мес. Эти исследования пока находятся на ранней стадии. Анализ аффиляции авторов публикаций и патентов показал, что в таких исследованиях активно участвуют военное ведомство США и сотрудничающие с ним организации (DTRA и др.). ***Заключение.*** Учитывая интерес на Западе к идеям модификации человека методами генной терапии, можно предположить, что это направление будет интенсивно развиваться в военных целях. В то же время сама идея заблаговременно созданной устойчивости к ФОВ востребована и в гражданской сфере в связи с широким применением органофосфатов в сельском хозяйстве. Автор считает, что более безопасным будет использование аллогенных мезенхимальных стволовых клеток, генетически модифицированных различными вариантами PON1. Эта устойчивость к ФОВ может защитить здоровье и спасти жизнь военнослужащих в реальном бою в случае использования противником ФОВ. Однако такие способы защиты от органофосфатов должны основываться на серьезных экспериментальных исследованиях, гарантирующих безопасность их применения. Дверь на новый уровень защиты от ФОВ открыта, технологии доступны.

Ключевые слова: *аденоассоциированный вирус; боевые отравляющие вещества нервно-паралитического действия; генетическая модификация; генная терапия; защита солдат; нервно-паралитические агенты; органофосфаты; параоксоназа 1; PON1; rePON1; химическое оружие.*

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In January 2020 the following statement of U.S. Army Medical Research Institute of Chemical Defense "Genetic Modification Could Protect Soldiers from Chemical Weapons"[1] has been published. This statement was based on a commentary in the journal Science[2]. Because this citation is not available always free to the reader in general, we cite some parts from the original Science comment. Here the author (Jocelyn Kaiser) writes: «Despite international bans, some countries, such as Syria, use deadly nerve agents against enemy soldiers and civilians[3]. Existing treatments for these chemical weapon attacks must be given quickly and don't always prevent convulsions or brain damage. Now, the U.S. Army researchers have created a gene therapy that allows mice to make their own nerve agent–busting proteins, providing protection against the toxicants for months. The strategy could theoretically be adopted for human soldiers, but it would have risks. A person could develop a harmful immune response to the introduced protein, for example. The statement follows: "There are a number of pros and cons"», says biochemist Moshe Goldsmith of the Weizmann Institute of Science, who was not involved with the research. Nerve agents are chemicals known as organophosphates. The most used type includes sarin, soman, cyclosarin, and tabun. All these block an enzyme that regulates levels of the neurotransmitter acetylcholine in muscles, causing muscle spasms, difficulty breathing, and sometimes death. Current treatments, such as atropine and diazepam, work by blocking acetylcholine receptors, but they must be administered right away and can't always prevent permanent neurological damage. Seeking a better solution, some researchers have injected lab animals with sped-up versions of human enzymes that spur organophosphates to break down before they can cause damage. For example, Goldsmith and collaborators have tweaked an enzyme called paraoxonase 1 (PON1) so that it can help the body defang nerve agents faster. But the Army would need to produce and store large quantities of such "bioscavengers" for injection into soldiers and might need to find a way to shield the proteins from the immune system for them to be effective. So, scientists at the U.S. Army Medical Research Institute of Chemical Defense took a different approach: Turn the liver into a factory for making a bioscavenger enzyme. Led by biochemist Nageswararao Chilukuri, they used a harmless virus called an adeno-associated virus to ferry DNA instructions into the liver cells of mice. The result was the mice's liver cells cranking out a potent version of PON1. Mice injected with the DNA-ferrying virus soon had high blood levels of the synthetic PON1 enzyme, which remained stable for the 5-month study. The rodents survived nine normally lethal injections of nerve agents over 6 weeks [1].

"We were surprised by how well this protein is expressed and how long it lasted," Chilukuri says. The team also showed the PON1 levels were just as high when the treatment was injected into muscles, a more practical delivery method on the battlefield. The gene therapy seemed to cause no harm to the mice. And although the animals made antibodies against the foreign PON1 protein, indicating an immune response, the antibody levels were too low to mute the protein's activity against nerve agents. Chilukuri's team suggests the therapy could protect soldiers, first responder medical staff, and military dogs, and could also protect farm workers at risk of being exposed to organophosphate pesticides. These are less toxic than nerve agents but can cause similar health effects at high doses. "It's a very nice paper, a nice advance in the field," says biochemist Oksana Lockridge of the University of Nebraska Medical Center. But she and others caution that the revved-up PON1 – which contains parts of the rabbit, rodent, and human versions of PON1 – is likely to provoke a stronger immune response in people, which could dull its effectiveness or cause severe health effects. People receiving the therapy might even make antibodies against standard human PON1, which the body uses to process harmful cholesterol, and could end up with an elevated risk of heart disease, Goldsmith says. Chilukuri acknowledges the caveats but notes his team didn't set out to solve all possible problems with the therapy. "It's kind of a proof of principle study," he says. "This is one way to keep the bioscavenger working for weeks and months in an animal."

The aim of the work is to identify the achieved level of research using gene therapy technologies creating living objects resistant to organophosphate toxic agents.

[1] Gunzinger M, RehbergC, Autenried L. *Five Priorities for the Air Force's Future Combat Air Force*. Published by Center for Strategic and Budgetary Assessments; Jan. 22, 2020. https://media.defense.gov/2020/Jan/24/2002238577/-1/-1/0/CSDS_Outreach1401.pdf (date: 12.11.2023).

[2] Kaiser J. Genetic modification could protect soldiers from chemical weapons. Gene therapy tested in mice turns liver into shield against deadly nerve agents. Science. 2020. Jan 22. https://doi.org/10.1126/science.abb0103 (date: 12.11.2023).

[3] Information about the use of nerve agents by Syrian troops "against enemy soldiers and civilians" is false information, disseminated in Western countries to discredit the Syrian and Russian leadership (**editor's note**).

The research method is analytical.

The source base of the research are publications in scientific journals and descriptions of patents.

Main

The key enzyme which is involved in the physiology (and of the acetylcholine action) is the acetylcholinesterase (AChE). The depiction of this process is shown in *Figure 1*.

We are not going in detail about the organophosphorus (OP) agents and mechanisms of their action. The interested reader can find the issue in literature[4] [3, 4].

Chemical warfare nerve agents (CWNAs) are colorless, odorless, and tasteless OP compounds being used as "invisible" lethal weapons in war zones and civilian societies worldwide. These toxic chemical compounds and their closely related pesticides are used in the form of gas, vapor, and liquid. Currently the stockpiles of several nerve agents are categorized into G series (GA, GD, GF, and GB), V series (VE, VG, VM, VP, VR, and VX), insecticides (malathion and parathion), and many more [1].

Pesticides are relatively less toxic than nerve agents. However, are believed to be responsible for nearly a quarter million annual deaths mainly in developing countries [5].

OP chemicals enter the bloodstream through the skin, by inhalation, food, and drink, cross the blood-brain barrier, and irreversibly inhibit acetylcholinesterase (AChE; EC 3.1.1.7), a key enzyme of the central nervous system. This disrupts normal communication between brain and muscles, causing miosis, hypersalivation, lacrimation, involuntary urination and defecation, seizures, and rapid death from respiratory failure [6].

Medical intervention with the available synthetic chemical regimen including atropine sulfate, 2-pyridine aldoxime methyl chloride (2-PAM), and diazepam is a method of choice and offers relief and remission and prevents death [7].

Nevertheless, these synthetic chemical therapeutics do not protect from postexposure complications such as convulsions, performance deficits, and permanent brain damage, pretreatment with pyridostigmine bromide (PB) appears advantageous to military and medical personnel. However, PB use has been suspected to be associated with Gulf War illness and to cause other medical issues such as diarrhea, vomiting, cold sweats, and blurred vision [8, 9].

Therefore, an alternative approach could be pretreatment with a natural or recombinant protein–based therapeutic capable of scavenging/hydrolyzing nerve agents into biologically inactive products before their escaping from blood circulation. Among protein-based scavengers of nerve agents, butyrylcholinesterase (BChE; EC 3.1.1.8), OP hydrolase (OPH; EC 3.1.8.1), and paraoxonase 1 (PON1; EC 3.1.8.1) appear promising

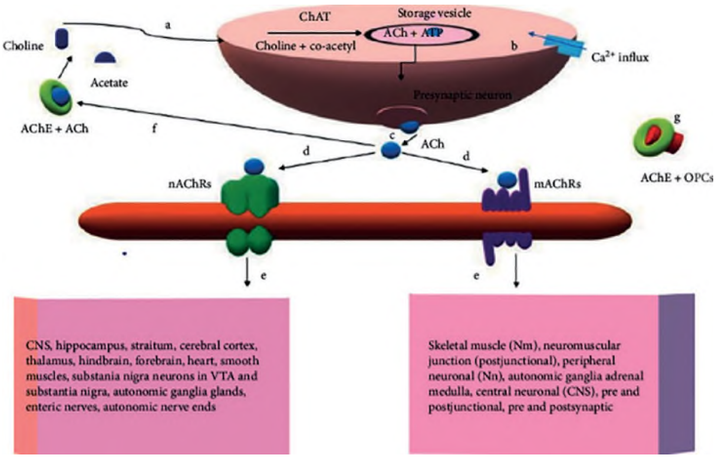

Figure 1 – *Schematic depiction of cellular interaction of AChE, acetylcholine (ACh), and OPs. (a) Choline released from ACh hydrolysis, moves to axon where it reacts with acetyl moiety of co-acetyl to from ACh by an enzyme ChAT (choline acetyltransferase) and gets stored in vesicles along with cotransmitters such as ATP. (b) The influx of calcium results in the fusion of membranes. (c) Fusion leads to release of ACh into neuron junctions. (d) Interaction of ACh with nicotinic (n) AChRs and muscarinic (m) AChRs. (e) Signaling to different physiological targets. (f) Excess ACh after signalling, interacts with AChE and degrades into choline and acetate. (g) OP binds with AChE and leads to increased ACh and endless signaling (https://www.hindawi.com/journals/jt/2020/3007984/)*
(OPC, nAChRs, mAChRs in the Figure are OPs, (n) AChRs, (m) AChRs in the text)

[4] Delfino K, Ribeiro T, Figueroa-Villar J. Organophosphorus compounds as chemical warfare agents: a review. *J Braz Chem Soc.* 2009;20(3). https://doi.org/10.1590/S0103-50532009000300003 (date: 10.12.2023).

in offering prophylactic protection in animal models [10].

However, BChE binds OP compounds at a one-to-one ratio. Therefore, this enzyme is required in large quantities to afford protection against CWNAs [11].

OPH and PON1 in their native forms or their variants hydrolyze OP in a catalytic manner. They are promising bioscavengers to offer prophylactic protection against OP nerve agents (*Figure 2*).

However, their short circulating half-lives, immunogenicity behavior, degradation, and rapid clearance from the bloodstream have become serious issues in developing them into prophylactics against CWNAs. Nanocapsulation, polysialylation, PEGylation, and polycarboxybetaine conjugation of these protein-based bioscavengers failed to resolve the issues of their poor circulation stability and immunogenicity [13–15].

In this study we would like further focus on the enzyme paraoxonase 1. Human serum paraoxonase 1 (PON1) is a Ca^{2+} dependent high-density lipoprotein (HDL) associated lactonase capable of hydrolysing a wide variety of lactones (including several pharmaceutical agents), thiolactones, arylesters, cyclic carbonates and organophosphate pesticides, nerve gases such as sarin and soman, glucuronide drugs and estrogen esters [16]. PON1 is currently classified as an aryldialkylphosphatase (EC 3.1.8.1) by the Enzyme Commission of the International Union of Biochemistry and Molecular Biology [17].

This enzyme is a glycoprotein with 354(5) amino acid residues with a molecular mass of 43 kDa [18].

The human *PON1* gene is a member of a multigene family consisting of three members in total. *PON1*, *PON2* and *PON3* are located next to each other on the long arms of chromosome 7 and share extensive structural homology. Interestingly, *PON1* can be differentiated from *PON2* and *PON3* by the three extranucleotide residues in exon 4 [19].

PON1 is synthesized mainly in the liver and secreted into the blood where it associates predominantly with HDL [20].

PON1 prevents atherosclerosis through its antioxidant activity, anti-inflammatory action, anti-apoptosis, anti-thrombosis, anti-adhesion, and lipid-modifying properties. PON1 stimulates cholesterol efflux, metabolizes oxidized phospholipids in high-density lipoprotein (HDL) and low-density lipoprotein (LDL), prevents lipid-peroxide accumulation on HDL and LDL, and preserves the anti-oxidative HDL function. Decreased PON1 activity contributes to elevated plasma levels of homocysteine and homocysteine-thiolactone. The latter can damage proteins by homocysteinylation and involve vascular damage pathology [21].

Schematic representation of the activity of the group of PON enzyme family is shown in *Figure 3*.

PON1 received its name from its ability to hydrolyze paraoxon, its first and most studied substrate. However, PON1 also hydrolyzes the active metabolites of other OP insecticides (e.g., chlorpyrifosoxon, diazoxon), as well as nerve agents such as sarin and soman. However, several other OP and carbamate insecticides are not hydrolyzed by PON1. Furthermore, only PON1 has OP esterase activity, while all three PONs are lactonases displaying overlapping but distinct substrate specificities for lactone hydrolysis [23].

For understanding of the "evolutionary process" mentioned in the part Introduction we will follow work of groups finally financed by the grants from the US DoD. The G-agents cyclosarin (known as GF also referred to as CMP-F) and soman (known as GD) are prime

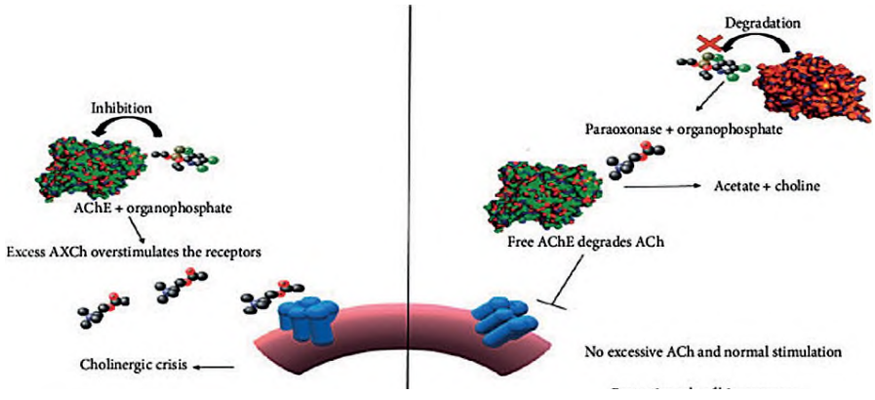

Figure 2 – *Diagrammatic representation of toxicity by OPs and their detoxification. During the toxicity by OPs, the compounds inhibit AChE and lead to excessive cholinergic effect, whereas the bioscavengers which can act as prophylactic agents neutralize the OPs before they reach their targets and result in normal physiological hydrolysis of ACh and thus control proper signalling process (https://www.hindawi.com/journals/jt/2020/3007984/) (AXCh – "toxic" – not degraded ACh)*

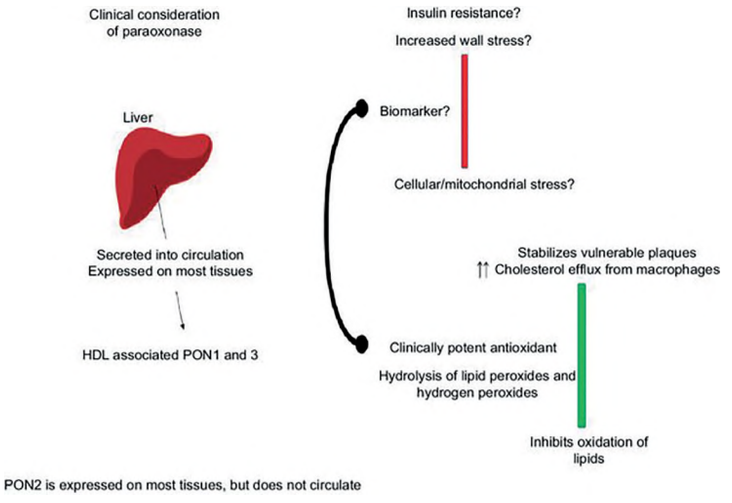

Figure 3 – *Three different PONs in humans. The liver secretes HDL-associated PON1 and 3 into the systemic circulation. PONs have antioxidant properties, stabilize vulnerable atherosclerotic plaques, and limit plaque rupture, with the potential limitation of atherosclerotic cardiovascular disease. PONs have the potential to serve as a biomarkers for cellular stress, vascular wall stress and insulin resistance. Abbreviations: HDL, high-density lipoprotein; PON, paraoxonase [22]*

targets for scavenger-based prophylaxis because of the low efficacy of pharmacological drugs used to counteract their toxicity. They are applied as racemates, but it is their S_p isomers that are the tangible threat. Unfortunately, enzymes tested thus far primarily hydrolyze the less toxic R_p isomer. In the publication [24], the authors report the engineering of enzyme variants with highly improved catalytic efficiencies toward the toxic S_p isomer of cyclosarin. They chosed the mammalian serum paraoxonase PON1 as starting point. It hydrolyzes G-agents at low catalytic efficiencies and reacts primarily with their R_p isomer [25].

The authors used a recombinant variant dubbed rePON1-G3C9 (referred to as rePON1 hereafter), which, unlike human PON1, is expressed functional and soluble in *Escherichia coli*. Its amino acid sequence is 94% and 85% identical to rabbit and human PON1s, respectively. The enzymatic specificity of rePON1 is essentially identical to human PON1, yet its stability is much improved. After an application of both random and targeted mutagenesis to rePON1 and screened mutants for organophosphate hydrolysis the authors enhanced PON1's activity toward the toxic S_p isomer of a coumarin analog of cyclosarin by ~10^5-fold. The authors were thus able to isolate variants that hydrolyze the S_p-coumarin analog of cyclosarin and cyclosarin itself with k_{cat}/K_M ~10^7 M^{-1} min^{-1}. They demonstrated the in vivo prophylactic activity of an evolved variant by showing, in mice, that it can provide considerable protection from a lethal dose of S_p-CMP-coumarin, and the newly developed screens provide the basis for engineering PON1 prophylactics against other G-type nerve agents [24, 26].

In the publication [27] in the part "Significance" the authors claim: "We describe the enzyme variants that hydrolyze the toxic isomers of G-type nerve agents with sufficiently high rates to detoxify these agents in vivo, such that prophylactic protection could be potentially achieved upon administering low enzyme doses. Following only four rounds of directed evolution, we obtained variants that were improved up to 340-fold relative to our previously described GF-hydrolyzing variant. Overall, these variants show 40- to 3,400-fold higher catalytic efficiencies than wild-type PON1 for hydrolyzing the toxic isomers of the three most toxic G-agents (GB, GD, and GF). Their k_{cat}/K_M values for GD and GF neutralization are particularly high: $3–5\times10^7$ M^{-1} min^{-1}. Although lower by about an order of magnitude, their catalytic efficiencies with GA and GB may still enable effective prophylaxis because of the lower toxicity of these agents. This work, therefore, demonstrates that, despite tradeoffs between different substrates, catalytically efficient broad-spectrum, generalist enzymes can evolve. Although the evolved variants show relatively broad specificity with respect to the substrate's O-alkyl group, their stereospecificity is remarkably high. Screening for variants that hydrolyze the toxic Sp-isomers resulted in possibly the highest recorded shift in enantiomeric preference between the wild-type enzyme and its evolved variants ($E^{evolved}/E^{wild}$-type > 10^6). Overall, his work describes promising candidates for in vivo tests of prophylaxis and postexposure treatment and a general scheme for the evolution of highly active OP hydrolyzing enzymes that could detoxify a broad range of nerve agents and pesticides. It demonstrates the powers of laboratory evolution

and its ability to completely reshape the catalytic activity and substrate specificity, as well as the stereoselectivity of enzymes."

In a 2016 publication [28] the authors evaluated the ability of evolved paraoxonase-1 (PON1) to afford broad spectrum protection against G-type nerve agents when produced in mammalian cells via an adenovirus expression system. The PON1 variants G3C9, VII-D11, I-F11, VII-D2 and II-G1 were screened in vitro for their ability to hydrolyze G-agents, as well as for their preference towards hydrolysis of the more toxic S_p(-) isomer. The variant I-F11 was found to be a leading candidate for further evaluation. The authors demonstrated broad-spectrum efficacy of I-F11 against G-agents, and a sequential 5 × LD_{50} dose of GD, GF, GB, and GA was administered to ten mice expressing I-F11 on days 3, 4, 5 and 6 following virus injection. At the conclusion of the experiment, 80% of the animals survived exposure to all four G-agents. Using the concept of stoichiometric efficacy, the authors determined that I-F11 affords protection from lethality against an administered dose of 10, 15, 90 and 80 molar equivalents of GA, GB, GD, and GF, respectively, relative to the molar equivalents of I-F11 in circulation. It appears that I-F11 can associate with high density lipoprotein in circulation, suggesting that I-F11 retained this function of native PON1. This combination of attractive attributes demonstrates that I-F11 is an attractive candidate for development as a broad-therapeutic against G-type nerve agent exposure.

In the "evolutionary" last, publication [1] the authors announced: "A onetime administration of AAV8 carrying PON1-IF11 gene (AAV8-PON1-IF11) resulted in high expression and secretion of PON1-IF11 recombinant protein in the circulation and conferred asymptomatic protection against multiple lethal doses of all G-type CWNAs for at least 5 months." The study clearly unfolds avenues to develop a one-time application of gene therapy to express a near-natural and circulating therapeutic PON1-IF11 protein that can potentially

Table 1 – *Authors of the publication [1]. Their affiliation (in 2020) and (if possible to find) research interests*

Author	Affiliation	Area of Interest	Source
Venkaiah Betapudi	Research and Development Division, Countering Weapons of Mass Destruction, U.S. Department of Homeland Security, 1120 Vermont Ave., Washington, DC 20005, USA	Demonstration of lentivirus and adeno-associated virus-mediated gene therapy to control tumor growth and confer protection against pesticides and toxic chemicals in mice	https://www.researchgate.net/profile/Venkaiah-Betapudi
Reena Goswami	Medical Toxicology Research Division, Biochemistry & Physiology Department, Agent Mitigation, United States Army Medical Research Institute of Chemical Defense, 8350 Ricketts Point Road, Aberdeen Proving Ground, MD 21010-5400, USA	–	https://pubmed.ncbi.nlm.nih.gov/?term=Reena+Goswami
Liliya Silayeva	ADS Federal, 4401N. Fairfax Dr., Suite 321, Arlington, VA 22203, USA	PhD PostDoc Position at US Army Medical Research Institute of Chemical Defense	https://www.researchgate.net/profile/Liliya-Silayeva
Deborah M. Doctor	Medical Toxicology Research Division, Biochemistry & Physiology Department, Agent Mitigation, United States Army Medical Research Institute of Chemical Defense, 8350 Ricketts Point Road, Aberdeen Proving Ground, MD 21010-5400, USA	–	https://pubmed.ncbi.nlm.nih.gov/31969483/
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protect humans against G-type chemical warfare nerve agents for several weeks to months.

For us it is interesting to check the Acknowledgement: "We thank … Deputy Director of Research, USAMRICD, for suggestions over the manuscript. The views expressed in this article are those of the author(s) and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government. ORISE participant L.S. was supported by an appointment to the Internship/Research participation program for the USAMRICD, administered by ORISE through an agreement between the U.S. Department of Energy and the U.S. Army Medical Research and Materiel Command. Funding: This research work was supported by the Defense Threat Reduction Agency (DTRA) (CB3945), Joint Science and Technology Office, Medical S&T Division, Department of the Army." Two authors declare to have competing interest in U.S. patent no. PCT/US2018/023746, titled "A method developing and employing recombinant adeno-associated virus-F11 particles for prophylactic protection against G-type chemical warfare nerve agents"[5]. The names of all authors [1], their affiliation and research interests are shown in the *Table 1*.

This patented construct is shown in detail[6] (and in *Figure 4* and *Figure 5*).

Here we would like to suggest another possible approach. The mesenchymal stem cells (MSC) are

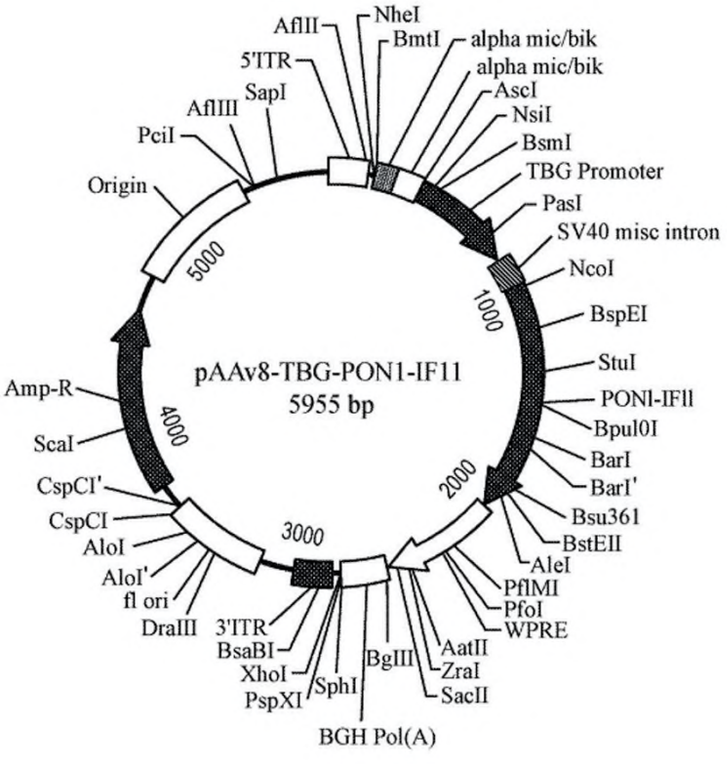

Figure 4 – *A circular map of viral plasmid expression vector carrying PON1-IF1 1 under TBG promoter. AAV8 expression vector carrying PON1-IF11 under a liver specific-thyroxine binding globulin (TBG) promoter was constructed. This vector carries viral sequences-5' inverted terminal repeat (5' ITR) and 3' inverted terminal repeat (3' ITR) to help in inserting the TBG-PON1-IF11 cassette in the viral genome. The figure is taken from Chilukuri N, Betapudi V. Recombinant adeno-associated virus-paraoxonase 1-ifii particles and the methods of making and using thereof. WO2018175712. Publication Date 27.09.2018. Priority Data 62/475,502. 23.03.2017 US. The Government of the United States of America as Represented by the Secretary of the Army [US]/[US]. https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2018175712&_cid=P11-LRWDGI-84299-1; date: 10.12.2023 (part drawings)*

[5] Chilukuri N, Betapudi V. Recombinant adeno-associated virus-paraoxonase 1-ifii particles and the methods of making and using thereof. WO2018175712. Publication Date 27.09.2018. Priority Data 62/475,502. 23.03.2017 US. The Government of the United States of America as Represented by the Secretary of the Army [US]/[US]. https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2018175712&_cid=P11-LRWDGI-84299-1 (date: 10.12.2023).

[6] Ibid.

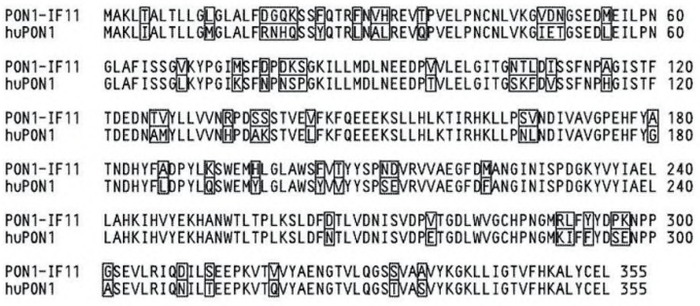

Figure 5 – *Amino acid sequence alignment of wild type human PON1 (SEQ ID NO: 7) with PON1 -IF11 (SEQ ID NO: 6). PON1-IF11 is a variant of the wild type human PON1. The figure is taken from Chilukuri N, Betapudi V. Recombinant adeno-associated virus-paraoxonase 1-ifii particles and the methods of making and using thereof. WO2018175712. Publication Date 27.09.2018. Priority Data 62/475,502. 23.03.2017 US. The Government of the United States of America as Represented by the Secretary of the Army [US]/[US]. https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2018175712&_cid=P11-LRWDGI-84299-1; date: 10.12.2023 (part drawings)*

rather well characterized cells [29]. The MSC have been genetically modified ("engeneered") (*Figure 6*) and used to treat cancer in experiments performed on animals, mainly rodents [30]. However, in humans these attempts failed due to lack of migration of (genetically modified) MSC into the tumors [31].

According to [1] PON1-IF11 gene (*Figure 5*) from pENT-CMV adenoviral vector was cloned into AAV8 shuttle plasmid containing three different types of promoters: TBG (AAV8-TBG-PON1-IF11) (*Figure 4*), synthetic CASI promoter (AAV8-CASI-PON1-IF11), and CMV (AAV8-CMV-PON1-IF11). The AAV8 vector used with the CMV promoter was a self-complementary type [32]. Further, in the experiments [1], mice were given 100 to 200 µl of phosphate-buffered saline (PBS) containing 5×10^{13} to 9.7×10^{13} GC/ml of either AAV8-PON1-IF11 or control AAV8 (lacking the PON1-IF11 gene) through tail vein injections. We are considering this approach in humans not reliable. First, the biggest concern of the authors in the study [32] using AAV8 vector has been the toxicity (hepatocellular injury). Two of the six patients required immunosuppressive steroid therapy [32]. Second, there has been a drop of the protein expression (factor IX) in all 6 patients. The levels remained in 2–11% range of the normal levels. In [33] the authors claim:

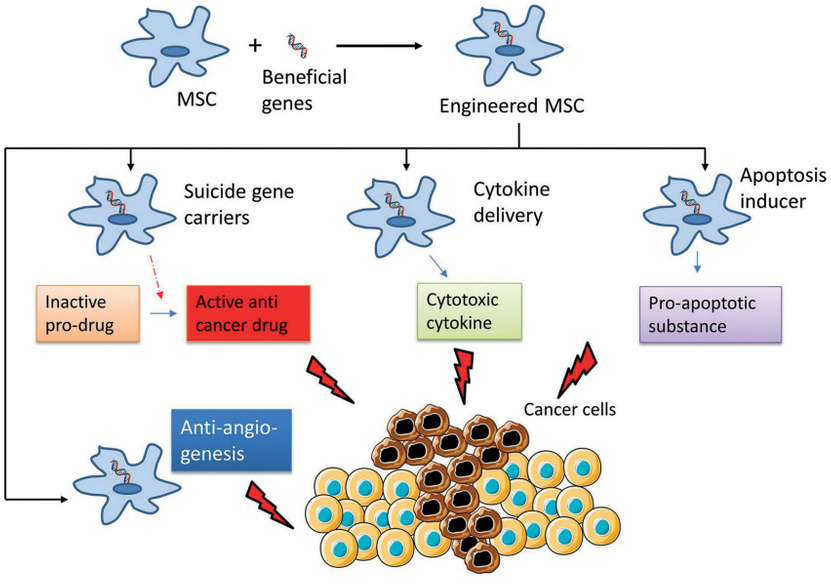

Figure 6 – *Mesenchymal Stem Cells for cancer treatment. Genetically modified MSCs are expressing "suicide genes" which can activate an inactive pro-drug to active anti-cancer drug; cytotoxic cytokines or apoptosis inducer that can kill cancer cells. The figure is taken from [30]*

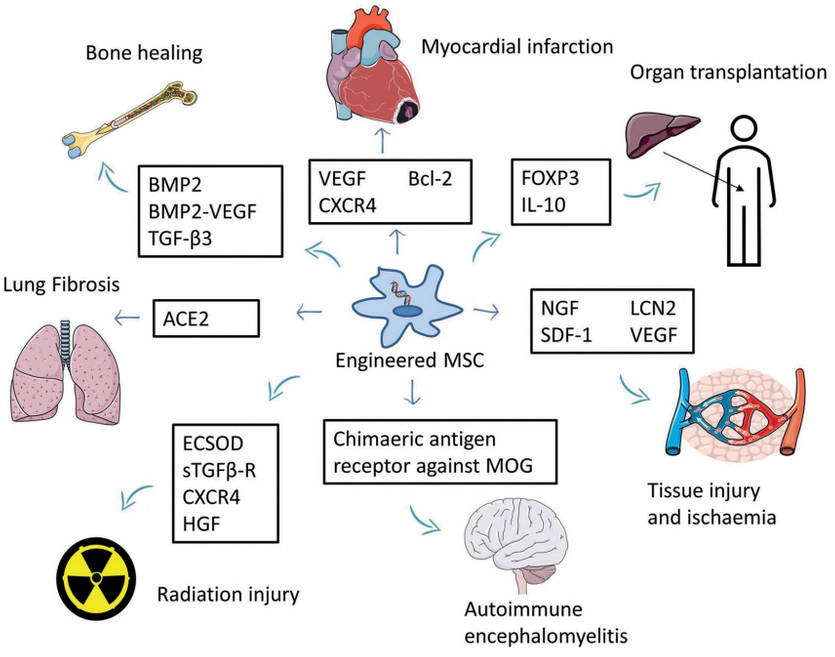

Figure 7 – *The use of engineered MSCs for the treatment of non-cancer diseases. MSCs overexpressing beneficial factors can be used to treat several conditions such as myocardial infarction, tissue injury, ischemia, and autoimmune encephalomyelitis. Engineered MSCs were also used to improve bone healing and reduce the side effects of organ transplantation. The figure is taken from [30]*

"However, ongoing challenges hamper wider use of rAAV vector-mediated therapies. These include immunity against rAAV vectors, limited transgene packaging capacity, sub-optimal tissue transduction, potential risks of insertional mutagenesis and vector shedding."

In our opinion these obstacles can be overcome with the use of MSC. The MSCs can be genetically modified to enhance their therapeutic effect in human medicine (*Figure 7*) [30]. It has been shown that the genetically modified MSCs in humans are dominantly homing in the bone marrow [34]. There are practically no immunological barriers in the use of allogeneic MSC [29]. So, for the further "human targeted" aims, the allogeneic MSC can be genetically modified with different variants of the enzyme paraoxonase 1. The stable expression and enhanced enzymatic activity of the secreted mutated PON1 must be proven by testing in vitro. Stable clones of MSCs expressing PON1 can be selected, grown up and applied to the volunteers to follow the long term PON1 expression profile. And finally, the "off shelf" genetically modified allogeneic MSCs can be applied to the civilians and soldiers to enhance their resistance to OPs. We find this approach more physiological and natural.

Conclusion

In the present paper we explained the current approach to neutralize the effects of OP agents in vivo. This approach is aimed to:

i) to enhance the activity of naturally occurring enzymes able to detoxicate OP agents;

ii) to clone these enzymes into "vectors," in our opinion in the MSCs;

iii) to introduce these stable gene constructs in human organism;

iv) and, finally to insure their stable (over) expression in men.

This resistance to OP agents can be health protective and lifesaving in soldiers in real combat when the enemy uses these agents. However, this approach must be based on a strong experimental background. The door is open, the technologies are available.

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Elaboration of the concept of the paper; collection, analysis, and systematization of scientific literature; writing

and edition of paper / Разработка концепции статьи; сбор, анализ и систематизация научной литературы; написание статьи.

Author's Statement / Заявление автора

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The article has been double blind peer reviewed by two experts in the respective field. Peer reviews are available from the Editorial Board and from Russian Science Citation Index database / Статья прошла двустороннее анонимное «слепое» рецензирование двумя рецензентами, специалистами в данной области. Рецензии находятся в редакции журнала и в РИНЦе.

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